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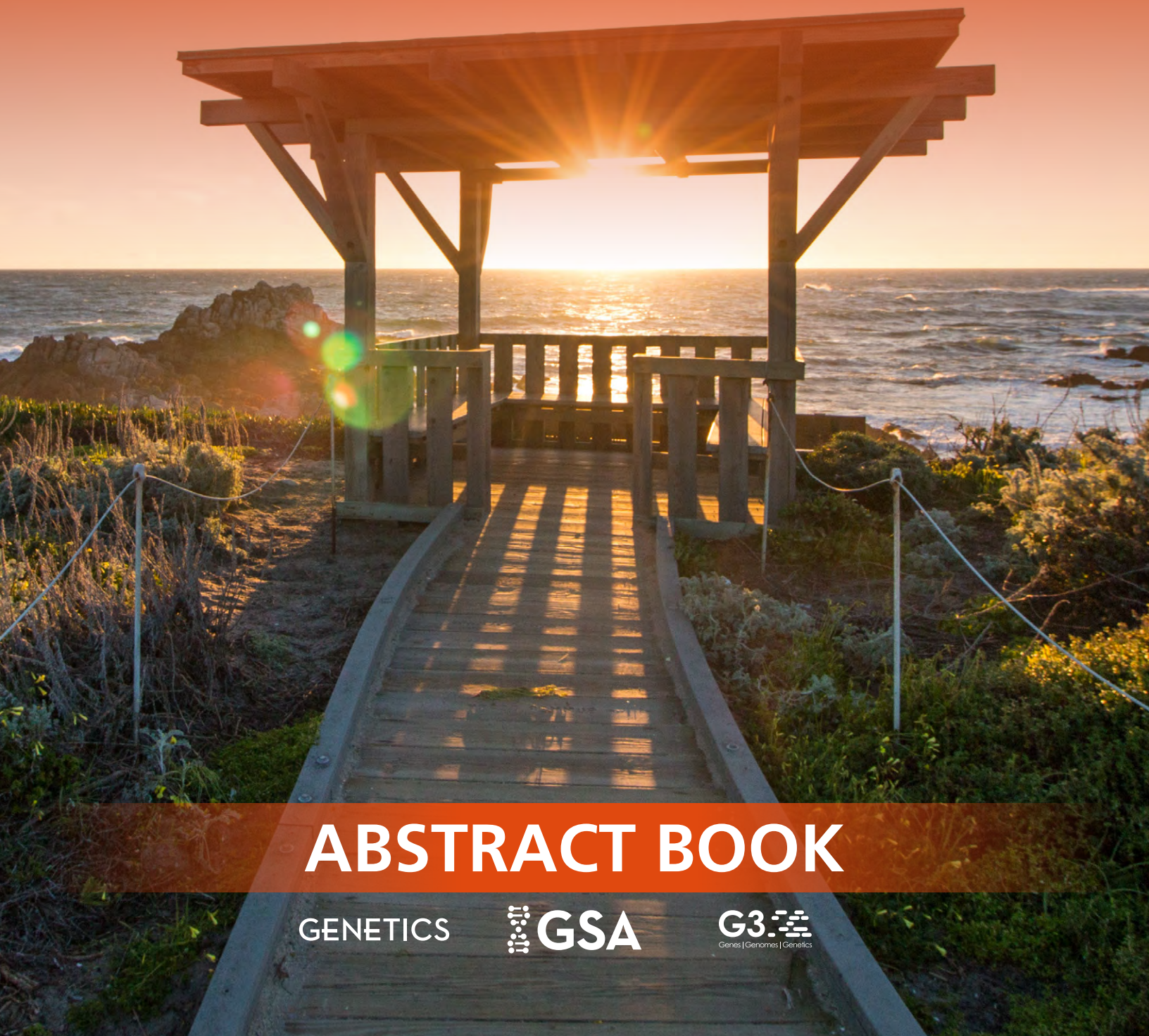

ABSTRACT BOOK

GENETICS GSA G3 Genes | Genomes | Genetics

1 Trade-off between Plasticity and Velocity in Mycelial Growth *Norio Takeshita*[1] 1) University of Tsukuba.

Tip-growing fungal cells maintain cell polarity at the apical regions and elongate by *de novo* synthesis of the cell wall. Cell polarity and tip growth rate affect mycelial morphology. However, it remains unclear how both features act cooperatively to determine cell shape. Here, we investigated this relationship by analyzing hyphal tip growth of filamentous fungi growing inside extremely narrow 1 µm-width channels of microfluidic devices. Since the channels are much narrower than the diameter of hyphae, any hypha growing through the channel must adapt its morphology. Live-cell imaging analyses revealed that hyphae of some species continued growing through the channels, whereas hyphae of other species often ceased growing when passing through the channels, or had lost apical polarity after emerging from the other end of the channel. Fluorescence live-cell imaging analyses of the Spitzenkörper, a collection of secretory vesicles and polarity-related proteins at the hyphal tip, in *Neurospora crassa* indicates that hyphal tip growth requires a very delicate balance of ordered exocytosis to maintain polarity in spatially confined environments. We analyzed the mycelial growth of seven fungal species from different lineages, including phytopathogenic fungi. This comparative approach revealed that the growth defects induced by the channels were not correlated with their taxonomic classification or with the width of hyphae, but, rather, correlated with the hyphal elongation rate. This report indicates a trade-off between morphological plasticity and velocity in mycelial growth and serves to help understand fungal invasive growth into substrates or plant/animal cells, with direct impact on fungal biotechnology, ecology, and pathogenicity.

2 The exocytic RAB11 pathway *Miguel Penalva*[1] 1) CSIC Centro de Investigaciones Biológicas.

RAB11 is crucial for the hyphal mode of growth. It governs the transition from Golgi to post-Golgi identity at the TGN, promoting the biogenesis of secretory vesicles (SVs) that will reach the apical plasma membrane by way of the Spitzenkörper (SPK). RAB11 gradually builds up on TGN cisternae until its levels are high enough to promote the budding of SVs, which engage molecular motors to be transported both to the SPK and to nascent septae. SVs are charged with dynein. myosin-5 (alias MyoE) and kinesin-1 (alias KinA). cooperate in a relay run-like mechanism to mediate the acropetal transport of SVs, whereas the role of dynein (alias NudA) and associates is not yet understood, although the fact that it moves vesicles departing from the SPK with basipetal trajectories appears as strongly supportive of Salomon Bartinicki-García's model of the SPK as a vesicle supply center. RAB11 accumulation at the TGN is determined by the previous and also gradual recruitment to the TGN of TRAPPII, the somewhat mysterious 1 MDa complex that mediates nucleotide exchange on the GTPase. As any other RAB, RAB11 exerts its roles by recruiting to membranes specific sets of proteins (denoted effectors) that are otherwise cytosolic, because they lack the means to be recruited to membranes by themselves. One of our aims is characterizing the whole set of effectors subordinated to RAB11. One such effector, BapH, connects the SPK with autophagy. Another is myosin-5 itself, which binds the GTPase loaded on SVs through its cargo-binding, globular C-terminal tail domain. This molecular research was inspired by seminal classical genetics work on hyphal morphology carried out by Steve Harris, John Hamer and Susan Kaminskyj at the end of the 20th century, ending in the identification of *hypA* as Trs120, the key component of TRAPPII.

3 A little key will open a large door: unexpected pleiotropic roles of fungal surface-active proteins in fungal cells *Irina Druzhinina*[1] 1) Nanjing Agricultural University.

The rapid modulation of the body surface hydrophobicity is essential for the fungal lifestyle because absorptive nutrition requires a hydrophilic surface, while nonmotile aerial spores need to be hydrophobic. The hydrophobicity of the spore or hyphal cell wall influences their biotic and abiotic interactions, such as adhesion to substrates and also symbiotic partnerships. One billion years of evolution of filamentous fungi has resulted in molecular adaptations to the physicochemical challenges associated with their lifestyle. For example, filamentous fungi are able to secrete the unique amphiphilic and superior surface-active small secreted cysteine-rich proteins (saSSCPs) – hydrophobins (HFBs), cerato-platanins (CPs), and the others – that self-assemble at hydrophobic/hydrophilic interfaces and thus modulate surface properties.

Using the saSSCP-enriched mold *Trichoderma* (Hypocreales, Ascomycota) and the HFB-free yeast *Pichia pastoris* (Saccharomycetales, Ascomycota), we studied functions, ultrastructure, and the cellular localization of HFBs and CPs. Our results demonstrated that the rapid release of HFBs by aerial hyphae shortly prior to conidiation is associated with their massive intracellular accumulation in vacuoles and/or lipid-enriched organelles. The tonoplast-like HFB-enriched vacuolar structures contribute to the maintenance of turgor pressure in aerial hyphae, supporting the erection of sporogenic structures (e.g., conidiophores) and providing intracellular force to squeeze out other HFB-enriched vesicles from the periplasm through the cell wall of aerial hyphae. This secretory mechanism results in the formation of an extracellular HFB-enriched matrix, which is required for the even spore coating by HFBs through a mechanism that involves microscopic water droplets. Furthermore, HFB4 in *T. guizhouense* controls spore dormancy and contributes to the water sensing mechanism required to detect optimal germination conditions. However, in *T. harzianum,* the functional loading of HFB4 is not apparent compared to that in *T. guizhouense*, but *hfb4* evolves under strong positive selection. The deletion of the *hfb4* gene in *T. harzianum* and *T. guizhouense*, respectively, increased the fitness scores of the first species and reduced it for the second one. Interestingly, the secretion of CPs that is associated with submerged growth surprisingly resembles HFBs (an intracellular retention step) indicating the possibility for a dedicated unconventional secretory pathway for saSSCPs in fungal hyphae.

Thus, saSSCPs essentially contribute to the control of fungal development and reproduction because they are involved in dispersal, stress resistance, and other fitness-related traits. In this talk, I will show that saSSCPs have a range of pleiotropic functions not only outside of fungal bodies but also inside hyphae and spores.

4 Role of the fission yeast NDR kinase Orb6 in the response to environmental stress *Fulvia Verde*[1] 1) University of Miami Miller School of Medicine.

The conserved NDR kinase plays a key role in the control of cell morphology and cell proliferation in organisms ranging from yeast and filamentous fungi to human cells. We have previously discovered that fission yeast NDR kinase Orb6 spatially regulates the activity of Cdc42 GTPase, a key morphology control factor, to promote cell shape emergence. Orb6 also inhibits the degradation of specific mRNAs, thereby promoting polarized cell growth. We find that Orb6 kinase activity is downregulated by a variety of stimuli, such as

nutritional deprivation or osmotic stress, suggesting that the Orb6 kinase pathway mediates cellular responses to environmental stress. Using genomic-scale and proteomic approaches we have identified novel targets of Orb6 kinase, and discovered a role for Orb6 kinase in promoting cell adaptation and chronological lifespan. We propose that an important role of NDR kinase in eukaryotic cells is to enable alternative physiological states, from active cell growth to cell quiescence, to promote cell resilience in the face of stress.

5 Temperature adaptation of biological phase separation *Amy Gladfelter*[1], Benjamin Stormo[1], Ian Seim[1], Ammon Posey[3], Fred Dietrich[2], Rohit Pappu[3] 1) UNC Chapel Hill; 2) Duke University; 3) Washington University at St. Louis.

Free-living microbes, plants and cold-blooded organisms survive in the face of temperature fluctuations that arise across many time scales. Climate change is increasing the amplitude and frequency of temperature variations in the natural world and biological phase separation may be a key mechanism of adaptation of the biosphere to climate change. The focus of this presentation will be on our recent work to identify how protein and RNA sequence encodes temperature sensitivity and how material properties of biomolecular condensates are maintained across temperatures fluctuations. For these studies, we focus on a model phase separation protein, Whi3, in the syncytial ascomycete fungus, *Ashbya gossypii.* This protein binds to and regulates specific RNAs important for cell cycle control and cell polarity. We have found natural sequence variation within the core protein/RNA components are sufficient to induce highly tunable temperature sensitivity for condensation. Sequence elements controlling protein-protein, protein-RNA and RNA-RNA interactions all contribute to modulating higher-order assembly and function in different temperature regimes. These studies indicate that small changes in protein and RNA sequences can promote organism adaptation to different climates providing potential mechanisms for adaptation of the biosphere to climate change.

6 Immune recognition of fungi: deciphering the writing on the wall *Neil Gow*[1] 1) University of Exeter .

The cell wall of a fungus is an exoskeleton composed of unique molecules that are unique to fungal cells and which are used by the immune system to induce antifungal responses. Immune surveillance and defence against potential fungal pathogens is based on the recognition of this suite of molecules in the fungal cell wall that are recognised by pattern recognition receptors of the innate immune system. Differences in the cell wall composition of different fungi and or the same fungus organisms growing in different morphologies and in differing environments generates a moving target for immune recognition. We have used a variety of microscopic, forward and reverse genetic and immunological tools to generate a new spatially accurate model of the cell wall and to explore how dynamic changes in the wall influence immune surveillance. We show that immune relevant epitopes can be diffuse or clustered, superficial or buried in the cell wall and they changed during batch culture and between yeast, hypha and other cellular morphologies. We have ask screened libraries of mutants with immune pattern recognition receptors (PRRs) to define the sub set of fungal genes that assemble and regulate immune epitopes. This is revealing novel processes that are important for the assembly of the PRR-ome. These experiments demonstrate that the fungal cell surface is ordered, complex and dynamically changing, making immune recognition a challenging process requiring the concerted action of multiple receptors operating singly and in combination. My presentation will focus on this work that demonstrates that describes recent advances that have generated a scaler model of the cell wall and show it behaves as an ordered and dynamically changing organelle that makes immune recognition a challenging process. Immune recognition requires the concerted action of multiple receptors operating singly and in combination.

7 Unmasking chitin in *C. neoformans*: Panic or protection? *Rajendra Upadhya*[1], Woei, C Lam[1], Jennifer, K Lodge[1] 1) Department of Molecular Microbiology, Washington University School of Meidicne, St. Louis.

Chitosan is an important component of the cell wall of *Cryptococcus neoformans*. It is essential for maintaining the integrity of the cell wall during *in vitro* growth and under a variety of environmental stress conditions. Most importantly, it is required for fungal virulence. Three distinct isoforms of chitin deacetylase (CDA) have been identified as being responsible for chitin «masking» via deacetylation to chitosan. The choice of a specific deacetylase for virulence in mammalian infection depends on the species of *Cryptococcus*; in *C. neoformans*, Cda1 is the major deacetylase, whereas in *C. gattii*, Cda3 plays an important role in the conversion of chitin to chitosan during infection. Furthermore, the involvement of specific deacetylases is dependent on the environment in which cryptococcal cells grow. While all three CDAs are dispensable during *in vitro* growth, coordinated activity of both Cda1 and Cda2 is required for fungal virulence in *C. neoformans*, whereas in *C. gattii*, Cda3 alone is sufficient for causing virulence. We show that the culture medium has a significant effect on chitosan biosynthesis. When compared to yeast extract, peptone and dextrose (YPD) grown cells, yeast grown in unbuffered yeast nitrogen base (YNB-U) medium had a 90% reduction in chitosan. As we discovered, *C. neoformans* also alters the pH of the medium during growth. When grown in unbuffered YPD, it raises the pH to alkalinity, but when grown in YNB-U, it lowers the pH to acidity. When YNB-U grown cells were compared to YPD or YNB, pH 7, the decrease in chitosan was associated with a significant increase in pathogen-associated molecular patterns (PAMPs) on the cell surface. When tested in a murine infection model, the altered cell wall architecture resulted in a significant reduction in virulence. Furthermore, when heat-killed cells were used for infection, KN99 grown in YNB-U caused an abnormal hyper-inflammatory response in the lungs, resulting in the death of the animals. Heat-killed KN99 cells grown in YNB, pH 7, on the other hand, caused little to no inflammatory response in the host lung, but when used as a vaccine, they conferred a robust protective response against a subsequent challenge infection with the virulent KN99 cells. These findings highlight the importance of chitin and its chitosan derivative in shaping the organization of the *C. neoformans* cell wall, impacting fungal virulence and pathogenicity.

8 *Candida albicans* and IL-17A stimulate cytokine production by oral epithelial cells via different mechanisms *Jianfeng LIN*[1], Quynh Trang[1], Hong Liu[1], Sarah Gaffen[2], Scott Filler[1,3] 1) The Lundquist Institute, Torrance, CA; 2) University of Pittsburgh, Pittsburgh, PA; 3) University of California- Los Angeles, Los Angeles, CA.

IL-17 signaling components (IL-17A, IL-17RA, IL-17RC, ACT1, etc.) are critical for the host defense against oropharyngeal candidiasis (OPC). Both IL-17A and *Candida albicans* stimulate oral epithelial cells to secrete pro-inflammatory cytokines. We investigated how these two different stimuli induced this pro-inflammatory response. Using indirect immunofluorescence, we found that the IL17RA and IL17RC co-localize with the epidermal growth factor receptor (EGFR) around the *C. albicans* hyphae on the OKF6/Tert-2 oral epithelial cell line. Although IL-17A and *C. albicans* cells both stimulated epithelial cells to secrete pro-inflammatory cytokines such as IL-8 and

GM-CSF, they do so through distinct mechanisms. Inhibition of EGFR with either gefitinib or an anti-EGFR antibody reduced IL-8 production in response to both *C. albicans* and IL-17A. However, knockdown of *IL-17RA* or *ACT1* with siRNA or knockout via CRISPR abolished IL-8 production induced by IL-17A, but not *C. albicans*. RNA-seq analysis revealed that IL17A and *C. albicans* induce the expression of distinct sets of genes. Strikingly, only *C. albicans* infection caused significant upregulation of genes encoding transcription factors encoding such as *c-FOS*, *c-JUN*, *NF-IL6*, *NFκB1*, and *NFκB2*, which in-turn resulted in massive up-regulation of pro-inflammatory cytokine gene mRNAs including *IL-6*, *IL-8*, *CSF2*, and *CSF3*. Although IL-17A induced low levels of pro-inflammatory cytokine transcripts relative to *C. albicans*, both stimuli induced similar levels of cytokine proteins. Intriguingly, transcripts of the mRNA binding proteins TTP and BRF1 were significantly upregulated in response to *C. albicans,* but not IL-17A. Knockdown of *BRF1,* dramatically increased *C. albicans*-induced IL-8 production without changing the *IL-8* transcript levels. By contrast, *BRF1* knockdown did not affect *IL-8* transcript or IL-8 protein levels in response to IL-17A. Single-molecule fluorescent in-situ hybridization (sm-FISH) analysis in oral epithelial cells infected with *C. albicans* demonstrated that *IL-8* mRNA does not associate with BRF1, indicating a yet unknown role of BRF1 in *C. albicans*-induced production of IL-8. Thus, *C. albicans* stimulates proinflammatory cytokine production in oral epithelial cells by inducing a strong transcriptional response that is modulated by BRF1 whereas IL-17A induces a weaker transcriptional response that is not affected by BRF1.

9 The ephrin tyrosine kinase receptor, EphA2, serves as a gateway for *Cryptococcus neoformans* into the central nervous system. Suvidha Menon[1], *Amelia Bennett*[1], Dylan Lanser[1], Kiem Vu[1], Angie Gelli[1] 1) University of California, Department of Pharmacology, SOM, Davis.

Systemic fungal disease can be life-threatening for individuals with a compromised immune system. Among the most devasting are fungal brain infections caused primarily by *Cryptococcus neoformans* (*Cn*). Following inhalation of its spores from the environment, *Cn* enters the lung where it can cause a pneumonia-like illness and subsequently disseminate to the brain. Several studies have demonstrated that *Cn* can move freely in the bloodstream (or co-opt monocytes), lodge within the lumen of capillaries and cross the blood-brain barrier (BBB). We initially examined the transcriptome of brain microvascular endothelial cells exposed to *Cn* in an *in vitro* model of the human BBB. Upon mapping the transcriptome to known canonical signaling, we identified the EPH-EphrinA1 (EphA2) tyrosine kinase signaling pathway and demonstrated that the EphA2 receptor mediated the migration of *Cn* across the BBB in a CD44-dependent manner. Silencing the EphA2 transcript or inhibiting EphA2 activity with an antibody or an inhibitor prevented *Cn* from crossing the BBB, whereas activation of EphA2 with the ephrinA1 ligand or an agonist (doxasozin) enhanced crossing of *Cn*. The EphA2 receptor was phosphorylated during *Cn* infection, but phosphorylation was prevented by dasatinib, consistent with less cryptococci crossing the BBB when treated with dasatinib. Localization studies of *Cn* and EphA2 in human brain endothelial cells, live-cell recording of HEK293T cells expressing EphA2, and protection assays demonstrated a clear association between *Cn* and EphA2, consistent with a role for EphA2 in internalizing *Cn*. Animal studies involving *EphA2-/-* knockout mice have demonstrated that the lack of EphA2 is protective consistent with our *in vitro* data. Our working model proposes that *Cn* associates with CD44 on the luminal side of the BBB and induces EphA2 phosphorylation via a CD44-mediated transactivation of EphA2. Once activated, EphA2 may promote signaling that reorganizes the actin cytoskeleton and internalizes *Cn* via endocytosis/macropinocytosis. We are currently identifying the EphA2 interactome by proximity-dependent labeling and investigating EphA2's role in regulating vesicular traffic of *Cn* across the BBB through the use of BBB spheroids and *EphA2-/-* knockout mice. These studies may identify novel drug targets to prevent fungal brain infections, and assist with better informed design strategies for technologies geared toward crossing the BBB and delivering cargo to the brain.

10 A ricin-like toxin derives tissue necrosis during invasive mucormycosis *Ashraf Ibrahim*[1,2] 1) The Lundquist Institute at Harbor-UCLA Medical Center, Torrance, CA; 2) David Geffen School of Medicine, Los Angeles, CA.

Mucormycosis, caused by Mucorales fungi, is the third most common invasive fungal infection in patients with hematological malignancies and organ transplants. Known to have high morbidity and mortality, mucormycoses are characterized by the propensity of fungi to angioinvasion and causing tissue necrosis. Our studies showed that CotH cell surface proteins are required for pathogenesis of mucormycosis by facilitating invasion to nasal and alveolar epithelial cells and vascular endothelial cells. Our more recent studies identified a hyphae-specific protein toxin that is also critical to driving host cell death and tissue necrosis. Specifically, purified or recombinantly expressed toxin cause host cell death and moribundity in mice. Furthermore, attenuating the expression of the toxin in *Rhizopus* (the most common cause of mucormycosis) results in reduced fungal virulence *in vitro* and *in vivo*. Importantly, antibodies targeting the toxin nullify its ability to cause host cell damage and protect mice from mucormycosis. The toxin has structural and functional features of ricin-like toxins including conserved ricin B chain, putative functional domains for ribosomal inhibition activity, vascular permeability and N-glycosylase activity. Consequently, we named this toxin "Mucoricin". In addition to its clinical importance and the potential of devising therapeutic strategies against this toxin, the discovery of Mucoricin demonstrates that ricin-like toxins can be produced by organisms beyond the plant and bacterial kingdoms.

11 Roles of *Candida albicans* chromosome instability in the host *Huijuan Yan*[1], Suzanne Noble[1] 1) University of California, San Francisco.

Candida albicans is a fungal pathobiont that colonizes the gastrointestinal tract of most healthy humans. It is also the most common cause of fungal infectious disease. Recently, our lab discovered that *C. albicans* maintains two histone-based systems (*H2A.1* and *H2A.2*) that promote chromosome instability under in vitro conditions. I hypothesize that *C. albicans* noncanonical *H2A.1* improves fungal fitness in at least one host niche. Consistent with this hypothesis, I found that WT *C. albicans*, which expresses two copies of noncanonical *H2A.1* and two copies of canonical *H2A.2*, is more virulent in a mouse bloodstream infection model than an isogenic all-*H2A.2* strain that exhibits more accurate chromosome segregation under in vitro conditions. In line with this, the all-*H2A.2* strain is also outcompeted by the WT strain in infected kidneys. However, WT and the all-*H2A.2* strains exhibit similar fitness in a mouse model of gut colonization, suggesting that moderate chromosome instability promotes virulence but not commensalism in *C. albicans*. In parallel with this work, I propose to test the hypothesis that *H2A.1* promotes virulence in the bloodstream model by facilitating the acquisition of adaptive aneuploidies in kidneys, the major target organ in this infection model. Monotypic infections will be performed with WT or the all-*H2A.2* strain, and *C. Albicans* recovered from dissociated kidneys will be eval-

uated using single-cell RNA-seq to identify aneuploidies. This study will reveal a role for chromosome instability in fungal adaptation to the host and will identify specific genetic changes that correlate with fitness.

12 Roles for microglia in *Cryptococcus* meningoencephalitis Jacquelyn Nielson[1], *J Muse Davis*[1] 1) University of Iowa.

Cryptococcal infection begins in the lungs, but yeast cells subsequently obtain access to the bloodstream, from which they can reach the central nervous system (CNS). The resulting meningoencephalitis is the most common disease presentation and is very difficult to treat. How this environmental fungus crosses the blood-brain barrier (BBB) and takes up residence in the brain remains a key question in human fungal pathogenesis. We and others have developed the zebrafish larva as a model host for cryptococcosis and demonstrated that hematogenous central nervous infection is replicated therein and rendered accessible to *in vivo* imaging. Here we have examined a large number of infected larvae to understand BBB crossing and the events immediately before and after. We have directly observed systemic macrophages carrying yeast into the brain, but we also find evidence for a novel mechanism in which microglia, the resident phagocytes of the CNS, obtain free yeast from within the vasculature, pulling them across the BBB. Yeast cells are also capable of crossing without the involvement of phagocytes, but this mechanism is not often seen. Regardless of how crossing takes place, we find that in the hours afterwards microglia are the predominant cell type containing *Cryptococcus* in the parenchyma. Depletion of microglia has complex impacts on BBB crossing and pathogenesis. These findings demonstrate previously underappreciated roles for microglia during cryptococcal pathogenesis.

13 Leveraging machine learning essentiality predictions and chemogenomic interactions to identify antifungal targets *Ci Fu*[1], Xiang Zhang[2], Amanda O. Veri[1], Kali R. Iyer[1], Emma Lash[1], Alice Xue[1], Huijuan Yan[3], Cassandra Wong[4], Zhen-Yuan Lin[4], BenJamin VanderSluis[2], Jing Hou[1,5], Yoko Yashiroda[6], Anne-Claude Gingras[1,4], Charles Boone[1,5,6], Teresa R. O'Meara[7], Matthew J.O'Meara[8], Suzanne Noble[3], Nicole Robbins[1], Chad L. Myers[2], Leah E. Cowen[1] 1) Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada; 2) Department of Computer Science and Engineering, University of Minnesota, Minneapolis, MN; 3) Department of Microbiology and Immunology, UCSF School of Medicine, San Francisco, CA; 4) Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada; 5) Donnelly Center, University of Toronto, Toronto, ON, Canada; 6) RIKEN Center for Sustainable Resource Science, Wako, Saitama, Japan; 7) Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI; 8) Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI .

Fungal pathogens pose a global threat to human health, with Candida albicans among the leading killers. Systematic analysis of essential genes provides a powerful strategy to discover potential antifungal targets. Here, we build a machine learning model to generate genomewide gene essentiality predictions for C. albicans and expand the largest functional genomics resource in this pathogen (the GRACE collection) by 866 genes. Using this model and chemogenomic analyses, we define the function of three uncharacterized essential genes with roles in kinetochore function, mitochondrial integrity, and translation, and identify the glutaminyl-tRNA synthetase Gln4 as the target of N-pyrimidinyl-β-thiophenylacrylamide (NP-BTA), an antifungal compound.

14 Alternative Transcription start sites in *Cryptococcus* Tuong Vi Dang Thi[1,2], Corinne Maufrais[1,2], Jessie Colin[1,2,3], *Guilhem Janbon*[1,2] 1) Institut Pasteur; 2) Unversité de Paris; 3) Ecole Pratique des Hautes Etudes, PSL University.

Pathogenic *Cryptococcus* species are responsible for nearly 200 00 death in the word every year. In recent years, we used different RNA sequencing strategies (RNA-Seq, TSS-Seq, 3UTR-Seq) to annotate the genome of three *Cryptococcus* species reference strains thus, characterizing the structure of a typical coding gene in these intron-rich yeasts. Thus, TSS-Seq-based coding gene 5'end annotation revealed the impact of the transcript leader sequence and length on both gene expression and proteome diversity (Wallace et al, NAR 2020). This analysis also revealed the existence of thousands additional transcription start site (TSS) clusters within the coding genes. We here performed additional bioinformatics analysis to characterize the structure of different types of TSS clusters in *Cryptococcus*. Our analysis also showed that several hundred genes bear at least two TSS clusters and the usage of these alternative TSS is largely controlled by growth conditions, thus regulating gene expression and protein diversity. We also identified a transcription factor regulating alternative TSS usage in *Cryptococcus*. Its function in regulating transcriptome structure, *Cryptococcus* biology and virulence will be discussed.

15 Endosomal mRNA transport *Michael Feldbrugge*[1] 1) Heinrich-Heine University.

Spatiotemporal expression is mostly achieved by transport and translation of mRNAs at defined subcellular sites. An emerging mechanism mediating mRNA trafficking is microtubule-dependent co-transport of mRNAs on shuttling endosomes. Although progress has been made in identifying various components of the endosomal mRNA transport machinery, a mechanistic understanding of how these RNA-binding proteins are connected to endosomes is still lacking. Here, I will present a structure function approach on the key RNA-binding protein Rrm4 linking mRNAs to endosomes during hyphal growth of *Ustilago maydis*.

16 The ribonucleoprotein complex components JSN-1 and GUL-1 are involved in asexual development in *Neurospora crassa* *Anne Yenewodage*[1], Inbal Herold[1], Oded Yarden[1] 1) The Hebrew University of Jerusalem .

RNA-binding proteins (RBPs) are critical for the spatial localization, translational regulation and overall fate of mRNAs. In *N. crassa*, the RBP GUL-1 has been shown to bind over 2000 mRNA species, many of which encode genes involved in cell wall integrity. *gul-1* is an extragenic suppressor of the colonial, hyperbranched, *cot-1*(ts) mutant. Despite the role played by GUL-1, only minor morphological changes are observed when *gul-1* is inactivated, suggesting that additional proteins may have overlapping functions with GUL-1. To identify other potential components of the GUL-1 ribonucleoprotein complex (RNP), we used a protein co-immunoprecipitation approach. Four of the over 100 proteins identified harbored hallmarks of RBPs. Inactivation of a gene encoding one of them, JSN-1, resulted in partial suppression of *cot-1* (ts). The epistatic nature of *cot-1* suppression by *gul-1* and *jsn-1* support the possibility of a functional overlap between the two RBPs. Furthermore, both RBPs were found to affect MAK-1 phosphorylation under stress conditions. The increased JSN-1::GFP association with nuclei under stress conditions, as previously observed with GUL-1::GFP, also supports their common presence and possible roles within an RNP. GUL-1 and JSN-1 have additional roles in fungal development. While strains in which either of the two genes had been deleted exhibited normal vegetative reproduction, the Δ*jsn-1*;Δ*gul-1* strain was

impaired in aerial hyphae formation and subsequent conidiation. We concluded that GUL-1 and JSN-1 are components of the same RNP, have partial overlapping functions within the COT-1 pathway and are jointly involved in asexual development.

17 An RNA-binding protein that evolved a change in function to control fungal growth: the surprising history, structure, and function of Ssd1 *Edward W. J. Wallace*[1], Rosemary A, Bayne[1], Elizabeth R. Ballou[2], Uma Jayachandran[1], Aleksandra Kasprowicz[1], Stefan Bresson[1], David Tollervey[1], Atlanta G. Cook[1] 1) The University of Edinburgh; 2) The University of Exeter.

Regulatory pathways evolve to enable organisms to adapt to their environment. In ascomycete fungi, homologous Ssd1/Sts5/gul-1 RNA-binding proteins regulate translation and affect cell growth, cytokinesis, and fungal pathogenicity. The domain structure of Ssd1 resembles that of proteins with a different function: the RNase II/Dis3 family of 3′-5′ exoribonucleases, which play essential roles in RNA degradation. Ssd1 itself has no nuclease activity, making it a “pseudonuclease”. However, the evolutionary origins of Ssd1-like pseudonucleases are unknown: what sequence of evolutionary and structural events led to their novel function, and when did these events occur?

Here, we show how Ssd1-like pseudonucleases are descended from active enzymes in the Dis3L2 subfamily. During fungal evolution, active site mutations in Dis3L2 homologs have arisen at least four times, in some cases following gene duplication. Our new crystal structure of Ssd1 shows that the ancestral RNA-binding “funnel” leading to the active site is blocked by loop insertions, implying emergence of a novel RNA-binding site. In contrast, N-terminal cold-shock domains and regulatory features are conserved across diverse dikarya and mucoromycota. We map the RNA-binding sites of Ssd1 by UV crosslinking and high-throughput sequencing. Our finding that Ssd1 binds near start codons at 5’ ends of mRNA emphasises a different mode of RNA binding from the 3’ terminal interaction reported for Dis3L2. We map the novel RNA-binding site to the cold-shock domains of Ssd1 by showing that mutations to a conserved surface reduce RNA binding *in vitro* and cause cell wall stress sensitivity *in vivo*.

We also show that in the basidiomycete pathogenic yeast Cryptococcus neoformans, the single Ssd1/Dis3L2 homolog is required for cell separation from polyploid “titan” growth stages. This phenotype is consistent with those of inactive fungal pseudonucleases, yet the protein retains an active site sequence signature. We propose that a nuclease-independent function for Dis3L2 arose in an ancestral hyphae-forming fungus, involving RNA-binding on the surface of the cold shock domains. Our work more generally indicates the power of fungal genetics for studying the evolution of proteins and their regulatory functions.

18 Extracellular vesicle-mediated cross-kingdom transport of plant mRNAs into fungal cells to suppress pathogenicity *Hailing Jin*[1], Shumei Wang[1], Baoye He[1], Huaitong Wu[1], Obed Ramirez Sanchez[2], Cei Goodger[2], Paul Birch[3] 1) University of California, Riverside, CA, USA; 2) National Laboratory of Genomics for Biodiversity (Langbio), Cinvestav, Irapuato, 36821 Guanajuato, Mexico.; 3) University of Dundee at James Hutton Institute, Invergowrie, Dundee DD2 5DA, United Kingdom.

Cross-kingdom small RNA trafficking plays a pivotal role in regulating gene expression in the interacting organisms during host and microbe interaction. However, whether messenger RNAs (mRNAs) are also transferred between hosts and microbes is unknown. Here, we discover that host plant Arabidopsis deliver a panel of mRNAs using extracellular vesicles (EVs) into fungal pathogen *Botrytis cinerea*. A fluorescent RNA aptamer reporter system allowed us to visualize the transferred host mRNAs in EVs and in fungal cells. By using Translating Ribosome Affinity Purification (TRAP) profiling in fungal cells isolated from the infected tissue, we observe the delivered host mRNAs associated with active fungal ribosomes, suggesting that they are likely to be translated in the fungal cells. Indeed, proteins of host mobile mRNAs were detected in *B. cinerea.* Arabidopsis knockout mutants of genes encoding these transferred mRNAs were more susceptible to *B. cinerea* infection. Ectopic expression of these mobile host genes in *B. cinerea* attenuates fungal pathogenicity whereas expression of mutated non-translatable versions of these genes failed to reduce infection, which further supports that these mobile host RNAs are translated to prevent disease. Thus, plants have evolved a novel and intricate means using mobile mRNAs, besides mobile small RNAs, to suppress fungal infection.

19 Exclusively RNAi-based antimicrobial drug resistance is inherited after meiosis in the mucormycosis pathogen *Mucor circinelloides* *Carlos Pérez-Arques*[1], María Isabel Navarro-Mendoza[1], Joseph Heitman[1] 1) Department of Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, NC, USA.

Heritable, epigenetic modifications that alter gene expression are a widespread phenomenon in eukaryotic organisms. These are known as epimutations and may arise from RNAi, DNA methylation, and/or heterochromatin modifications, often resulting in gene silencing. Recently, epimutations were identified as a novel mechanism conferring antimicrobial drug resistance, one of the gravest threats to public health. Epimutations were discovered for the first time in two species of the early-diverging fungus *Mucor* and result in small RNA (sRNA) silencing of the gene *fkbA* encoding the FK506 target FKBP12. This silencing results in transient, unstable drug resistance that reverts after several mitotic growth cycles in the absence of FK506. We have discovered that epimutations conferring drug resistance in *Mucor* species are exclusively RNAi-based and post-transcriptional, as demonstrated by the absence of cytosine DNA methylation (5mC) or histone H3 lysine 9 (H3K9) di- or trimethylation but the presence of RNA polymerase II and complementary antisense sRNAs directed against epimutationally-silenced loci.
Mucor epimutations are sufficiently stable to be trans-generationally inherited following sexual reproduction and meiosis, despite lacking heterochromatin marks frequently associated with epigenetic inheritance. We have identified new, RNAi-based epimutants in the pathogenic *Mucor circinelloides* phylogenetic species 15 (PS15) that is able to undergo a complete sexual cycle including the production of viable meiotic progeny after zygospore germination. Epimutations were found to be inherited stochastically in the progeny of the *fkbA*-epimutant and an opposite mating-type wildtype parent, in contrast to the mendelian ratio (1:1) observed in the progeny from an *fkbA*$^{-}$ mutant and wildtype cross. Similar to the FK506-resistant epimutant parental isolate, the epimutant progeny are resistant to FK506 and harbor antisense sRNAs targeting *fkbA*, and following passage in the absence of drug both FK506-resistance and sRNAs targeting *fkbA* of the epimutant progeny were lost. Our findings demonstrate that epimutations are broadly present across the *Mucor* species complex and act exclusively through post-transcriptional gene silencing to control gene expression. Although epimutations are stable through both mitosis and meiosis, their detection may pose a challenge to typical culture methods employed in clinical

diagnostics given that these involve growth in the absence of drug selective pressure. Understanding how epimutations arise and the mechanisms via which they confer resistance may enable their detection in clinical settings and provide solutions to combating the challenge of antimicrobial drug resistance.

20 How important is cross-kingdom RNA interference in nature? *Arne Weiberg*[1], Florian Dunker[1], Antoine Porquier[1], Constance Tisserant[1] 1) Ludwig-Maximilians University - LMU.

RNA communication across kingdoms has emerged as a new frontier in host-pathogen infection research. In a pioneering work, the fungal plant pathogen *Botrytis cinerea* was found to send small RNAs into its host plants to establish infection. These fungal small RNAs hijack the plant`s own RNA silencing machinery to suppress important plant immunity genes. That is why this virulence mechanism was termed cross-kingdom RNA interference (ckRNAi). Once this fascinating phenomenon was discovered in a fungal pathogen infection, an obvious question arose: ***how important in ckRNAi in nature?***
To address this question, our lab is exploring ckRNAi in two different plant pathogen species, *Botrytis cinerea* and *Hyaloperonospora arabidopsidis*; these two species belong to the kingdoms of fungi and oomycetes, respectively. We have now found evidence that both species use ckRNAi to infect their host plants. Further on, we uncovered high natural diversity in small RNAs produced by six *B. cinerea* field strains isolated from different geographical and host origins. Hereby, we revealed a class of gypsy-type retrotransposon-associated small RNAs significantly contributed to virulence of this fungal pathogen. Based on our observations, we conclude that ckRNAi is a widespread and important phenomenon in nature.

21 Identification of a stage-specific co-factor required for A-to-I mRNA editing during sexual reproduction in fungi Chanjing Feng[1], Kaiyun Xin[1], Zhuyun Bian[2], Jingwen Zou[1], Yanfei Du[1], Jin-Rong Xu[2], *Huiquan Liu*[1] 1) State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, China; 2) Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, United States of America.

Adenosine-to-inosine (A-to-I) editing of mRNAs catalyzed by double-stranded RNA-specific adenosine deaminase (ADAR) enzymes is an important post-transcriptional modification in animals. In the filamentous ascomycete *Fusarium graminearum* that lacks ADARs but has genome-wide A-to-I editing, two genes orthologous to yeast *TAD2* and *TAD3* encoding two subunits of tRNA-specific adenosine-34 deaminase, respectively, have been implicated in catalyzing A-to-I mRNA editing specifically during sexual reproduction. RNA immunoprecipitation sequencing analysis revealed that edited RNAs were more likely to be bound by FgTad2 during sexual reproduction. By characterizing 34 genes expressed specifically during sexual reproduction, we found one of them is essential for A-to-I mRNA editing in *F. graminearum* (named *AME1* for activator of mRNA editing). *AME1* is highly conserved in Sordariomycetes but not present in other fungi. It encodes a protein with a domain of unknown function (DUF). Deletion of *AME1* had no effects on growth, conidiation, and pathogenesis but resulted in the abolishment of A-to-I mRNA editing during sexual reproduction. Perithecia formed by the Δ*ame1* mutant had no asci/ascospores and no detectable RNA editing events. Interestingly, when the *AME1* gene was expressed with a constitutive promoter, approximately two-thousands of mRNA editing sites were detected in vegetative hyphae. The physical interaction between Ame1 and FgTad2/FgTad3 and their functional relationship during A-to-I mRNA are being characterized and will be presented. In addition, we identified a gene encoding an RNA-binding protein that represses mRNA editing during sexual reproduction. Taken together, our results showed that Ame1 functions as a stage-specific co-factor of FgTad2 and FgTad3 for A-to-I mRNA editing in fungi during sexual reproduction.

22 Endohyphal bacteria modulate tissue colonization, saprotrophy, and thermotolerance by endophytic fungi *in vitro* and under field conditions *A. Elizabeth Arnold*[1], Rachel Gallery[1], David Baltrus[1] 1) The University of Arizona.

Endohyphal bacteria (EHB) are increasingly recognized for modulating the phenotypes of plant-symbiotic fungi under diverse conditions, but the scope and context-dependency of such phenotypic modulation is not well understood. We examined the influence of facultative EHB on plant tissue infection and leaf biomass degradation by three fungal species isolated as foliar endophytes with saprotrophic life phases. *In vitro* assays demonstrated that EHB increased cellulase and ligninase activity relative to fungi without bacterial symbionts. EHB enhanced fungal growth on media with only cellulose or indulin as nutrient sources, and increased thermotolerance under nutrient-poor conditions consistent with those on the phylloplane. Strains with EHB consistently grew more rapidly on dried leaf tissue than strains without EHB, and fungal growth was consistent with mass loss from plant tissue over time. Biolog characterization revealed that EHB altered the breadth and efficiency of use of diverse carbon substrates, often in a partnership-specific manner. Together, these findings were mirrored by field experiments confirming that (1) EHB can shape the capacity of endophytes to colonize living plant tissue, and (2) mass loss, enzyme activity, and tissue degradation observed *in vitro* scale readily to natural environments. Contextualized by preliminary investigations of genomic and metatranscriptomic data, these experiments highlight how EHB modulate ecological modes of plant-associated fungi, with potential implications for evolutionary dynamics across two domains of life.

23 How do plants deploy smRNAs to engage with beneficial microorganisms while fighting pathogens? *Maitree Pradhan*[1], Ian Thomas Baldwin[2], Shree Prakash Pandey[2], Natalia Requena[1] 1) Molecular Phytopathology, Karlsruhe Institute of Technology - KIT, Karlsruhe, Germany; 2) Department of Molecular Ecology, Max Planck Institute for Chemical Ecology, Jena, Germany.

Plants constantly encounter fungi that are pathogenic as well as beneficial in nature. The host plants must mount discriminant and specific responses towards foes and friends. These interactions involve a sophisticated molecular mechanism of signalling and a large-scale regulation of gene expression. Small RNAs (smRNAs), including the microRNAs (miRNAs) offer such an essential layer of regulators of organismic processes that direct the symbiotic and pathogenic outcomes. We have investigated how these non-coding players of gene-expression regulation are deployed for modulation of signal transduction and response towards invading fungi, and in the process help the hosts to use them as communication tools to discriminate friends and foes. Further, we describe a split-root system in tomato that is appropriate for studying host components of mobile signals across kingdoms of interacting partners, and to discover such communication as well as regulatory tools by host and microbes.

24 Mycangial colonization in the laurel wilt (*Raffealea lauricola*)-Ambrosia beetle symbiosis *Ross Joseph*[1], Kamaldeep

Bansal[1], Nemat Keyhani[1] 1) University of Florida.

Ambrosia symbioses are reciprocally obligate mutualisms between a group of specialized wood-boring weevils (*Curculionidae*: *Scolytinae* and *Platypodinae*) and their associated fungal symbionts. Beetles rely on fungal symbionts as their sole food source and the fungi rely on their beetle hosts for dispersal and cultivar maintenance. These beetles are unique among fungus-farming insects in their development of specialized fungal transport organs called mycangia**,** which promote the growth of their fungal partners and dispersal to new environments, allowing for vertical symbiont transmission across beetle generations. Mycangia vary in size, shape, complexity, and location, and the more complex of these organs house glandular cells and display morphological plasticity upon symbiont recognition, suggesting that sustained chemical crosstalk occurs between microbe and host. Despite the critical role that mycangia play in these sustained, high-fidelity, interactions, very little is known regarding the factors contributing to the establishment and maintenance of mycangial symbioses. Here, we report a model preoral mycangia colonization system between the laurel wilt pathogen, *Raffaelea lauricola*, and its symbiotic beetle vectors in the *Xyleborus* group. Aposymbiotic beetles were reared in the presence of specific reporter strains of *R. lauricola*, and mycangia were colonized through beetle feeding. Aspects of mycangial colonization including rate, dynamics, fungal morphology, and physical interactions between the beetle host and the fungal microbe were subsequently examined by electron microscopy, cryosectioning and fluorescence microscopy, and counting of colony forming units. Specific hypotheses regarding mycangial symbiosis were tested to address questions concerning the stability of colonization over time, the dynamics of mycangial change, and competition between different fungal species during mycangial colonization. Our data provides new insights into the nature of ambrosia symbioses that are important in light of recent ambrosia fungi emerging as devastating agriculture and forest pathogens. These data also establish a framework for genetically probing insect-fungal symbioses, ubiquitous but understudied natural systems.

25 Testing the role of the transcription factor TvSom1 in adhesion of *Trichoderma virens* germlings Ariella Alperovitch-Lavy[1], Tri-Thuc Bui[2,3], Harting Rebekka[2], Braus Gerhard[2], *Benjamin Horwitz*[1] 1) Technion - IIT, Haifa, Israel; 2) Institute of Microbiology and Genetics, University of Göttingen, Germany; 3) College of Agriculture and Forestry, Thai Nguyen University , Vietnam.

Trichoderma-root interactions prime the plant immune response, attenuating disease upon later challenge with a pathogen. Relatively little is known about the molecular details of this opportunistic fungal-plant symbiosis. Attachment of hyphae or germlings to the root, however, is likely to be a critical early step. Like other microorganisms, Trichoderma may adhere to the plant host with adhesive molecules found on the hyphal surface. In the soilborne pathogen *Verticillium dahliae*, three transcription factors controlling the network underlying adhesion have been isolated by a yeast expression strategy [1]. TvSom1 is a candidate *T. virens* ortholog of one of these, Som1. The 2668 bp predicted coding region of TvSom1 in the *T. virens* database (Joint Genome Institute [2]) is interrupted by 4 introns and encodes a 795 amino acid protein. Alignment of TvSom1 with *V. dahliae* Som1 gives 63.9% identity and 82.4% similarity. The sequences of the LisH domain, nuclear localization signal (NLS) in the N-terminal half of the protein, and SSDP domain are well-conserved in the alignment, while the SnAPC domain is less so with disconnected regions of identity, and the NLS in the C-terminal region differs at 3 residues. TvSom1 is expressed in germinating conidia. In the most abundant transcript, the first two introns are spliced. Transcripts in which either the first or the second intron is spliced were detected at the order of 1%, and we are testing whether their expression is developmentally regulated. Adhesion-related genes can eventually be targeted for both understanding of, and agriculturally-relevant manipulation of, the Trichoderma-root interaction. New insights can be obtained into biocontrol of fungal pathogens in the soil, and the trade-off between fungal-fungal and fungal-root interactions in the rhizosphere. This balance is agriculturally relevant.
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26 The combined activity of two secreted fungal enzymes is implicated in fungal accommodation in the roots and triggers cell death in different host species Nick Dunken[1], Patrizia Zecuara[1], Pia Saake[1], *Alga Zuccaro*[1,2] 1) University of Cologne, Germany; 2) Cluster of Excellence on Plant Sciences (CEPLAS), Cologne, Germany.

Intracellular colonization of plant roots by the beneficial fungal endophyte *Serendipita indica* (syn. *Piriformospora indica*) follows a biphasic strategy. After an early biotrophic phase the interaction switches to a host cell death phase restricted to the root epidermis and cortex layer. This host cell death is required for fungal accommodation and the establishment of a long-lasting beneficial interaction in barley and *Arabidopsis thaliana*. However, how this cell death is activated and regulated is largely unknown. Here we show that two fungal enzymes, the ecto-5'-nucleotidase *Si*E5NT and the nuclease *Si*NucA act synergistically in the apoplast at the onset of cell death to produce deoxyadenosine (dAdo), a potent cell death inducer in animal systems. Uptake of extracellular dAdo, but not of the structurally related adenosine (Ado), activates a previously undescribed cell death mechanism in *A. thaliana* as well as in the liverwort *Marchantia polymorpha*, suggesting that a conserved cell death response to dAdo exists across plant lineages. Mutation of the root-expressed *A. thaliana* equilibrative nucleoside transporter *ENT3* confers resistance to extracellular dAdo-induced cell death and leads to decreased fungal-mediated cell death during root colonization. Additionally, in an attempt to identify downstream components mediating dAdo cell death, we performed a mutant screening of 6800 Arabidopsis T-DNA insertion lines. A gene encoding a nucleotide-binding leucine-rich repeat protein (NLR) was identified and proven to be implicated in dAdo-mediated cell death. Taken together our data show that the combined activity of two secreted fungal enzymes leads to the production of a metabolite, which is sufficient to trigger regulated cell death in different host species.

27 Deciphering the potential niche of novel black yeast fungal isolates in a biological soil crust based on genomes, phenotyping, and melanin regulation *Erin Carr*[1], Quin Barton[1], Sarah Grambo[2], Mitchell Sullivan[1], Cecile Renfro[1], Alan Kuo[3], Jasmyn Pangilinan[3], Anna Lipzen[3], Keykhosrow Keymanesh[3], Emily Savage[3], Kerrie Barry[3], Igor Grigoriev[3,4], Wayne Riekhof[1], Steven Harris[2] 1) University of Nebraska - Lincoln, Lincoln, NE; 2) Iowa State University, Ames, Iowa; 3) US Department of Energy Joint Genome Institute Lawrence Berkley National Laboratory, Berkley California; 4) Department of Plant and Microbial Biology, University of California Berkeley, Berkeley, California .

Black yeasts are polyextremotolerant fungi that contain high amounts of melanin in their cell wall and maintain a primarily yeast form. These fungi grow in xeric, nutrient deplete environments which implies that they require highly flexible metabolisms and the ability to form lichen-like mutualisms with nearby algae and bacteria. However, the exact ecological niche and interactions between these fungi and their surrounding community is not well understood. We have isolated and described two novel black yeast fungi of the genus *Exophiala*: JF 03-3F "Goopy" *E. viscosium* and JF 03-4F "Slimy" *E. limosus*, which are from dryland biological soil crusts. A combination of whole genome sequencing and various phenotyping experiments have been performed on these isolates to determine their fundamental niches within the biological soil crust consortium. Our results reveal that these *Exophiala* spp. are capable of utilizing a wide variety of carbon and nitrogen sources potentially from symbiotic microbes, they can withstand many abiotic stresses, and can potentially provide UV resistance to the crust community in the form of secreted melanin. Besides the identification of two novel species within the genus *Exophiala*, our study also provides new insight into the production and regulation of melanin in extremotolerant fungi.

28 Establishment of functional symbioses between *Epichloë* endophytes and the modern cereals rye (*Secale cereale*) and hexaploid wheat (*Triticum aestivum*). Wayne Simpson[1], Hisashi Tsujimoto[2], S Kato[2], David Hume[1], Wade Mace[1], Joanne Drummond[3], Phil Rolston[3], *Richard Johnson*[1] 1) AgResearch Limited, Grasslands Research Centre, Palmerston North, New Zealand.; 2) Arid Land Research Center, Tottori University, Tottori, Japan.; 3) Foundation for Arable Research, Templeton, New Zealand.

Asexual *Epichloë* endophytes have been used successfully in pastoral systems to enhance the agronomic performance of important temperate grasses such as perennial ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*) via improved tolerance to biotic and abiotic stresses.

Modern cereals such as wheat (*Triticum aestivum*) and rye (*Secale cereale*) within tribe Hordeae (Triticeae) do not naturally host *Epichloë*, but some wild relatives (e.g., *Elymus* spp.) have been shown to be infected with *Epichloë bromicola.* Inoculation of *E. bromicola* into both rye and wheat has shown that successful symbiosis depends on the genetics of both the host and the endophyte strain deployed. Endophyte compatibility in rye has been improved through recurrent selection of desirable phenotypes, which is possible due to its outcrossing nature and broad population based genetic variation. However, wheat is a selfing species and populations are genetically narrow, making recurrent selection for improved phenotype impractical. Until recently the phenotypes of *Epichloë* – wheat associations have been compromised, including host death and stunting. We have overcome this barrier by utilising experimental wheat lines containing alien chromosome additions or substitutions from wild species and have identified symbioses whereby infected wheat plants are phenotypically comparable to uninfected controls. These plants completed their full lifecycle including the transmission of *Epichloë* into the next generation of grain.

Assessment of *Epichloë* infected rye has demonstrated increased resistance to both insect pests and fungal diseases with field trials yielding between 39% to 95% more grain than the endophyte-free lines. Whilst research in wheat has not yet progressed to field trials, glasshouse experiments have shown that *Epichloë* infected wheat is more resistant to a number of insect pests including aphids.

29 Genetic determinants of endophytism in the *Arabidopsis* root mycobiome *Fantin Mesny*[1], Shingo Miyauchi[1,2], Thorsten Thiergart[1], Brigitte Pickel[1], Lea Atanasova[3,4], Magnus Karlsson[5], Bruno Hüttel[6], Kerrie Barry[7], Sajeet Haridas[7], Elodie Drula[8,9], Bernard Henrissat[10], Annegret Kohler[2], Igor Grigoriev[7,11], Francis Martin[2,12], Stéphane Hacquard[1,13] 1) Max Planck Institute for Plant Breeding Research - Cologne, Germany; 2) UMR Interactions Arbres/Microorganismes, Centre INRAE Grand Est-Nancy - Champenoux, France; 3) Institute of Chemical, Environmental and Biological Engineering, Vienna University of Technology - Vienna, Austria; 4) Institute of Food Technology, University of Natural Resources and Life Sciences - Vienna, Austria; 5) Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences - Uppsala, Sweden; 6) Max Planck Genome Centre - Cologne, Germany; 7) U.S. Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory - Berkeley, CA, USA; 8) INRAE, USC1408 Architecture et Fonction des Macromolécules Biologiques - Marseille, France; 9) Architecture et Fonction des Macromolécules Biologiques (CNRS, Aix-Marseille Univ.) - Marseille, France; 10) Department of Biological Sciences, King Abdulaziz University - Jeddah, Saudi Arabia; 11) Department of Plant and Microbial Biology, University of California Berkeley - Berkeley, CA, USA; 12) Beijing Advanced Innovation Centre for Tree Breeding by Molecular Design (BAIC-TBMD), Institute of Microbiology, Beijing Forestry University - Beijing, China; 13) Cluster of Excellence on Plant Sciences (CEPLAS), Max Planck Institute for Plant Breeding Research - Cologne, Germany.

The roots of *Arabidopsis thaliana* host diverse fungal communities that affect plant health and disease states. We sequenced the genomes of 41 fungal isolates representative of the *A. thaliana* root mycobiota for comparative analysis with 79 other plant-associated fungi. Our analyses indicated that root mycobiota members evolved from ancestors with diverse lifestyles and retained large repertoires of plant cell wall-degrading enzymes (PCWDEs) and effector-like small secreted proteins. We identified a set of 84 gene families associated with endophytism, including genes encoding PCWDEs acting on xylan (family GH10) and cellulose (family AA9). Transcripts encoding these enzymes were also part of a conserved transcriptional program activated by phylogenetically-distant mycobiota members upon host contact. Recolonization experiments with individual fungi indicated that strains with detrimental effects in mono-association with the host colonized roots more aggressively than those with beneficial activities, and dominated in natural root samples. Furthermore, we showed that the pectin-degrading enzyme family PL1_7 linked aggressiveness of endophytic colonization to plant health.

30 *Candida* biofilms: importance, regulation, and evolution *Clarissa Nobile*[1] 1) University of California, Merced.

An infection is often treated as if it is composed of a single microbial species in isolation, yet in reality, infections are immensely complex ecosystems composed of many interacting microbes. Research in the Nobile lab is directed towards understanding the molecular and mechanistic bases of biofilm microbial communities. We are most interested in investigating how transcriptional networks underlie the regulation of gene expression during the development of biofilms. Much of this work is carried out in the *Candida* clade species, consisting of some of the most prevalent fungal pathogens of humans. Questions we are currently pursuing include: How are *Candida* biofilms regulated? How are they built? How are their unique and specialized properties maintained? How have they evolved?

31 Targeting the fungal cell wall *Carol Munro*[1] 1) University of Aberdeen.

The fungal cell wall is a dynamic polysaccharide-rich organelle which maintains cellular integrity. The polysaccharides, chitin and β-1,3-glucan, give the wall its mechanical strength and an outer fibrillar layer of highly glycosylated mannoproteins include important virulence factors such as adhesins. Embedded in the wall are carbohydrate active enzymes that act upon cell wall polysaccharides and generate cross links. Fungi can alter the composition, structure and properties of their outer coats in response to different internal and external stimuli such as changes in environmental conditions.
Many fungal cell wall components are fungal specific and attractive targets for the generation of novel therapeutics and diagnostics to combat life-threatening invasivefungal infections. Antifungal drug resistance is an ever present threat to the treatment of patients with fungal infections and there is a clear clinical need for a larger reportoire of classes of antifungal agents, currently limited to 3 major classes.
We have compared the cell wall structure, composition and proteomes of drug sensitive and drug resistant clinical isolates of *Candida* species. In addition we have examined the genetic variation in genes with cell wall associated functions in a diverse set of clinical isolates. Our aims are to gain a better understanding of cell surface variation and the role of the cell wall in host interactions, drug tolerance and resistance mechanisms to identify potential antifungal targets and drug resistance biomarkers.
Our analyses have highlighted changes in cell wall composition such as elevated chitin levels and the increased expression of cell surface proteins in response to antifungal drug treatment and in drug resistant isolates. We have examined the influence of antifungal-induced cell wall remodelling on host interactions and developed monoclonal antibodies that target specific cell surface epitopes as a new generation of biologics-based antifungals and potential theranostics.

32 Multi-omics Profiling Reveals New Pathways Regulating Hyphal Morphogenesis in *Candida albicans* *Kyunghun Min*[1], Thomas Jannace[1], Haoyu Si[1], Krishna Veeramah[2], John Haley[3,4], James Konopka[1] 1) Department of Microbiology and Immunology, Renaissance School of Medicine, Stony Brook University (SUNY), Stony Brook, NY; 2) Department of Ecology and Evolution, Stony Brook University (SUNY), Stony Brook, NY; 3) Department of Pathology, Renaissance School of Medicine, Stony Brook University (SUNY), Stony Brook, NY; 4) Biological Mass Spectrometry Shared Resource, Renaissance School of Medicine, Stony Brook University (SUNY), Stony Brook, NY.

Fungi grow in a wide range of different morphologies that provide distinct advantages for survival and virulence. The human fungal pathogen *Candida albicans* switches between budding and filamentous hyphal morphologies in the host. Long hyphal filaments promote invasion into tissues, biofilm formation, and escape from macrophages. Adenylyl cyclase (Cyr1) has long been thought to be the master regulator of hyphal growth, through activation of cAMP signaling. However, it is difficult to define the roles of the cAMP pathway because *cyr1*Δ/Δ mutants grow very poorly and expresses abnormally low levels of genes needed for hyphal growth. Surprisingly, we discovered that faster growing *cyr1*Δ/Δ pseudorevertant (PR) mutants form hyphae in the absence of Cyr1 and cAMP. Genome analysis of multiple PR mutants revealed that their improved growth was due to loss of one copy of *BCY1*, the negative regulatory subunit of protein kinase A. Furthermore, hyphal morphogenesis was improved in some of PR mutants by multigenic haploinsufficiency resulting from loss of large regions of the left arm of chromosome 2, including global transcriptional regulators. Interestingly, the mutant cells were also able to induce hyphal associated genes in the absence of cAMP that are needed for virulence. This indicates that basal protein kinase A activity is required for hyphal induction, but further stimulation of PKA is not needed. Integrating information from different omics approaches identified cAMP-independent mechanisms that promote hyphal growth. Phosphoproteomic analysis indicated that the Cdc28 cyclin-dependent kinase and the casein kinase Yck2 play key roles in promoting polarized growth. In addition, integrating transcriptomic and proteomic data reveals that hyphal induction increased protein translation rate of the key transcription factors that are important for hyphal growth.

33 Nanoscale imaging of dynamic cell wall formation in fission yeast Pascal Odermatt[1, 2, 3], Amilcar Perez[1,4], Kerwyn Huang[2], *Fred Chang*[1] 1) UCSF, San Francisco, CA, USA; 2) Stanford U., Stanford, CA, USA; 3) EPFL, Lausanne, Switzerland; 4) Johns Hopkins, Baltimore, MD, USA.

The cell wall is a critical structural element responsible for cell shape and mechanical properties. Many walled cells-- including fungi, bacteria, and plants– derive their shape by tip growth, in which new cell wall is assembled at the cell tip. Similarities in the shapes of tip growing cells across kingdoms and over several magnitudes in size suggest that these organisms utilize common physical mechanisms. Here, we used atomic force microscopy to visualize for the first time the dynamic behaviors of individual glucan fibers of the cell wall during tip growth in the fission yeast *Schizosaccharomyces pombe*. We embedded cells in a porous matrix to stand the cell on end, which allowed us to probe the growing tip through an entire cell cycle (hours) at minute time resolution with < 10-nm spatial resolution. Analyses reveal a mesh-like structure composed of filaments (likely to be glucan bundles) that reorganize continuously at the growing cell tip. Our findings provide evidence for insertion at discrete sites, followed by directed movement, with apparent stretching and remodeling of these fibers as they progress outwards towards the lateral walls. We further used single molecule imaging to track the behavior of the major cell wall synthase Bgs4. Single molecules of halo-tagged Bgs4 exhibited processive movements at sites of cell wall synthesis. These movements were not dependent on actin but were inhibited by caspofungin, an inhibitor of Bgs4 activity, suggesting that movement of the synthase is powered by glucan assembly. Together, these findings represent promising new avenues to probe dynamic mechanisms of cell wall assembly at the nanoscale.

34 A myosin light chain, linking fungal morphology and filament extension, is critical for *Candida albicans* growth robustness Charles Puerner[1,2], Antonio Serrrano[1], Rohan Wakade[1,3], Martine Bassilana[1], *Robert Arkowitz*[1] 1) University Cote d›Azur/CNRS/INSERM, Nice, France; 2) Present address: Dept. Microbiology & Immunology, Geisel School of Medicine, Dartmouth, Hanover, NH, USA; 3) Present address: Dept. Pediatrics, Carver College of Medicine, University of Iowa, Iowa City, Iowa, USA.

Apical growth is critical in a range of fungal pathogens for invasion into animal and plant tissues. In a number of elongated cells, such as fungal hyphae, a vesicle cluster referred to as a Spitzenkörper is observed at the growing apex [1], including in the human fungal pathogen *Candida albicans*. This structure is thought to function as a vesicle supply center and a central prediction of the vesicle supply center model is that the filament diameter is proportional to the extension rate [2-4]. Analyses of the Spitzenkörper function has been

challenging, as a majority of components identified thus far are essential for growth. Here, we probe the function of the Spitzenkörper in the human fungal pathogen *C. albicans*, using genetics and synthetic physical interactions. We show that the *C. albicans* Spitzenkörper is comprised principally of secretory vesicles. Mutant strains lacking the Spitzenkörper component myosin light chain 1 (Mlc1) or having a synthetic physical interaction between Mlc1 and either another Spitzenkörper component, the Rab GTPase Sec4, or prenylated GFP, are viable and still exhibit a Spitzenkörper during filamentous growth. Strikingly, all three of these mutants formed filaments, yet are defective in growth regulation, exhibiting a range of growth rates and sizes, indicating that Mlc1 negatively regulates the activity of the myosin V, Myo2. Our quantitative analyses reveal a strong correlation between filament diameter and extension rate, consistent with the vesicle supply center model for fungal tip growth, and suggest that the Spitzenkörper is important for growth robustness.

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35 Structural base of the cell wall diversity of *Candida glabrata* *Lars-Oliver Essen*[1], Hans-Ulrich Mösch[2], Piet de Groot[3] 1) Department of Chemistry, Philipps University, Marburg, Germany; 2) Department of Biology, Philipps University, Marburg, Germany; 3) Regional Center for Biomedical Research, Castilla-La Mancha Science & Technology Park, University of Castilla–La Mancha, Albacete, Spain.

Candida glabrata is a commensal of human mucosal tissues, but acts increasingly as an opportunistic pathogenic yeast by being the second most frequent cause of candidiasis after *Candida albicans*. In contrast to the latter *C. glabrata* lacks hyphal development as virulence factor. Instead *C. glabrata* exerts a high tolerance to antifungals and contains two batteries of cell wall adhesins, the Epa family and the Awp1 family, whose members are mostly encoded at subtelomeric regions. This diversity is crucial for its success as pathogen and resembles the antigenic variation mechanisms of *Trypanosoma* and *Plasmodia*.
Our analysis of putative adhesins from homology clusters III, V and VI showed that their A-domains adopt a parallel right-handed β-helix domain that is linked to a C-terminal α-crystallin domain. The A-regions of Awp1, Awp3b and Awp14 show structural similarity to pectate lyases but binding to neither carbohydrates nor Ca^{2+} was observed. Accordingly, phenotypic analysis of *awp1*Δ, *awp3*Δ, *awp1,3*Δ and awp14Δ mutants did not confirm a role as adhesins. In contrast, deletion mutants of the cluster V adhesin Awp2 in the hyperadhesive clinical isolate PEU382 demonstrated its importance for adhesion to polystyrene or glass, biofilm formation, cell aggregation and other cell surface-related phenotypes. *C. glabrata* CBS138 hence relies not only on 27 adhesins with PA14-like domains (Epa, Pwp), but also on 42 Awp1-related adhesins with β-helix/α-crystallin domain architecture for modifying the surface characteristics of its cell wall.

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36 Study of the physiological role of amyloid structures in the pathogenic yeast *Candida albicans*. *Thierry Mourer*[1], Sophie Bachellier-Bassi[1], Christophe d'Enfert[1] 1) Institut Pasteur, Université de Paris, INRA USC2019, Fungal Biology and Pathogenicity Unit, F-75015 Paris, France..

The human commensal fungus *Candida albicans* can, under some conditions, cross the digestive mucosa, disseminate into the bloodstream, and cause invasive candidiasis. *C. albicans* can also form structured communities, namely biofilms, attached on epithelia or indwelling medical devices. This yeast is a huge burden for healthcare systems worsened by the fact that *C. albicans* biofilms are strongly resistant to classical antifungal drugs and can also evade the immune system. Because of these issues, curing patients with invasive candidiasis remains challenging. A better understanding of biofilm formation at the molecular level could lead to new therapeutic strategies. Glycosylphosphatidylinositol anchored proteins (GAPs) are heavily glycosylated proteins associated to the cell wall. Over the past decade, many GAPs of *C. albicans* have been shown to contribute to biofilm establishment, although their precise molecular functions need to be elucidated. Overexpression of some genes encoding GAPs have been shown to affect biofilm formation. Among them, overexpression of *PGA59* increases adhesion forces to the substrate and between cells, and results in an increase of biofilm dry weight. Recently, our laboratory has uncovered that Pga59 displays amyloid properties. We have produced recombinant His-tagged Pga59 using *Escherichia coli* as a host, and using electron microscopy and Thioflavin T staining (a specific dye of amyloid fibers), we demonstrated that *in vitro* recombinant Pga59 was able to adopt an architecture reminiscent of beta amyloid fibers. We then investigated if Pga59 is a part of cell surface amyloid fibers in *C. albicans* and whether parietal amyloids impact cellular adhesion and biofilm formation. *In vivo* Thioflavin T staining allowed us to visualize amyloid structures in the cell wall of *C. albicans*. Interestingly, we showed that a mutant impairing Pga59 amyloid assembly impacts biofilm formation in a continuous-flow microfermentor system. Moreover, our results strongly suggest that amyloid properties of Pga59 are required to mediated cell-cell interactions. Altogether, information gathered on amyloid fibers formation in response to adhesion and on their functions could lead to the discovery of an unsuspected mechanism to regulate biofilm formation in *C. albicans*.

37 Defining the septin interactome and its role in appressorium-mediated plant infection by the rice blast fungus *Mag-*

naporthe oryzae *Iris Eisermann*[1], Andrew J. Foster[1], Paul Derbyshire[1], Frank L.H. Menke[1], Nicholas J. Talbot [1] 1) The Sainsbury Laboratory, Norwich, UK.

Rice blast disease is initiated by formation of a specialized infection cell by the blast fungus, called the appressorium. The appressorium generates turgor of up to 8 MPa, enabling the fungus to develop a rigid penetration peg to breach the rice cuticle. *Magnaporthe oryzae* possesses six septin GTPases, which play major roles during plant infection. The four core septins Sep3, Sep4, Sep5 and Sep6 collectively form a hetero-oligomeric ring at the appressorium pore, which is essential for plant infection. Two non-core septins, Sep7 and Sep8, belonging to the class 5 group of septins, which includes AspE from *Aspergillus nidulans*, also form a range of membrane and cytoskeleton-associated structures. To reveal the role of each septin during appressorium-mediated plant-infection we have carried out high throughput yeast two hybrid assays, coupled with *in vivo* immunoprecipitation mass spectrometry (IP-MS) experiments, and phosphoproteomics, to define the septin interactome. We have identified a wide range of interaction partners of each septin during appressorium development, including polarity determinants, cytoskeletal components and a range of regulatory proteins. We observed that Sep7 interacts with Sep3, Sep4, Sep5 and Sep6 specifically during early appressorium formation, 4h after conidial germination, forming a plasma membrane-associated complex. Sep8, which contains a transmembrane helix, also interacts with each septin and may link septins to the plasma membrane. We present a model for how septins organise the appressorium pore and deploy polarity determinants to facilitate cuticle rupture and invasive fungal growth.

38 Stable parasexuality – a novel fungal reproductive strategy uncovered by population genomics *Cene Gostinčar*[1], Nina Gunde-Cimerman[1] 1) University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia.

Population genomic studies suggest that the extremotolerant black yeasts *Hortaea werneckii* and *Aureobasidium melanogenum* employ a previously unrecognized reproductive strategy that we call «*stable parasexuality*". The fusion of cells normally followed by return to the original ploidy through meiosis or, in the case of parasexuality, random loss of genetic material, in these two species appears to result in highly heterozygous diploid hybrids that are stable over long periods of time and distributed over large geographic areas.
The age of genomics has helped to disprove the once widely held assumption that a large proportion of fungi are exclusively clonal. However, the clonality of some species has also been confirmed with the precise tools available today. These include *H. werneckii* and *A. melanogenum*, two yeasts from the group Dothideomycetes. Their clonality was confirmed by whole-genome sequencing and analysis of 115 wild isolates. No decay of linkage disequilibrium was observed between pairs of polymorphic loci, even at large genomic distances, and a high degree of concordance was observed between phylogenies of different genomic regions. The discovery that about two-thirds of the isolates of *H. werneckii* and *A. melanogenum* are diploid and the rest are haploid was therefore unexpected. Heterozygosity of diploid strains was high in both species, with some genome distances between hybridizing haploid genome pairs exceeding the threshold that typically separates different fungal species. The origin of the diploid strains collected worldwide could be traced to only a handful of hybridization events.
The mechanism of these apparently rare hybridization events, which are not followed by meiosis or haploidization, remains unknown, as does their possible role in the extremotolerant phenotypes of both investigated species. It is also unclear how widespread this "stable parasexuality" is. We sequenced whole genomes of over 200 wild isolates belonging to six fungal species from extreme environments. Three of them were strictly clonal and stable parasexuality was found in two. While this sample is too small to draw any conclusions, the fact that the phenomenon can only be identified through targeted comparative analyzes and is easy to miss even after whole genome sequencing raises the possibility that it may be more widespread than is currently known.

39 A cystic fibrosis patient lung environment allowed for coexistence of multiple *Exophiala dermatitidis* clades over time *Tania Kurbessoian*[1], Daniel Murante[2], Alex Crocker[2], Jason Stajich[1], Deborah Hogan[2] 1) University of California, Riverside, Riverside, CA; 2) Geisel School of Medicine, Dartmouth, Hanover, NH.

It is vital to understand how microbes persist in the lungs of individuals with cystic fibrosis (CF) and cause inflammatory responses and irreversible lung damage. While most respiratory infections that occur in CF are dominated by bacteria, some are dominated by fungi such as the slow-growing black yeast *Exophiala dermatitidis*. Here, we report that isolates of *E. dermatitidis* cultured from two samples, collected two years apart, from a single subject. One isolate was sequenced using long-read Nanopore technology to assemble an in-population reference for comparative single nucleotide polymorphism (SNP) and insertion-deletion (INDEL) variant analysis. We then used population genomics and phylogenomics to compare 23 strains to the in-population reference and type strain *E. dermatitidis NIH/UT8656.* Three *E. dermatitidis* clades were detected, each with varying mutation rates. Additionally, all strains are MAT 1-1, which was consistent with the absence of evidence for mating or recombination between isolates. Phylogenetic analysis grouped sets of strains into clades that contained isolates from both early and late timepoints indicating there are multiple persistent lineages had adapted to the host lung environment. Functional assessment of variants unique to each clade identified alleles in genes that encode transporters, cytochrome P450 oxidoreductases, iron acquisition and DNA repair processes. The persistent population heterogeneity identified in lung-derived strains is an important factor to consider in treatment and indicates for further investigation, and the analysis of changes in fungal pathogens over time in chronic infections may provide important insights into the physiology of black yeasts and other slow-growing fungi in vivo.

40 Surviving in the brine: a multi-omics approach for understanding the physiology of the halophile fungus *Aspergillus sydowii* *Ramon Alberto Batista Garcia*[1] 1) Universidad Autónoma del Estado de Morelos (RFC UAE671122G49).

Halophilic *Aspergillus sydowii* is a model organism for the study of molecular adaptations of filamentous fungi to hypersaline conditions. An omics approach (transcriptomics and metabolomics) was used to compare the growth of *A. sydowii* at optimal salinity (1 M NaCl) to saturated concentration (5.13 M NaCl). Analyzing the mRNA profile at saturated NaCl showed 1,842 genes significantly differentially expressed, of which 704 were overexpressed. As revealed by GO analysis, the enriched biological process reflected extensive physiological adaptation to high salt concentrations, mainly on metabolism and signal transduction. Processes identified previously in other halophilic fungi as crucial for adaptations to hypersaline conditions, were restructuring of the cell wall, synthesis of compatible solutes and phosphorylation of the signal transduction system. Major changes at the transcriptional level included the high-osmolarity glycerol (HOG) signal transduction pathway, ion transporters and cell wall ultrastructure, and morphology. Interestingly, genes encoding

chitin synthesis were repressed, exposing the important role on cell growth and increased energy requirements at saturated NaCl of ß-1,3 glucans, Ca^{2+} transporters and gene products related to polarized growth, morphogenesis and the cell cycle. This study is the first attempt to clarify the role of lncRNAs in response to stress caused by high NaCl concentrations. The metabolomic profiling described the adaptation of a halophilic fungus to saturated NaCl conditions by changing the consumption of media nutrients, including a metabolic switch towards non-lipid sources and differences in the production of secondary metabolites. We also applied high resolution solid-state NMR to characterize the fungal cell wall, redefining our understanding of the molecular architecture and dynamics of this organelle at hypersaline conditions. The cell wall modification clearly showed an increase in cell wall thickness in extreme salinity. We also observed a noticeable increase in chitin composition from 0 M to 2 M salt concentrations, but the carbohydrate structural integrity is retained. The *A. sydowii* inner cell wall consists of β-1,3 glucan and chitin, whereas the outer cell wall consists of mobile β-1,3 glucan, galactosaminogalactan, and galactomannan. Also, a phenotypic microarray provided a high throughput characterization of the *A. sydowii* physiology at saturated NaCl concentration. This analysis showed the efect on the fungal metabolism of the extremely water deprivation by NaCl, KCl and sorbitol. In summary, this study signals the beginning of an "omic", and molecular understanding of how halophilic fungi adapt to most extreme salinities that are hostile to most eukaryotic microorganisms.

41 The Ess1 prolyl isomerase and its target, the CTD of RNA polymerase II, in cold-adapted fungi. *Steven Hanes*[1], Ryan Palumbo[1], Nathan McKean[1], Erinn Leatherman[1], Kevin Namitiz[1,5], Laurie Connell[2], Aaron Wolfe[3], Kelsey Moody[3], Cene Gostinčar[4], Nina Gunde-Cimerman[4], Alaji Bah[1] 1) SUNY-Upstate Medical University; 2) School of Marine Sciences, University of Maine, Orono, ME USA; 3) Ichor Therapeutics, Lafayette, NY, USA; 4) Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia; 5) Current address... Penn State University.

Most of the world's biological diversity is adapted to living in cold, often below freezing temperatures (-1°C to 4°C). We are interested in understanding the mechanisms of transcription by RNA polymerase II (RNAPII) in the cold. Toward this end, we cloned the Ess1 gene from fungal species that survive in the extreme environments of the polar regions. Ess1 is an essential prolyl isomerase that regulates transcription by inducing conformational changes in the carboxy-terminal domain (CTD) of the large subunit of RNAPII. Ess1 enzymes from cold-adapted fungi (Arctic and Antarctic) were active in the model organism *Saccharomyces cerevisiae*, and sequence substitutions may provide clues as to how they function in the cold. More striking was that the CTDs from cold-adapted fungi are highly-divergent from the near consensus repeat sequence ($YSPTSPS_{26}$) found in *S. cerevisiae* and other model organisms. This divergence profoundly affected the ability of the CTD, which is intrinsically-disordered, to undergo liquid-liquid phase separation (LLPS) *in vitro* and to localize and function *in vivo*. We propose that one mechanism for cold-adaptation (and other environmental tolerance) is altered LLPS behavior via sequence divergence within the intrinsically disordered regions of otherwise globular proteins. Indeed, phylogenetic analyses revealed that most fungal species that live outside the laboratory carry sequence-divergent CTDs. Our findings lay the groundwork for future detailed studies on a new and highly-tractable model for evolutionary cold-adaptation of a globular enzyme (Ess1/Pin1), as well as for uncovering the link between evolutionary selection for sequence divergence in intrinsically disordered regions, LLPS properties and the CTD Code.

42 Developing genetic tools to unlock the biotechnological potential of anaerobic gut fungi *Radwa Hanafy*[1], Casey Hooker[1,2], Ethan Hillman[2], Javier Muñoz[2], Kevin Solomon[1,2] 1) University of Delaware, Newark, DE; 2) Purdue University, West Lafayette, IN.

Anaerobic Gut Fungi (AGF) (phylum Neocallimastigomycota) are native to the digestive system of large herbivores and play a prominent role in anaerobic degradation of untreated lignocellulosic plant materials. AGF have evolved into efficient plant biomass colonizers and degraders through secretion of powerful lignocellulolytic enzymes, and production of secondary metabolites to compete with other microbes. Such remarkable degradation capacities and vast enzymatic repertoire make them promising candidates for enzymes and bioactive molecules that may be used in many biotechnological applications. However, AGF are not yet genetically tractable and require major advances in their genetic manipulation protocols. Primarily guided by available genomic and transcriptomic data, we have identified genetic elements regulating gene expression (e.g. promotors and terminators) and are beginning to assemble a nascent genetic toolbox for manipulation. In this study, we have established a transformation protocol leveraging the natural competency of juvenile zoospores. We have developed methods to introduce selectable markers such as hygromycin resistance gene (*hph*) and validated two fluorescent reporters, flavin-based iLOV and heme-dependent iRFP. In addition, heterologous protein expression may be directed to specific cellular compartments such as the nucleus through the use of localization tags. Efforts to expand this genetic toolkit to include alternative selection genes, reporters, and promotors are currently underway. Important elements such as autonomously replicating sequences and centromere binding sequences are being identified to help stabilize expression vectors. Ultimately, the developed genetic toolkit provides a platform for sustainable bioprocess development, enabling us to exploit the full potential of these non-model anaerobic fungi.

43 *Knufia petricola* – a model for exploring the biology of black rock-inhabiting fungi *Julia Schumacher*[1,2], Romy Breitenbach[1], Eileen Erdmann[1,2], Ruben Gerrits[1], Felix Heeger[1], Sarah Nitsche[1,2], Chiara Tonon[1], Oliver Voigt[1], Anna Gorbushina[1,2] 1) Bundesanstalt für Materialforschung und -prüfung (BAM), Berlin, Germany; 2) Freie Universität Berlin, Germany.

Black fungi also called black yeasts, rock-inhabiting fungi or microcolonial fungi are a group of Ascomycetes [Eurotiomycetes, Arthoniomycetes and Dothideomycetes] that exhibit high stress tolerance, yeastlike or meristematic growth, and constitutive 1,8-dihydroxynaphthalene (DHN) melanin formation. They dominate a range of hostile natural and manmade environments – from desert rocks and salterns to dishwashers, roofs, and solar panels. Due to their slow growth and the lack of sexual cycles and genetic tools, the underlying mechanisms of black fungi's phenotypic traits have remained largely unexplored. We consider the rock inhabitant *K. petricola* [Eurotiomycetes, Chaetothyriales] a suitable model for studying the phenotypic characteristics of black fungi. With *K. petricola* the regulation of pigment synthesis, general stress responses and the unusual modes of cell division can be dissected by advanced reverse and forward genetics approaches. The genome of *K. petricola* strain A95 was sequenced using a combination of short high quality Illumina reads and long PacBio reads. The final assembly consists of twelve contigs: five complete chromosomes and six contigs with one telomer each. Gene annotation supported by transcriptomics and proteomics data was manually curated. Recently, we developed a set of genetic tools to manipulate the genome for analyzing gene functions and studying the cell biology. This set includes CRISPR/

Cas9-based genome editing and live-cell imaging using genetically encoded fluorescent proteins, as well as protocols for -omics approaches and for simulation of mineral weathering in the laboratory. Mutants defective in DHN melanogenesis, carotenogenesis or both processes are currently studied to elucidate the role of these protective pigments in tolerance of natural and man-made stresses, weathering of olivine, penetration of marble, and adhesion to surfaces. Further, the established protocols and knowledge gained from *K. petricola* form a starting point for making other extremotolerant black fungi accessible to genetic manipulation.

44 Diversity of genomic adaptations to post-fire environment in higher fungi points to a crosstalk between charcoal tolerance and sexual development *Andrei Stecca Steindorff*[1], Kyungyong Seong[1,2], Akiko Carver[1,2], Sara Calhoun[1], Monika Fischer[2], Kyra Stillman[2], Haowen Liu[2], Elodie Drula[3,4], Bernard Henrissat[5,6], Hunter Simpson[7], Jonathan Schilling[8], Anna Lipzen[1], Guifen He[1], Mi Yan[1], Jasmyn Pangilinan[1], Kurt LaButti[1], Vivian Ng[1], Matthew Traxler[2], Thomas Bruns[2], Igor Grigoriev[1,2] 1) US DOE Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA, USA; 2) Plant and Microbial Biology, UC Berkeley, Berkeley, CA, USA; 3) Architecture et Fonction des Macromolécules Biologiques (AFMB), CNRS, Marseille, France; 4) INRAE, Architecture et Fonction des Macromolécules Biologiques (AFMB), Marseille, France; 5) Department of Biological Sciences, King Abdulaziz University, Jeddah, Saudi Arabia; 6) DTU Bioengineering, Technical University of Denmark, Lyngby, Denmark; 7) Department of Bioproducts and Biosystems Engineering, University of Minnesota, St. Paul, MN, USA; 8) Department of Plant and Microbial Biology, University of Minnesota, St. Paul, Minnesota, USA.

Understanding post-fire soil systems are essential because they have significant direct and indirect effects on global carbon storage. Soil microbes are likely involved in the degradation of pyrolyzed organic matter (PyOM), yet little is currently known about the organisms or metabolic processes involved. So far, we have sequenced and annotated four pyrophilous Basidiomycetes and seven Ascomycetes genomes. In our previous work on Basidiomycetes fungi, we found expansion of genes potentially involved in the degradation of the hydrophobic layer, pyrolyzed organic matter, and mushroom formation. In this work, we focused on the seven ascomycetes genomes and compared them with the other 12 non-pyrophilous in the same order and 124 genomes at a larger scale, including pyrophilous Basidiomycetes and other organisms with heterogenous lifestyles. Additionally, we explored enriched Pfam domains and CAZymes to identify patterns associated with these organisms' "fire-loving" lifestyle. Our analyses uncovered gene families related to the degradation of pyrolyzed organic matter, but these gene families were distinct from those expanded in the pyrophilous fungi in Basidiomycota. The enrichment analysis revealed families like peritrophin-A, arthropod defensin, β-glucosidases, heat shock proteins, and fungal fucose-specific lectin. These families might be involved with the pyrophilous fungi' capacity to survive in a toxic environment like post-fire soil. We found a CAZyme CBM14 expanded exclusively in the Pyronemataceae family. This family is mainly found in insects and some fungi. Since it is a chitin-binding domain, this suggests that secreted CBM14 domain proteins might protect the fungus from microbial attacks in its soil habitat. Another interesting finding is that pyrophilous fungi have significantly larger proteins and higher GC3 content than non-pyrophilous, being in an intermediate state to thermophiles. Pyrophilous fungi are commonly found fruiting after fire events, passing through their sexual stages in this process. To make an in-depth comparison of these conditions, we analyzed the available transcriptomic data of *Pyronema domesticum* grown in charcoal and during sexual development. We performed a co-expression network analysis and found two modules with the most differentially expressed genes in charcoal and sexual development. Gene Ontology categories like chitin/carbohydrate/lipid/superoxide metabolism and transport were found in both modules, showing that such processes are likely required to grow in the presence of charcoal and sexual development. Also, the transcription factors STE12, LreA, LreB, VosA, and EsdC involved in mating response and environmental cues in yeasts and filamentous ascomycetes were up-regulated in charcoal, revealing a crosstalk between charcoal tolerance and sexual development.

45 Resistance and adaptation of the melanized yeast *Exophiala dermatitidis* to ionizing radiation exposure *Zheng Wang*[1] 1) US Naval Research Laboratory.

The melanized yeast *Exophiala dermatitidis* is resistant to many environmental stresses and is used as a model for understanding the radiation resistance mechanism in fungi. We describe the extent of resistance of *E. dermatitidis* to acute γ-radiation exposure and the major mechanisms it uses to recover from this stress. We find that environmental factors such as nutrient availability, culture age and culture density are much greater determinants of cell survival after exposure. We also observe a dramatic transcriptomic response to γ-radiation that mobilizes pathways involved in morphological development, protein degradation and DNA repair, and is unaffected by the presence of melanin. Moreover, through adaptive laboratory evolution, we demonstrate that resistance to γ-radiation can be greatly increased through repeated rounds of irradiation and outgrowth. This enabled the identification of genetic mutations in genes encoding proteins with a broad range of functions from 10 evolved strains. Specifically, we find that greatly increased resistance to γ-radiation is achieved through disruption of the non-homologous end-joining pathway, with three individual evolutionary paths converging to abolish this DNA repair process. This result suggests that non-homologous end-joining, even in haploid cells where homologous chromosomes are not present during much of the cell cycle, is an impediment to repair of radiation-induced lesions in this organism, and that the relative levels of homologous and non-homologous repair in a given fungal species may play a major role in its radiation resistance.

46 Antifungal Potential of the Skin Microbiome *Lindsay Kalan*[1], Shelby Sandstrom[1], Isabelle Ludwikoski[1], MacKinnley Rybolt[1], Caitlin Carlson[1], Cameron Currie[1], Nasia Safdar[1], James Gern[1] 1) University of Wisconsin-Madison.

Bacteria have the genomic capacity to produce an array of small molecule metabolites, encoded by specialized biosynthetic gene clusters (BGC). In human-associated microbes, the structure and function for the vast majority of these metabolites are unknown. Focusing on the skin, our *central hypothesis* is that skin microbiota harbor BGC encoding for functionally important molecules. We have built a large library of diverse bacterial isolates (>2100) that span the major phyla of the skin microbiome, including low abundant genera (n=36), for testing in functional bioassays. We screened ~1200 isolates against a panel of 24 pathogens including Gram-positive and Gram-negative bacteria and fungi (>28,000 total pairings). After scoring, Euclidian hierarchal clustering revealed that isolates from distinct body sites group together based on antimicrobial activity profiles. We found selective and broad inhibition of phylogenetically diverse fungi, with particularly strong inhibitory activity against the human pathogens in the genera *Candida*, *Cryptococcus*, and *Aspergillus.* We compared the inhibitory profiles to soil *Streptomyces*, the most important historical source of antimicrobials, and found that the percentage of isolates from skin and nares able to completely inhibit the growth of pathogenic fungi such as *C. albicans* is

higher (27% vs 15% of isolates). We then chose a subset of isolates for genome sequencing (n=162) and identified BGC families using antiSMASH and BiG-SCAPE. Using Clinker to examine shared genomic content between each BGC family, we identified genetically distinct families in isolates shown to produce unknown and unique metabolites by paired untargeted metabolomics. This approach permits us to target specific strains for downstream chemical characterization to identify novel antimicrobial molecules. Overall, our work suggests that skin taxa are producing bioactive metabolites targeting fungi and that by characterizing these metabolites, we address a critical gap in knowledge regarding the role of metabolite-mediated interactions in the skin microbiome.

47 Horizontal transmission and loss-driven evolution in *Mycoavidus*, a *Mortierella*-associated endohyphal bacterium *Kevin Amses*[1], William Davis[2], Nicole Reynolds[3], Rasheed Adeleke[4], Teresa Pawlowska[3], Timothy James[5], Greg Bonito[6], Jessie Uehling[1] 1) Department of Botany and Plant Pathology, Oregon State University, Corvallis OR 97333 USA; 2) Department of Biological Sciences, Kent State University, Kent, Ohio 44319 USA; 3) School of Integrative Plant Science, Cornell University, Ithaca, NY, 14853 USA; 4) Unit for Environmental Science and Management, North-West University, Potchefstroom Campus, Private Bag X6001, Potchefstroom, South Africa; 5) Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor MI 48109 USA; 6) Department of Plant Soil and Microbial Sciences, Michigan State University, East Lansing MI 48824 USA.

Endohyphal bacteria (EHB) are those capable of colonizing the fungal cytosol. EHB are comprised of a polyphyletic assemblage that spans a spectrum from free-living bacteria that can transiently inhabit fungal cells (i.e., facultative) to obligate endosymbiotic bacteria that are dependent on their hosts. The widespread prevalence and biological importance of EHB associated with diverse hosts is becoming increasingly appreciated. *Burkholderia*-related endohyphal bacteria, or BRE, are known as associates of soil fungi in the Mucoromycotina. Transmission between hosts is thought to be predominantly vertical, where BRE are heritable via passage in the cytosol of sexual or asexual reproductive propagules. Genomes of sequenced BRE, such as *Mortierella*-associated *Mycoavidus*, exhibit hallmarks of vertically transferred, obligate endosymbionts such as the loss of genes in key metabolic pathways and genome contraction consistent with adaptation to the intracellular environment. However, current phylogenetic analyses of BRE separate these bacteria into several major lineages that exhibit different degrees of host specificity, and do not rule out the influence of horizontal transmission in BRE evolution. Evolutionary theory predicts different outcomes for strictly vertically transmitted, obligate endosymbiont populations, which are bottlenecked and isolated from genetic exchange, versus those that are more promiscuous (i.e., via horizontal transmission). Despite an increasingly diverse pool of rDNA sequences generated by PCR-based screening, BRE genome sequences remain few, and their transmission biology remains poorly understood. In this work, we use genome-scale data for 16 newly sequenced genomes from BRE allied to *Mycoavidus* to (i) clarify evolutionary relationships within BRE, (ii) investigate the evolutionary patterns of loss that tie extant BRE to their free-living ancestors, and (iii) assert the importance of horizontal transmission in the evolutionary history of BRE.

48 A shelter from the elements: understanding requirements for fungal chlamydospore formation and bacterial invasion *Isabelle Ludwikoski*[1], Nancy Keller[1] 1) University of Wisconsin - Madison, Madison, WI.

Bacterial-fungal interactions (BFIs) drive microbiome dynamics from environmental to healthcare settings, impacting survival and dispersal of the interacting partners. Previous work from our lab established that the plant pathogen *Ralstonia solanecearum* induces formation of swollen, overwintering spores in *Aspergillus* spp. through production of a cyclic lipopeptide called ralsolamycin (1). With deletion of *rmyA*, the polyketide synthase in the ralsolamycin biosynthetic cluster, chlamydospores are not induced. Further, in co-culture *R. solanecearum* can invade the chlamydospores (1). Recent work in the lab identified survival benefits for bacterial invasion under starvation and cold stress conditions compared to mutants unable to invade chlamydospores (2). Additionally, several Gram-negative bacteria unable to invade chlamydospores independently can invade when co-cultures are supplemented with ralsolamycin (2). Thus far most of the work on this system has been done using WT *Aspergillus flavus* and we have little understanding of mechanistic components required by the fungus and the bacterium to undergo the chlamydospore formation and invasion processes. To dig deeper into the mechanisms underlying this process, we performed an RNA-seq analysis which indicated numerous aberrantly regulated proteins involved in cell wall biosynthesis and secretion. We found *fleA,* a gene encoding a lectin that binds fucosylated structures, to be upregulated in the condition with chlamydospore production. Initial data suggests that the loss of *fleA* leads to changes in chlamydospore density. Additional preliminary data suggests that chlamydospore formation in response to ralsolamycin is dependent on density of the spore inoculum, where more chlamydospores are produced at higher density, which may also impact bacterial invasion dynamics.

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49 From iron to antibiotics: Bacterial-fungal interactions revealed by genome-wide mutational analyses *Emily Pierce*[1], Manon Morin [1], Jessica Little[4], Roland Liu[1], Joanna Tannous[2], Nancy Keller[2], Kit Pogliano [1], Benjamin Wolfe[3], Laura Sanchez[4], Rachel Dutton[1] 1) University of California San Diego, La Jolla, CA, USA; 2) University of Wisconsin-Madison, Madison, Wisconsin, USA; 3) Tufts University, Medford, Massachusetts, USA; 4) University of Illinois at Chicago, Chicago, Illinois, USA.

Intermicrobial interactions are key aspects of the biology of microbiomes. Recently, there has been a shift towards studying interactions in more representative contexts, whether using multispecies model microbial communities or by looking at interactions *in situ*. Cheese rind biofilms have been developed as experimentally tractable systems to study microbiomes. Although many studies of microbiomes focus solely on the bacterial community, previous work in the cheese rind system has revealed the importance of fungi in shaping this microbiome via interactions with bacteria. Leveraging this model system, we identified a diversity of bacterial genes involved in, and the associated fungal contributors to, bacterial-fungal interactions. To achieve this, we combined bacterial cytological profiling, RNA-Seq, metabolomics, and random barcode transposon site sequencing, a high-throughput genetic screen of 120000+ bacterial mutants. We characterized bacterial-fungal interactions across 16 bacterial-fungal pairs made up of 8 cheese-associated fungi and *E. coli* or a cheese-associated *Pseudomonas psychrophila*. We observed broad changes in bacterial mutant fitness in the presence of fungi com-

pared to growth alone. The strongest and most widespread bacterial-fungal interaction that we observed suggests that fungal species can dramatically modulate bacterial access to iron through the provision of fungal hydroxamate siderophores, such as ferrichrome and coprogen. It has long been known that bacteria grown in isolation are able to uptake purified fungal siderophores, but the ecological relevance of this putative interaction had not been demonstrated. Our results demonstrated that this exchange takes place between bacteria and filamentous fungi growing in a biofilm and that this exchange can have impacts on the competitive fitness of bacteria. Due to the importance of iron in bacterial physiology and the prevalence of fungi in microbial ecosystems, we expect that iron-based bacterial-fungal interactions are important in other microbiomes. In addition to filamentous fungi, we showed that the basidiomycete skin yeast *Malassezia pachydermatis* alleviated bacterial iron limitation. Moreover, fermented foods are known to contain fungal siderophores, which could be a source of fungal siderophores in the gut in addition to potential siderophore production by gut-resident species.

50 Elucidating Fungal Immune Receptors and Testing the Potential Role of Nucleotide-binding Domain Leucine-rich Repeat-like Proteins (NLR-like) Against Bacterial Antagonists. *Frances Stark*[1], Ksenia Krasileva[1], N. Louise Glass [1] 1) University of California, Berkeley .

Filamentous fungi are hosts to pathogens such as viruses, bacteria, parasitic fungi, and grazing nematodes. Besides RNAi to protect fungal genomes from mycoviruses, a fungal inducible defense upon recognition of bacteria has yet to be fully described. Genes encoding nucleotide-binding domain Leucine-rich repeat-like (NLR-like) proteins are present in abundance in the genomes of filamentous fungi. NLRs are intracellular receptors known to mediate cross-kingdom, antagonistic communication in plants and metazoans. Although a role for NLR-like proteins in fungi has been described for allorecognition known as heterokaryon incompatibility, evidence of cross-kingdom surveillance of fungal NLR-like proteins is lacking. In order to investigate if fungal NLR-like proteins participate in an inducible response like plant and animal NLRs, I utilize Neurospora crassa and various bacteria with a primary focus on the seventeen putative NLR-like proteins encoded in the N. crassa genome. I show that exposure of N. crassa to bacteria and bacterial secretions results in an environmental-dependent response including growth defects, increased growth rate, macroconidia production, and cell death. These results suggest that N. crassa is initiating many physiological changes, including programmed cell death upon recognition of bacteria that might be constituting a putative immune response. In order to investigate these responses, I plan on conducting RNAseq, reverse genetics of NLR-like genes, and Genome Wide Association studies of N. crassa environmental isolates. The discovery of genes underlying an immune-like response within the kingdom of fungi will not only lead to a better understanding of basic fungal biology but possibly identify novel tiggers of programmed cell death pathways to target destructive fungi or bacterial/fungal relationships.

51 Characterization of internal ribosomal entry sites in fungal RNA viruses and their potential use in multiple gene expression in filamentous fungi Kanoko Murata[1], Matteo Calassanzio[1], Akane Ueda[1], Atif Jamal[2], Nobuhiro Suzuki[2], *Sotaro Chiba*[1] 1) Nagoya University, Japan; 2) Okayama University, Kurashiki, Japan.

The reverse-genetics approach in filamentous fungi has fully relied on the availability of expression vector plasmids with a limited number of selective markers (antibiotics and their resistance genes) that are effective in a given fungal species. Hence, multiple gene expression in fungi is considered reflective of this circumstance; the number of genes for transgenic expression is generally restricted. Some unique multiple expression systems are used mostly in animal systems, including the internal ribosome entry site (IRES)-based non-canonical translation system. However, these techniques are not widely used in eukaryotic cell research except for integrating multiple promoter/terminator cassettes.

In this study, the IRES-mediated gene expression in the model fungus, *Cryphonectria parasitica*, was assessed by seeking effective IRES elements from fungal viruses (mycoviruses) by a dual-luciferase reporter system. In total, 30 fungal virus sequences and three known IRESs from animal viruses were tested. As a result, we identified sequences having the IRES activity in 5' untranslated region (5'-UTR) and following 72 nt coding regions (5'-UTR+72nt) of fungal RNA viruses that belong to seven viral lineages (families *Hypoviridae*, *Chyrsoviridae*, and *Totiviridae*, and genera *Botybirnavirus*, Fusagravirus, Yadokarivivirus, and Phlegivirus). IRES-positive viruses commonly possessed long 5'-UTRs that ranged from 395 nt to 1088 nt. Although 5'-UTR+72nt sequences of a megabirnavirus scored high IRES activities, we could not prove whether this result is true or false positive. None of the animal viral IRESs were functional in *C. parasitica*.

Furthermore, we tried to use IRES elements for multiple gene expressions in *C. parasitica*. Minimally functional regions from 5'-UTR+72nt of Rosellinia necatrix fusagravirus 3 (RnFGV3) and Alternaria alternata botybirnavirus 1 (AaBV1) 4a dsRNA2 segment were applied to two separated experiments: 1) dual fluorescent protein (YEP and RFP) expression with the AaBV1 IRES, and 2) bimolecular fluorescent complementation (BiFC) assay with the RnFGV3 IRES. Both experiments revealed that IRES-mediated multiple gene expression with a single transformation vector plasmid is applicable in *C. parasitica*. On the other hand, it also highlighted expression levels limitations. Based on the obtained results, prospects of IRES-mediated expression systems in fungi will be discussed.

52 A GPI-anchored protein gene from the chestnut blight fungus *Cryphonectria parasitica* is a hypovirus-specific virulence factor and a tolerance factor against hypovirus infection Jeesun Chun[1], Yo-Han Ko[1], Kum-Kang So[1], Su-Hwan Cho[1], *Dae-Hyuk Kim*[1] 1) Department of Molecular Biology, Department of Bioactive Material Sciences, Institute for Molecular Biology and Genetics, Jeonbuk National University, Jeonju, Jeonbuk, Korea.

The chestnut blight fungus, *Cryphonectria parasitica*, and its interaction with hypovirus, Cryphonectria hypovirus 1 (CHV1), is a model to study the fungus-virus interaction. Our previous transcriptomic analysis identified a transcript that encodes a glycosylphosphatidylinositol (GPI)-anchored protein (GPI-AP) was differentially expressed by sectorization and CHV1 infection. Sequence analysis of a deduced amino acid of the cloned gene (*CpGap1*) showed a high similarity to and phylogenic clustering with known fungal GPI-APs with canonical N-terminal leader peptide and C-terminal GPI-anchoring signal. Functional analysis comparing the *CpGap1*-null mutant with the wild type resulted in no observed phenotypic changes in growth rate, sporulation, and pigmentation. The mutant showed no changes in colonial growth in response to osmotic and temperature stresses observed. However, the *CpGap1*-null mutant showed

an increased sensitivity to the cell-wall disturbing agent SDS, but not CR and CFW. Hypersensitivity of the *CpGap1*-null mutant was observed in response to ROS. *In silico* analysis of a matured peptide of the protein product of the *CpGap1* gene (CpGAP1) suggested five motifs with antioxidizing properties, and three of these five synthesized peptides showed a strong radical scavenging capacity. Interestingly, virulence was significantly reduced in the *CpGap1*-null mutant. Phytotoxic activity, as measured by leaf discs assay using synthetic peptides, was observed in specific peptides. These results suggest that CpGAP1 functions as a protective barrier against host defenses such as ROS, even as it acts as a virulence factor that places stress on host cells. Moreover, when the CHV1 was transferred to the *CpGap1*-null mutant, severely retarded colonial growth was observed. In addition, virus-titer in the mycelia of CHV1-infected *CpGap1*-null mutant was significantly higher than those in the CHV1-infected isogenic strain (UEP1). These results indicate that CpGAP1 functions as a protective barrier against host defenses such as ROS, but also act as a virulence factor that stress host cells. Moreover, our study demonstrates that the *CpGap1* gene is a host-tolerating antiviral factor that helps maintain fungal growth and suppress viral titer after infection of *C. parasitica* with CHV1.

53 Deciphering the mycovirome of *Botrytis cinerea* *Ana Ruiz-Padilla*[1], Julio Rodríguez-Romero[1, 2], Irene Gómez-Cid[1], Davide Pacífico [3], Maria A Ayllón[4, 2] 1) Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid/Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Pozuelo de Alarcón, Madrid, Spain.; 2) Departamento de Biotecnología-Biología Vegetal, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid, Spain.; 3) Institute of Bioscience and Bioresources, National Research Council of Italy, Palermo, Italy.; 4) Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid/Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Pozuelo de Alarcón, Madrid, Spain mariaangeles.ayllon@upm.es..

The ascomycete necrotroph *Botrytis cinerea* Pers.: Fr. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel) produces gray mold or gray rot in more than 200 crops spread throughout the world and it is considered the second most significant fungal plant pathogen. It causes economically significant losses in winter crops of tomato, cucumber, bean, pepper, etc.; in grapevine it develops mainly in mature grapes in autumn; and it significantly affects both strawberry plants in field and fruits in post-harvest.
Fungal control strategies include cultural practices, biological control agents, host resistance, and fungicides. Nevertheless, traditional chemical fungicides are not a sustainable solution as a treatment anymore, especially due to the gene plasticity of the fungal genomes, which makes them easily resistant. To date, several botrycide products, based on microorganisms as active ingredients, have been developed for *B. cinerea* biocontrol. The discovery of mycoviruses that decrease the virulence of their fungal hosts could provide another alternative as biological control agents. Several mycoviruses have been already associated to hypovirulence in *B. cinerea* indicating that it is feasible to use mycoviruses in biocontrol strategies of this fungus.
In this line, we have explored the mycovirome of 248 *B. cinerea* field isolates from grapevine of Italy and Spain to increase the knowledge about mycoviral diversity and evolution, and to search for new widely distributed mycoviruses that could be active ingredients in biological products to control this hazardous fungus. A total of 92 viruses were identified, 62 of them constituting putative novel viral genera and families. Of these mycoviruses, 57 had a positive-sense single-stranded RNA (ssRNA) genome, 19 contained a double-stranded RNA (dsRNA) genome, 15 had a negative-sense ssRNA genome, and 1 contained a single-stranded DNA (ssDNA) genome. Some of the identified mycoviruses belong to genera that have previously been associated with hypovirulence, as for instance, *Mitovirus*, *Hypovirus*, *Partitivirus*, etc. Moreover, some of them, as the ssDNA mycovirus, has been already proved to decrease the virulence of *B. cinerea*. This study not only have expanded our knowledge of mycoviral diversity, horizontal transfers, and putative cross-kingdom events; but also, it has generated a collection of mycoviruses, some of which could be potential candidates of biological control agents of *B. cinerea*.
Ruiz-Padilla et al.. Novel Mycoviruses Discovered in the Mycovirome of a Necrotrophic Fungus. mBio. 2021 May 11;12(3):e03705-20. doi: 10.1128/mBio.03705-20
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54 Circadian Clock Control of mRNA Translation *Deborah Bell-Pedersen*[1], Teresa Lamb[1], Kathrina Castillo[1], Ebimobowei Preh[1] 1) Texas A&M University.

A longstanding puzzle in circadian biology is the existence of proteins that show a robust rhythm despite no rhythm in the levels of the corresponding mRNA. Although some of this effect may be due to circadian regulation of protein degradation, much of this circadian regulation of protein levels is due to rhythmic mRNA translation. In *Neurospora crassa*, at least 15% of mRNAs are rhythmically translated from mRNAs that don't cycle in abundance. We discovered that clock control of translation can be regulated in at least three different ways.

In one mechanism, the clock regulates the phosphorylation, and thus inactivation, of up to half of the conserved translation initiation factor eIF2a. Rhythms in the levels of phosphorylated eIF2a are caused by daytime activation of the eIF2a kinase CPC-3, and nighttime activation of PPP1 phosphatase. This mechanism mediates rhythmic translation initiation of up to half of rhythmically translated mRNAs.

The second mechanism involves targeting mRNAs to evolutionarily conserved cytoplasmic ribonucleoprotein granules, called p-bodies, which contain translationally repressed mRNAs. Among the 67 most robust rhythmically translated mRNAs, 93% contain a sequence motif that targets these mRNAs to p-bodies to repress translation. Loss of either the p-body component SNR-1, or deletion of the p-body target sequence, validated that this process is necessary for rhythmic translation of specific mRNAs.

The third mechanism involves clock control of ribosome composition. In *N. crassa*, the clock regulates levels of at least six ribosomal proteins, and 1 ribosome interacting protein, in intact ribosomes, some of which are not essential for growth. One of the non-essential clock-controlled ribosomal proteins is RPL-31. Loss of RPL31 in ribosomesabolished rhythms a large fraction of the rhythmically translated mRNAs and led to an increase in translation stop codon readthrough.

Disruption of clock control of mRNA translation reduces the fitness of *N. crassa* cells. Clock control of mRNA translation, as opposed to mRNA levels, provides several opportunities to increase fitness, including partitioning the energy demanding process of translation for highly expressed genes to times of day when energy levels are high, and sequestering mRNAs to p-bodies to preserve mRNAs that encode proteins needed to overcome stress to allow for a rapid response to an acute stress. Because rhythmic translation is a powerful way for the clock to control biological functions, it is not surprising that several different mechanisms have evolved to carry out this regulation.

55 Insights into biological responses to light from baker's yeast *S. cerevisiae*, an organism lacking established photoreceptors *Mikael Molin*[1] 1) Chalmers University of Technology.

Unlike other fungi, baker's yeast *Saccharomyces cerevisiae* lacks the established photoreceptors cryptochromes/photolyases, phytochromes, opsins and LOV-domain containing proteins. However, blue light still causes pronounced oscillations of the stress-associated Zn-finger transcription factor Msn2 into and out of the nucleus. We have shown that this poorly understood phenomenon is initiated by a peroxisomal oxidase that converts light into a hydrogen peroxide (H_2O_2) signal, sensed by the peroxiredoxin Tsa1, which in turn counteracts cAMP-dependent protein kinase (PKA)-dependent Msn2 phosphorylation. Interestingly, a homologous peroxisomal oxidase was previously implicated in blue light toxicity in mammalian cells suggesting that the pathway may be conserved. Our data show that upon H_2O_2 unconventional redox-signaling involving both peroxiredoxin catalytic cysteine residues attenuates the ability of the catalytic subunit of the conserved nutrient signaling kinase PKA to bind substrates independently of the second messenger cAMP, allowing Msn2 nuclear accumulation. The messenger role of H_2O_2 in signaling appears to be common both to yeast Msn2 oscillations and to light-induced entrainment of circadian rhythms. In this regard it is highly interesting that peroxiredoxins have been proposed to constitute the first identified circadian clock regulators/components conserved across the three kingdoms of life. Taken together with our data a picture in which peroxiredoxins and redox signaling appear to play key roles in endogenous rhythms emerges.

Nevertheless, the growth rate of yeast cells exposed to white visible light of moderate intensity is slowed down significantly in a manner independent on the blue-light reactive peroxisomal oxidase. Via high-throughput, ordered genetic screening for genes required for yeast to grow at a light-intensity corresponding to a fraction of that on a sunny day we identified intracellular processes involved in visible light resistance. This effort pin-pointed, among other things, e.g. proteins linked to multiple aspects of ribosome biology and translation, cellular functions impinging on the morphogenetic PKA pathway and oxidative stress management/oxidant signaling as enriched among genes required for growth in the light.

I will discuss these data in the contexts of, firstly, H_2O_2 playing key roles as a second messenger in endogenous signaling pathways e.g. in balancing translation, protein secretion and metabolism and, secondly, roles of peroxiredoxins in protein homeostasis.

56 A role for gene expression and mRNA stability in the mechanism underlying circadian nutritional compensation in *Neurospora crassa* *Christina Kelliher*[1], Jennifer Loros[1], Jay Dunlap[1] 1) Geisel School of Medicine at Dartmouth, Hanover, NH.

Circadian clocks maintain a period length of approximately 24 hours across a range of physiologically relevant external conditions, including temperature and nutrient levels. This defining circadian principle of period length maintenance is called compensation. Compensation effectors play an active role in buffering the core clock machinery from the external environment, and compensation mechanisms have been primarily solved using the circadian model system *Neurospora crassa*. Phosphorylation of the core negative element of the clock, FREQUENCY in *Neurospora* or PER in insects and mammals, contributes to Temperature Compensation (TC). However, Nutritional Compensation (NC) remains understudied.

Here, we show that mutant strains of *Neurospora* with defective TC have normal NC period maintenance. This suggested that NC effectors are distinct from TC effectors. Therefore, we performed a reverse genetic screen for alterations in the circadian period length upon glucose and amino acid starvation using ~500 single gene knockouts from the *Neurospora* deletion collection. Novel regulators were identified with disrupted or complete lack of nutritional compensation. Taken together with four NC mutants that have been characterized in previous work (Δ*csp-1*, Δ*rco-1*, *prd-1*, and Δ*ras-2*), a model emerges where normal NC of the circadian clock is maintained by regulation at the levels of transcription, chromatin regulation, and mRNA stability, which is distinct from the current phosphorylation-centric model for TC mechanism. Fascinatingly, our genetic screen uncovered one hit where NC is altered under amino acid starvation but unaffected by changes in glucose availability, consistent with multiple independent nutritional controls for period length.

Previous studies and preliminary data suggest that both the clock's positive arm (the White Collar Complex) and negative arm (FRQ/FRH/CKIa) are regulated to achieve nutritional compensation of the ~24-hour circadian period length. Furthermore, we have begun to establish a human cell culture model for investigating conservation of NC mechanism between the fungal and mammalian circadian clock. We have identified mammalian orthologs of genes that emerged as NC hits in our screen and found that knocking down those genes in mammalian cells also results in period length changes. We anticipate that one or more NC regulators in *Neurospora* may play a conserved role in maintaining the mammalian clock period length across nutrient levels, reminiscent of Casein Kinases I and II controlling TC across circadian model systems.

57 Dark stipe mutants in fruiting body development of *Coprinopsis cinerea* *Shanta Subba*[1], Botond Hegedüs[2], Laszlo G. Nagy[2], Chee Seng Man[1], Ursula Kües[1] 1) Molecular Wood Biotechnology and Technical Myycology, University of Goettingen, Goettingen, Germany; 2) Institute of Biochemistry, Biological Research Center, HAS, Szeged, Hungary.

Fruiting body formation in *Coprinopsis cinerea* is a complex morphological process employing successively > 30 different cell types in cap and stipe development. It follows a conserved developmental pathway defined by day and night phases, with well predictable distinct stages over the time. The differentiation process starts with loose aggregate formation primary hyphal knots (Pks) in the mycelium in the dark. Upon a light signal, these primary hyphal knots (Pks) turn into compact secondary hyphal knots (Sks) in which

stipe and cap tissues start to differentiate. Primordial tissue development takes 4 days (stepwise recognized as primordia stages P1 to P4) until all basic tissue formation and differentiation is completed, with probasidia in the hymenia on the gills of the P4 primordia ready to receive a light signal to induce karyogamy. Nuclear fusion is finished on day 6 of development P5 stage primordia and culminates in meiosis and basidiospore production. Basidiospore production parallels stipe elongation and cap expansion for fruiting body maturation. Mature fruiting bodies autolyze on day 7 of the fruiting pathway to release the spores in liquid droplets. Development is strictly regulated by environmental conditions including nutrients, alternating light and dark phases, temperature and aeration (CO_2). Failure in daily illumination at the Sk to the P4 stages leads to the formation of so-called 'dark stipes', under proliferation of stipe tissues and blocks in cap development. Failure in dark signaling at night phases at the Sk to the P4 stages leads to the formation of 'dwarf primordia' with short stipes and bulky caps with retarded gill differentiation. 'Dark stipe' phenotypes are further formed from P3 and P4 stages under non-aerated conditions with normal light-dark changes. Scavenger experiments of CO_2 with KOH recovered the normal phenotypes in fruiting body development. 'Dark stipe' mutants with different genetic defects are available. *dst1* and *dst2* mutants form 'dark-stipes' from secondary hyphal knots and are defective in light regulation by mutations in the WC1 light receptor and in a FAD/FMN-binding enzyme of the GlcD superfamily. *dst3* and *dst4* mutants turn into dark-stipe phenotypes at stages P3 and P4, respectively, and appear to be defective in CO_2 signaling. The *dst3* and *dst4* mutants are defective in regulation at these later stages of primordial development while they are not blind with respect of light-induced oidiation. Genome sequencing identified defective candidate genes involved in CO_2 metabolism and the Cop9 signalosome. Functions in metabolic pathways producing CO_2 and CO_2 signaling pathways will be discussed.

58 Conformational Changes in the Circadian Negative Arm Correlate with Dynamic Interactomes Involved in Diverse Biological Processes *Jacqueline Pelham*[1], Alexander Mosier[1], Samuel Altshuler[1], Christopher Kirchoff[1], William Fall[1], Lisa Baik[2], Joanna Chiu[2], Jennifer Hurley [1,3] 1) Department of Biological Sciences, Rensselaer Polytechnic Institute, Troy, NY; 2) Department of Entomology and Nematology, University of California Davis, CA; 3) Center for Biotechnology and Interdisciplinary Sciences, Rensselaer Polytechnic Institute, Troy, NY.

The widely conserved circadian clock employs a transcriptional/translational negative feedback loop (TTFL) to anticipate environmental changes due to the Earth's diurnal cycle. While an astounding amount of physiology is coordinated by this feedback loop, the intricacies of the conserved molecular oscillator are poorly understood. In *Neurospora crassa* the source of circadian output has been canonically accepted as transcriptional activation by the positive arm. However, over 40% of oscillating *Neurospora* proteins do not have rhythmic mRNA, establishing circadian post-transcriptional regulation through unknown sources, a phenomena which is conserved in higher eukaryotes. To this end we are investigating the *Neurospora*negative arm protein FREQUENCY (FRQ) as a potential source of this regulation. Given the pervasive conservation of the intrinsically disordered protein (IDP) nature of negative-arm clock proteins, we hypothesized that post-transcriptional regulation may stem from conformational shifts in negative-arm proteins that time vacillations in the constituents of negative-arm macromolecular complexes to time cellular physiology. Our investigation of the negative arm clock protein FRQ demonstrated temporal conformational fluidity correlated with daily changes in physiologically diverse macromolecular complex components. FRQ interactors that are classified as IDPs were more likely to interact with FRQ at their nadir, suggesting FRQ may tune post-transcriptional regulation via the control of interactor stability. An analogous investigation of the macromolecular complexes centered around *Drosophila melanogaster* PERIOD (dPER) and human PERIOD (hPER2) found a similar number and physiological diversity of interacting partners in higher eukaryotes. Short linear motifs (SLiMs) associated with the interactors localized to disordered and phosphorylated regions on the PERs and FRQ, with disordered interactors oscillating in the macromolecular complexes over circadian time. This oscillation correlated with oscillations in post-transcriptionally regulated proteins, suggesting the negative arm may tune cellular physiology and proteostasis post-transcriptionally via oscillations in the circadian negative-arm macromolecular protein complexes.

59 Casein kinase 1 and disordered clock proteins form functionally equivalent phospho-based circadian modules in fungi and mammals Daniela Marzoll[1], Fidel Serrano[1], Anton Shostak[1], Carolin Schunke[1], *Axel Diernfellner*[1], Michael Brunner[1] 1) Heidelberg University Biochemistry Center, Heidelberg, Germany.

Circadian clocks adjust physiology and metabolism to the 24-h day-night cycle. Eukaryotic circadian clocks are based on transcriptional-translational feedback loops. Core components, such as FRQ in *Neurospora crassa* and PERs in animals are not conserved. We show that CK1 is sufficient to promote hyperphosphorylation of FRQ and mPER2 on a circadian timescale. CK1 targets a large number of low affinity sites. The slow phosphorylation relies on site-specific recruitment of CK1 and access of intrinsically disordered segments of FRQ or mPER2 to the bound kinase, and on CK1 autoinhibition. We propose that the clock proteins FRQ or PERs and CK1 form functionally equivalent phospho-based timing modules in the core of the circadian clocks of fungi and animals.

60 Genome wide insights into signal integration by the G-protein pathway for regulation of carbon- and secondary metabolism *Miriam Schalamun*[1], Wolfgang Hinterdobler[1], Tiziano Benocci[1], Nicole Wanko[1], Johann Schinnerl[3], Monika Schmoll[1,2] 1) Austrian Institute of Technology GmbH, Department Health and Bioresources, Konrad Lorenz Strasse 24, 3430 Tulln, Austria; 2) University of Vienna, Department of Microbiology and Ecosystem Science, Division of Terrestrial Ecosystem Research, Djerassiplatz 1, 1030 Vienna, Austria; 3) Department of Botany and Biodiversity Research, University of Vienna, Vienna, Austria.

Nutrient sensing is of utmost importance for gene regulation in fungi, with the heterotrimeric G-protein pathway as prototypical transmission machinery. Previously we could show an interrelationship between light response and cellulase gene regulation in the filamentous fungus *Trichoderma reesei*. Recently we found that also secondary metabolism is regulated in a light dependent manner in *T. reesei*. In both cases, G-protein coupled receptors (GPCRs) exemplified these connections: the glucose sensors CSG1 and CSG2, which are responsible for posttranscriptional regulation of cellulase expression as well as GPR8, a GPCR associated with the sorbicillin cluster. To gain further insight into the balance between carbon- and secondary metabolism along with its dependence on light, we performed functional, transcriptome analyses and network analysis. We used deletion mutants Δ*gna1*, Δ*gna2* and Δ*gna3,* Δ*gnb1* and Δ*gng1* as well as strains expressing constitutively activated versions of the G-alpha proteins, GNA1QL, GNA2QL and GNA3QL in *T. reesei* QM6a. We found characteristic alterations in biomass formation, enzyme production and growth as well as an influence on sexual and asexual

development. Cellulase gene transcription was differentially regulated between the investigated G-protein mutant strains with important differences between light and darkness. Analysis of secondary metabolite production revealed an impact on regulation of several compounds hence substantiating the link between cellulase regulation and secondary metabolism.
We conclude that the G-protein pathway integrates signals relevant for carbon- and secondary metabolism to optimally balance enzyme biosynthesis and growth with metabolite production.

61 The evolution of DNA repair: how a cryptochrome photoreceptor became a CPD photolyase in mucoral fungi *Luis Corrochano*[1], Eusebio Navarro[2], Nils Niemann[3], Dennis Kock[3], Tamila Dadaeva[3], Gabriel Gutiérrez[1], Timo Engelsdorf[3], Stephan Kiontke[3], Alfred Batschauer[3], Victoriano Garre[2] 1) Universidad de Sevilla, Spain; 2) Universidad de Murcia, Spain; 3) University of Marburg, Germany.

Cryptochromes and photolyases are blue-light photoreceptors and DNA-repair enzymes, respectively, with conserved domains and a common ancestry. Photolyases use UV-A and blue light to repair lesions in DNA caused by UV radiation, photoreactivation, and cryptochromes have specialized roles ranging from the regulation of photomorphogenesis in plants, to clock function in animals. A group of cryptochromes (cry-DASH) from bacteria, plants, and animals has been shown to repair in vitro cyclobutane pyrimidine dimers (CPDs) in single-stranded DNA (ssDNA), but not in double-stranded DNA (dsDNA), presumably due to the lack of an efficient flipping of the dsDNA lesion into the catalytic pocket. We show that cry-DASH from *Phycomyces blakesleeanus* can repair CPD lesions in dsDNA as a bona fide photolyase, and that cry-DASH of a related fungus, *Mucor circinelloides*, not only repairs CPDs in dsDNA in vitro but is the enzyme responsible for photoreactivation in vivo. A structural model of the *M. circinelloides* cry-DASH suggests that the capacity to repair lesions in dsDNA is an evolutionary adaptation from an ancestor that only had the capacity to repair lesions in ssDNA.

62 Cross-kingdom interactions in arbuscular mycorrhizal symbiosis *Maria Harrison*[1] 1) Boyce Thompson Institute.

Arbuscular mycorrhizal (AM) fungi, of the sub-phylum Glomeromycotina, are obligate symbionts that live in mutualistic associations with land plants. Current data indicate that their dependency on their plant hosts arises from their inability to synthesize fatty acids *de novo* and their lack of hydrolytic enzymes required to release sugars from complex macromolecules; they receive both lipid and sugars from their plant host and in return, assist plants with the acquisition of mineral nutrients, particularly phosphate from the soil (1). Development of the symbiosis is complex and requires reciprocal signal exchange between the plant and fungus to enable hyphal growth into the root, and ultimately differentiation of highly- branched hyphae, called arbuscules within the root cells. Substantial rearrangement of the root cell, including the development of the periarbuscular membrane which surrounds the arbuscule, is also essential to enable arbuscule development. The mechanisms coordinating arbuscule and periarbuscular membrane development are not known but plant mutants in which arbuscule development is impaired, have led to the identification of key players within the plant cell. Lipid and phosphate transfer between the plant and fungus occurs over the arbuscule/periarbuscular membrane interface and several plant proteins involved nutrient transport have been identified. Loss of function mutants reveal that both phosphate and carbon exchange are essential to maintain the mutualism.
Fueled with carbon from its plant host, the AM fungus also develops an extraradical mycelium in the soil, and this is exposed to, and interacts with, the soil microbiota. Recent 16S rRNA profiling revealed a community of microbes tightly associated with the extraradical mycelium that is conserved across soils and two fungal species (2). The roles of this community remain to be determined but it has the potential to enhance fungal access to phosphate. Progress towards an understanding of AM fungal development within the plant cell and AM fungal interactions with the soil microbiota will be discussed.
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63 *Metarhizium*: jack of all trades, master of many *Raymond St. Leger*[1] 1) University of Maryland.

Metarhizium is a genus of highly abundant globally distributed fungi with representatives that can transition between long-term beneficial associations with plants to transitory pathogenic associations with protozoans, nematodes, insects or even lizards. These species provide tractable models to address how new mechanisms for econutritional heterogeneity, host switching and virulence are acquired and relate to diverse sexual life histories, genomic variation and speciation. The commonest species trade nitrogen extracted from their insect hosts for plant carbohydrates, thereby boosting plant growth as well as their own. They are usually specialized to particular soil and plant ecologies, but can protect and nourish their plants by overpowering a wide spectrum of insects with numerous enzymes and toxins. Some of these effectors apparently derived from horizontal gene transfer events. In addition, extensive duplication of effector-encoding genes has been facilitated by loss of meiosis associated genome defense mechanisms. Broad host range species use parasexuality instead of sex to combine beneficial mutations from separate clonal individuals into one genome (Vicar of Bray dynamics). Species which kill a narrow range of insects retain sexuality to facilitate host-pathogen coevolution (Red Queen dynamics). Some narrow-host range strains specifically target important pests such as mosquitoes, and these can be genetically engineered to produce highly effective bioinsecticides. Generalist species have multiple beneficial effects on plant growth but can also be engineered with customized properties to potentially replace chemical pesticides and fertilizers.

64 Chemical interactions between fungi and nematodes *Reinhard Fischer*[1] 1) Karlsruhe Institute of Technology (KIT).

Nematode-trapping fungi, such as *Duddingtonia flagrans*, are fascinating predatory microorganisms (1). In a nutrient-rich environment they live as saprotrophs, but if nutrients are scarce and nematodes are present, they can switch to a predatory lifestyle. The switch is characterized by the formation of adhesive trapping networks. The interaction requires complex interspecies communication involving pheromones, secondary metabolites, and virulence factors.
D. flagrans trap formation is repressed at nutrient-rich conditions by fungal arthrosporols and 6-methyl salicylate. The spatial control of the expression of the genes of the arthrosporol polyketide gene cluster leads to production of 6-MSA at the tip of hyphae and arthrosporols at the rear. Both inhibit trap formation and 6-MSA is an attractant for *C. elegans* (2). If nematodes are present and the nematode population reaches a certain level, nematode-derived ascarosides cause the downregulation of the arthrosporol gene cluster.

The decrease of the arthrosporol concentration leads to induction of trap formation.
Trap formation requires intracellular signaling through the STRIPAK signaling complex and a cell-communication system at the tip to allow ring closure (3).
Shortly after *C. elegans* is trapped by *D. flagrans,* hyphae penetrate into the worm body and secrete small proteins as virulence factors to overcome the worm defense and lytic enzymes to digest the organic material. For one of such virulence factors, we showed that it is secreted at a bulbous structure close to the entry point of the hypha (4).

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65 A structure for effector delivery in smut fungi? *Regine Kahmann*[1] 1) Max Planck Institute for Terrestrial Microbiology.

Plant pathogenic fungi colonizing living plant tissue secrete a cocktail of novel effector proteins to suppress plant immunity and reprogram host cells. Although many of these effectors function inside host cells, delivery systems related to the ones used by pathogenic bacteria to translocate effectors into host cells have not been detected in fungi. During the course of studying novel effectors in *Ustilago maydis,* a biotrophic fungus causing smut disease in corn, we have identified five unrelated effectors and two membrane proteins, which form a stable protein complex. The corresponding genes appear co-regulated, and are only expressed during colonization. Single mutants in any of these seven genes arrest in the epidermal layer, fail to suppress host defense responses and fail to induce non-host resistance, two reactions likely depending on translocated effectors. The complex is anchored in the fungal membrane, protrudes into host cells and contacts channel forming plant plasma membrane proteins, suggesting that we identified an effector translocation system. The system could be reconstituted in a surface-exposed form in cultured *U. maydis* cells. As orthologs of all complex-forming proteins are conserved in smut fungi, I will also present how we have development the complex as potential novel fungicide target.

66 A *Ralstonia pickettii* endosymbiont allows *Rhizopus microsporus* to evade amoeba and cause opportunistic virulence in animals Herbert Itabangi[1], Poppy Sephton-Clark[1], Diana Tamayo[2], Xin Zhou[1], Georgina Starling[3], Zamzam Mahamoud[3], Ignacio Insua[4], Mark Probert[1], Joao Correia[1], Patrick Moynihan[1], Teclegiorgis Gebremariam[5], Yiyou Gu[5], Ashraf Ibrahim[5,6], Gordon Brown[2], Jason King[3], *Elizabeth Ballou*[1,2], Kerstin Voelz[1] 1) Institute for Microbiology and Infection, School of Biosciences, University of Birmingham, UK; 2) Centre for Medical Mycology, University of Exeter, Exeter, UK; 3) School of Biosciences, University of Sheffield, Western Bank, Sheffield, UK; 4) School of Chemistry, University of Birmingham, Edgbaston, Birmingham, UK; 5) The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, California, U.S.A; 6) David Geffen School of Medicine, UCLA, Los Angeles, California, U.S.A.

Opportunistic infections by environmental fungi are a growing clinical problem, driven by an increasing population of people with immunocompromising conditions. Spores of the Mucorales order are ubiquitous in the environment but can also cause acute invasive infections in humans through germination and evasion of the mammalian host immune system. How they achieve this, and the evolutionary drivers underlying the acquisition of virulence mechanisms, are poorly understood. Here we show that a clinical isolate of *Rhizopus microsporus* contains a *Ralstonia pickettii* bacterial endosymbiont required for virulence in both zebrafish and mice, and that this endosymbiosis enables secretion of factors that potently suppress growth of the soil amoeba *Dictyostelium discoideum,* as well as their ability to engulf and kill other microbes. As amoebae are natural environmental predators of both bacteria and fungi, we propose this tri-kingdom interaction contributes to establishing endosymbiosis and acquisition of anti-phagocyte activity. Importantly, we show this activity also protects fungal spores from phagocytosis and clearance by human macrophages, and endosymbiont removal renders the fungal spores avirulent *in vivo.* Together, these findings describe a new role for a bacterial endosymbiont in *Rhizopus microsporus* pathogenesis in animals, and suggests a mechanism of virulence acquisition through environmental interactions with amoebae.

67 Functional diversification of effectors in smut fungi Weiliang Zuo[1], Jasper Depotter[1], Janina Werner[1], Georgios Saridis[1], Anna Rybecky[1], Andrea Passarge[1], *Gunther Doehlemann*[1] 1) University of Cologne.

The biotrophic smut fungi are one of the largest groups of fungal plant pathogens. While symptoms of most smut pathogens are restricted to the floral organs, the maize smut *Ustilago maydis* causes the formation of plant tumors in all aerial organ, locally at sites of infection. Formation of these symptoms requires a fine-tuned effector repertoire with a stage-, organ- and even cell-type specific transcriptional regulation of effector genes during plant colonization. The closely related fungus *Sporisorium reilianum*causes head smut in maize, which is characterized by systemic colonization of the plant with symptoms being mainly restricted to the inflorescences. Comparative transcriptomics followed by CRISPR-Cas9 mediated genome editing demonstrated that both the differential transcriptional regulation, as well as functional divergence of effectors contribute to the different pathogenic development of the two related species (1).
Based on these findings, we are investigating how diversifying effector repertoires of smut fungi contribute to their distinct pathogenic life-styles. This study identified the effector protein Sts2, which is translocated to the host nucleus, where it triggers the formation of tumorigenesis via a yet unknown mechanism. The tumor-inducing activity of Sts2 is linked to a sequence motif which present in *U. maydis*, but not in *S. reilianum*. Collectively, our study provides insights to the different mechanisms of host-pathogen co-evolution in related fungal pathogens.

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68 Fungal Pathogens Utilize Extracellular Vesicles for Transport of Effector Proteins into Plant Host Cells *Claire Whitaker*[1,2], Baoye He[2], Hailing Jin[2] 1) Department of Plant Biology, University of California, Riverside, CA, USA; 2) Department of Microbiology & Plant Pathology, Center for Plant Cell Biology, Institute for Integrative Genome Biology, University of California, Riverside, CA, USA.

Effectors, small, secreted proteins which modulate plant immune response, are critical for successful fungal infection. While the effector proteins of biotrophic and hemibiotrophic fungi have been well characterized, little research has been done on the effector proteins of necrotrophic fungal plant pathogens. Beginning in 2016, research on necrotrophic fungal effectors began to illuminate the vast collection of effector proteins found in the genomes of necrotrophic fungi. Many of these predicted effectors lack a N-terminal signal peptide targeting them for the traditional secretion pathway, so it remains unclear how these effectors are delivered to the apoplastic space, and subsequently, plant cells. Recent discoveries have shown that *Arabidopsis thaliana* utilizes extracellular vesicles (EVs) to deliver sRNAs to its fungal pathogen, *Botrytis cinerea*. This exchange of EVs is potentially bidirectional, with fungal EVs packaging and delivering fungal effectors into plant cells. In fact, EVs have been shown to transport the effectors of human fungal pathogens into human cells, though no such research has been done on necrotrophic fungal plant pathogens. With this in mind, our goal is to examine the EVs of *Botrytis cinerea* to determine if they are trafficking effector proteins. We have identified potential effector proteins and through the generation of mutant *B. cinerea* strains with the effectors knocked out or tagged with YFP we will determine the transport mechanism of the identified effectors. Preliminary data has indicated that our chosen proteins are in fact effector proteins, as the deletion mutants show a decreased ability to infect and kill *A. thaliana* cells. Our research will greatly expand the understanding of the roles of extracellular vesicles in fungal pathogenesis as well as identify a non-conventional secretion pathway of protein effectors.

69 Multiple mutagenesis of *Botrytis cinerea* by an improved CRISPR/Cas9 protocol reveals high redundancy of phytotoxic proteins for necrotrophic infection Thomas Leisen[1], Janina Werner[2], Patrick Pattar[1], Nassim Safari[1], Edita Ymeri[1], Frederik Sommer[1], Michael Schroda[1], Ivonne Suárez[3], Isidro Collado[3], David Scheuring[1], *Matthias Hahn*[1] 1) Kaiserslautern Univ; 2) University of Cologne; 3) Universidad de Cádiz.

Botrytis cinerea is a necrotrophic plant pathogen characterized by a wide range of host plants. During invasion, it quickly kills the host cells and colonizes the dead tissue. Mechanisms that contribute to host killing during host invasion include secretion of CWDE, release of phytotoxic proteins and metabolites, tissue acidification and the activation of PTI-related defence responses culminating in plant hypersensitive cell death. The precise role of the individual components during infection is not well understood. We have established CRISPR/Cas9 genome editing in *B. cinerea* (Leisen et al. 2020, PLoS Pathogens, 16, 1-32). The protocol was further improved by using a double sgRNA-RNP strategy in combination with transiently selected telomere vector for repetitive marker-free gene deletions, which resulted in highly efficient generation of homokaryotic, marker free single or double mutants within three weeks. By this means, we have constructed and characterized a series of up to 18-fold knockouts of genes encoding cell death inducing proteins (CDIPs) and metabolites, including Spl1, Nep1/2, Xyn11A, Xyg1, Hip1, IEB1, Xyl1, Gs1, PG1/PG2, botrydial and botcinin. Genome sequencing of a 12x mutant confirmed the deletions and revealed only few off-target mutations, and MS/MS analysis of the secretomes produced by the mutants verified the loss of the deleted CDIPs. The mutants showed generally decreased virulence with increasing numbers of deleted genes, but dependent on the infected host tissue (leaves of beans, tomatoes, maize, Arabidopsis; apple fruit), different effects of gene deletions were observed. Mutants deleted in 16 genes for CDIPs and two metabolites were still able to form necrotic lesions and to sporulate on diseased tissue, and their secretomes had substantial remaining phytotoxic activity. Our data document one of the first systematic approaches to address functional redundancy of virulence factors of a pathogenic fungus, and the apparent absence of single major virulence proteins in *B. cinerea*. While searching for and deleting further CDIPs, we are investigating the role of activation of PTI-related immune receptors by these proteins for cell-death induction and necrotrophic pathogenesis. Infection of *Nicotiana benthamiana sobir1* mutants lacking a coreceptor of LRR-RP receptor proteins did not reveal major differences to WT plants in susceptibility against *B. cinerea* WT and 12x mutants.

70 Pyricularia HAG effector family interactions with rice candidate target proteins *Nicholas Farmer*[1], Meilian Chen[2], Guodong Lu[3], Zonghua Wang[2,3], Daniel Ebbole[1] 1) Texas A&M University; 2) Minjiang University; 3) Fujian Agriculture and Forestry University.

Plant pathogen effectors play important roles in parasitism, including countering plant immunity. However, investigation of the diversification of fungal effectors is limited. Previously we described a 21-member gene family of the rice blast fungus Pyricularia oryzae that we named host-adapted genes (HAGs). Most AVR/effector genes of P. oryzae are either unique or have few paralogs. The presence of such a high number of paralogous HAG effectors suggests the potential for both redundancy and diversification in effector function. Redundancy may allow for the loss of some gene family members without loss of virulence activity. In fact, most members of the gene family display presence/absence polymorphism in the rice infecting population. Redundancy may also allow for more precise regulation of effector expression or adaptation to allelic variation of host targets. On the other hand, divergence can allow for expansion of effector target repertoires that can also lead to increased fitness. Closely related Pyricularia species contain orthologous gene family members. However, in many cases the sequence divergence of orthologs is as great as is found between paralogs. One view is that orthologs would display conservation of host target interactions and paralogs would display diversification. We have begun to test these assumptions using Yeast Two-Hybrid assays to identify candidate rice target proteins that interact with members of the HAG effector family. Putative targets identified via Yeast Two-Hybrid were cross tested with the paralogous effectors from P. oryzae as well as orthologous effectors from other closely related Pyricularia species, allowing us to define overlap in the target repertoires of these effectors.

71 Appressorium-mediated plant infection by *Magnaporthe oryzae* is regulated by a Pmk1-dependent hierarchical transcriptional network *Miriam Oses-Ruiz*[1,3], Neftaly Cruz-Mireles[2], Magdalena Martin-Urdiroz[3], Darren M. Soanes[3], Alice Bisola Es-

eola[2], Bozeng Tang[2], Paul Derbyshire[2], Mathias Nielsen[4], Jitender Cheema[4], Vincent Were[2], Iris Eisermann[2], Michael J. Kershaw[3], Xia Yan[2], Guadalupe V aldovinos-Ponce[6], Camilla Molinari[2], George R. Littlejohn[7], Barbara Valent[5], Frank L. H. Menke[2], Nicholas J. Talbot[2] 1) Public University of Navarre, Navarre, Spain; 2) The Sainsbury Laboratory, Norwich, United Kingdom; 3) University of Exeter, Exeter, United Kingdom; 4) John Innes Centre, Norwich Research Park, Norwich NR4 7UH; 5) Kansas State University, Manhattan, Kansas , USA; 6) Colegio de Postgraduados, Montecillo, Texcoco, Mexico ; 7) University of Plymouth, Plymouth, United Kingdom.

Rice blast disease is caused by the ascomycete fungus *Magnaporthe oryzae* and is the most destructive disease of cultivated rice world-wide. In response to surface signals from the rice leaf, the fungus produces a specialised infection cell called the appressorium, that enables host cuticle penetration through the plant cell wall. The regulation of appressorium formation and plant infection, are still not well understood. By using a combinatorial approach of transcriptomics, genetics, phosphoproteomics and cell biology we defined a network of temporally co-regulated transcription factors that act downstream of the Pmk1 mitogen-activated protein kinase pathway to regulate gene expression of more than 6000 genes during appressorium-mediated plant infection. We show that this is a two-tiered regulatory mechanism involving Pmk1-dependent phosphorylation of the Hox7 homeobox transcription factor and Mst12 transcription factor. Upon phosphorylation, Hox7 regulates more than 4000 genes associated with induction of major physiological changes required for appressorium development, including cell cycle control, autophagy-mediated cell death, turgor generation and melanin biosynthesis, as well as controlling a further set of virulence-associated transcription factor-encoding genes. We also show that Pmk1-dependent phosphorylation of Mst12 regulates more than 25000 genes, with functions involved in tissue invasion such as septin-dependent cytoskeletal re-organisation, polarised exocytosis, and effector gene expression. Identification of this regulatory cascade provides new potential targets for disease intervention.

72 Alternative sulfur scavenging and host colonization by the plant pathogen *Raffaelea lauricola* *Joshua Konkol*[1], Qiang Wang[1,2], Jeffrey Rollins[1] 1) University of Florida, Plant Pathology Department, Gainesville, FL 32611; 2) Northwest A&F University, State Key Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Yangling, Shaanxi, China.

Raffaelea lauricola (Ascomycota, Sordariomycetes, Ophiostomatales), causal agent of laurel wilt disease, is an invasive pathogen to North America where it has decimated populations of native Lauraceae trees in the eastern US and threatens commercial avocado production. Its acute lethality is unusual for an ambrosia beetle nutritional symbiont with the ecological role of sustaining larval development and beetle growth in natal galleries. Pathogenicity is hypothesized to be a trait that co-evolved in its native habitat in Asia where it is reported to cause mild (non-lethal) symptoms on Lauraceae trees. The rapid killing of Lauraceae trees in North America is thought to be the result of an evolutionary mismatch between the fungus and naïve populations of related tree species. We have explored the temporal-spatial dynamics of host colonization using a GFP-labeled *R. lauricola* strain, transcriptomic analysis and gene-specific gene deletion. Transcriptomic analyses of susceptible, infected trees revealed a strong up-regulation of genes encoding sulfur compound uptake and assimilation that included 7 methionine and 3 cysteine permeases, 1 sulfate transporter, 13 alternative sulfur transporters and more than 50 genes for sulfur assimilation. A screen of sulfur compounds determined that *R. lauricola* grows on a large number of organic and inorganic sulfur compounds. By contrast, gene deletion mutants of the *cyc3/metR* homolog (*RlmetR*), a positive regulator of sulfur uptake and assimilation genes, could grow only on growth media supplemented with methionine or cysteine, and, to a lesser extent, glutathione. Genetic complementation of the *RlmetR* mutation restored wild-type utilization of diverse sulfur compounds. Infection studies with the susceptible host *Persea palustris* (swampbay) determined that *RlmetR* is essential for pathogenicity. Its requirement for beetle symbiosis has not yet been established. The myriad factors that have evolved to permit colonization of natal galleries as well as pathogenicity are certain to be intertwined as in the case of sulfur scavenging. The dissection of these processes, and particularly the identification of pathogenicity-specific factors, is being pursued through comparative biology of susceptible and resistant hosts with pathogenic and non-pathogenic *Raffaelea* species and via analysis of gene-specific mutations.

73 Oomycete RXLR effectors enter plant cells by clathrin-mediated endocytosis *Paul Birch*[1], Haixia Wang[1], Steve Whisson[2], Petra Boevink[2] 1) University of Dundee; 2) James Hutton Institute, Dundee.

Oomycetes, such as the infamous potato late blight pathogen *Phytophthora infestans*, deliver RXLR effectors into host cells to suppress immunity and facilitate disease. How RXLR effectors enter plant cells is unknown. One possible route involves clathrin-mediated endocytosis, which is activated during infection by defence-associated pattern recognition receptors (PRR) when they detect pathogen-derived ligands. We transiently silenced genes encoding clathrin, and also the plant-specific rab5, Ara6, and demonstrated that endocytosis of the PRR FLS2 was compromised in each case. Silencing *clathrin* and *Ara6* attenuated *P. infestans* infection and prevented translocation of RXLR effectors into plant cells. In contrast, whereas silencing a susceptibility factor that is not required for endoytosis, PP1c, also reduced infection, it failed to prevent uptake of RXLR effectors into host cells. We performed endosome enrichment and immunoprecipitated Ara6- and clathrin-associated vesicles during infection. Ara6-and clathrin-associated vesicles co-immunoprecipitated RXLR effectors, but not apoplastic effectors that act outside plant cells. MS/MS analyses of proteins co-immunoprecipitated with Ara6 during infection revealed an enrichment of host proteins associated with endocytic vesicles alongside multiple pathogen RXLR effectors, indicating that these *P. infestans* virulence determinants are taken into the plant cell via endocytosis.

74 Pathotypes of *Fusarium oxysporum f. sp. fragariae* express discrete repertoires of accessory genes and induce distinct host transcriptional responses during root infection. Bradley Jenner[2], *Peter Henry*[1] 1) United States Department of Agriculture; 2) University of California, Davis.

Isolates classified as *Fusarium oxysporum* f. sp. *fragariae* are genetically diverse and cause one of two syndromes on strawberry. One syndrome includes symptoms of wilting and chlorosis and is caused by the "yellows-*fragariae*" pathotype, whereas only wilting symptoms are caused by the "wilt-*fragariae*" pathotype. Past work differentiated these pathotypes by symptoms and comparative genomics, but their effects on host transcription and the genomic organization of wilt-*fragariae* pathogenicity genes remain unexplored. To address these knowledge gaps, we challenged susceptible strawberry (*Fragaria* × *ananassa*) plants to root infection by five fungal isolates: three yellows-*fragariae*, one wilt-*fragariae*, and one that is not pathogenic to strawberry. The host and fungal transcriptomes were characterized at 6- and 13-days post inoculation and contrasted with non-inoculated plants or *in vitro* fungal growth. On average, >6 times more strawberry genes were differentially expressed (DE) in response to yellows-*fragariae* isolates than the other two isolates

at each timepoint. Responses to yellows-*fragariae* infection were characterized by early induction of genes related to the jasmonic acid phytohormone pathway and widespread reprogramming of carbohydrate metabolism. The wilt-*fragariae* isolate induced few transcriptional responses at 6-days post-inoculation, when plants remained asymptomatic, but strongly induced ethylene production and response factors by the later timepoint. Pathotypes were not differentiated by conserved, fungal effector gene expression and few pathotype-specific differences were observed in the expression of other conserved fungal genes. By contrast, fungal DE genes on accessory chromosomes were almost entirely distinct between pathotypes. An ~150 kbp 'pathogenicity island' on a wilt-*fragariae* accessory chromosome was enriched with DE genes, many of whose predicted functions were related to plant infection. Sequence conservation suggests this region was horizontally transferred between two wilt-*fragariae* lineages. There were 15 accessory genes expressed by all yellows-*fragariae*isolates during root infection, and only one of these genes was also DE by the wilt-*fragariae* isolate. These results support the conclusion that wilt- and yellows-*fragariae* cause physiologically distinct syndromes by the expression of discrete repertoires of genes on accessory chromosomes. Implications for our understanding of classification by '*forma specialis'* will be discussed.

75 Honor by association, leveraging global gene co-expression networks for specialized metabolic pathway discovery *Jennifer Wisecaver*[1] 1) Purdue University.

Specialized metabolites serve myriad biological functions that allow organisms to interact with and manage their environment (e.g., resist abiotic stress, combat negative ecological interactions and promote beneficial ones). These metabolites are synthesized in response to dynamic ecological pressures, and as a consequence, the pathways involved in metabolite biosynthesis are often fast-evolving, lineage-specific, and remain uncharacterized at the genetic level. To address this challenge, we've developed the mutual ranks to modules workflow, a method for identifying small, overlapping modules of co-expressed genes in global co-expression networks. These modules serve as the basis for high-throughput prediction of specialized metabolic pathways. Using the model plant Arabidopsis, modules accurately recovered the enzymatic genes of functionally characterized specialized pathways as well as genes involved in pathway regulation and metabolite transport. Importantly, a co-expression network approach can straightforwardly be applied to any species, model and non-model, so long as the organism's transcriptome can be sampled under a range of ecologically relevant conditions. The utility of this approach is illustrated by ongoing work in our lab to characterize various pathways for the production of specialized metabolites, from plant allelochemicals to fungal mycotoxins.

76 Lichen-like consortia and multicellular structures protect algae against bacterial toxins *Mario Krespach*[1, 3], Maria Stroe[1], Anna Komor[2, 3], Sandor Nietzsche[4], Volker Schroeckh[1], Severin Sasso[5], Maria Mittag[6], Christian Hertweck[2, 3], Axel Brakhage[1, 3] 1) Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany; 2) Department of Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany; 3) Institute for Microbiology, Friedrich Schiller University, Jena, Germany; 4) Electron Microscopy Center, University Hospital Jena, Jena, Germany; 5) Department of Plant Physiology, Institute of Biology, Leipzig University, Leipzig, Germany; 6) Matthias Schleiden Institute of Genetics, Bioinformatics, and Molecular Botany, Friedrich Schiller University, Jena, Germany.

Soil is a densely populated habitat housing between 10^3 – 10^5 fungi, 10^7 – 10^9 bacteria, and 10^3 – 10^6 algae (1). Recently, the prominent soil alga *Chlamydomonas reinhardtii* and the fungus *Aspergillus nidulans* were found to physically attach and exchange nutrients (2). We could show that *A. nidulans* attracts motile *C. reinhardtii* and together they form a lichen-like consortium. As lichens are often colonized by potentially harmful bacteria, we introduced the algicidal soil bacterium *Streptomyces iranensis* into the system. We found that *S. iranensis* killed *C. reinhardtii* in dual co-culture by secretion of azalomycin F (3). In a tripartite fungal-algal-bacterial co-culture, however, *C. reinhardtii* survived the presence of *S. iranensis*. We showed that azalomycin F binds to lipid membranes. As a filamentous microorganism, *A. nidulans* offers an immense lipid surface and sequesters a large proportion of azalomycin F. Thus, fewer azalomycin F molecules are available to harm *C. reinhardtii* (3). When no fungal partner is present, *C. reinhardtii* is also able to protects itself against azalomycin F. This is achieved by the formation of a novel multicellular structure that we named gloeocapsoid (4). Gloeocapsoids are characterized by a spacious extracellular polysaccharide matrix and several cell membranes. We suggest that these additional layers are produced to sequester azalomycin F and thereby protect the algal cells. These structures are on the verge of true multicellularity and suggest that natural products played a role in the evolution of multicellularity.
Collectively, we discovered an unprecedented dynamic tripartite biosystem consisting of the ascomycetous fungus *A. nidulans*, the unicellular green alga *C. reinhardtii*, and the bacterium *S. iranensis*. This system involves production of toxins, protection of mutualistic partners, and induction of protective multicellular structures from a unicellular alga.

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77 CRISPR-based transcriptional activation tool for silent genes in filamentous fungi *Laszlo Mozsik*[1,2], Mirthe Hoekzema[2], Niels A W de Kok[2], Roel A L Bovenberg[3,4], Yvonne Nygård[5], Arthur F J Ram[1], Arnold J M Driessen[2] 1) Leiden University, Dept. of Molecular Microbiology and Biotechnology, Groningen, the Netherlands; 2) University of Groningen, Dept. of Molecular Microbiology, Groningen, the Netherlands; 3) University of Groningen, Dept. of Synthetic Biology and Cell Engineering, Groningen, the Netherlands; 4) DSM Biotechnology Center, Delft, the Netherlands; 5) Chalmers University of Technology, Department of Biology and Biological Engineering, Gothenburg, Sweden.

Filamentous fungi are historically known to be a rich reservoir of bioactive compounds that are applied in a myriad of fields ranging from crop protection to medicine. The surge of genomic data available shows that fungi remain an excellent source for new pharmaceuticals. However, most of the responsible biosynthetic gene clusters are transcriptionally silent under laboratory growth conditions.

Therefore, generic strategies for the activation of these clusters are required. We constructed a genome-editing-free, transcriptional regulation tool for filamentous fungi, based on the CRISPR activation (CRISPRa) methodology. Herein, a nuclease-defective mutant of Cas9 (dCas9) was fused to a highly active tripartite activator VP64-p65-Rta (VPR) to allow for sgRNA directed targeted gene regulation. dCas9-VPR was introduced, together with an easy-to-use sgRNA "plug-and-play" module, into a non-integrative autonomously replicating AMA1-vector, which is compatible with several filamentous fungal species. The AMA1 sequence is known to self-replicate within a number of fungal species within the genera of *Aspergillus*, *Penicillium*, *Giberella*, and *Trichoderma*. To demonstrate its potential, this vector was used to transcriptionally activate a fluorescent reporter gene under the control of the transcriptionally silent synthetic *penDE* core promoter in *Penicillium rubens* (previously identified as *P. chrysogenum*). Subsequently, we activated the transcriptionally silent, native *P. rubens* macrophorin biosynthetic gene cluster by targeting dCas9-VPR to the promoter region of the transcriptionally silent, *macR* positive transcriptional regulator. This resulted in the transcriptional activation of the complete biosynthetic gene cluster of the antimicrobial macrophorins. This CRISPRa technology can be used for the rapid and convenient activation of silent fungal biosynthetic gene clusters, and thereby aid in the identification of novel compounds such as antimicrobials.

78 The fungal battery: A redox flow battery containing the biosynthesised quinone phoenicin from *Penicillium astrosanguineum* Charlotte Overgaard Wilhelmsen[1], Sebastian Birkedal Kristensen[1], Oliver Nolte[2], Ivan Volodin[2], Johan Vormborg Christiansen[3], Thomas Isbrandt[3], Trine Sørensen[4], Celine Petersen[4], Thomas Ostenfeld Larsen[3], Jens Christian Frisvad[3], Martin Hager[2], Ulrich S. Schubert[2], Kåre Lehmann Nielsen[4], Teis Esben Sondergaard[4], Jens Muff[1], *Jens Laurids Sørensen*[1] 1) Aalborg University, Esbjerg; 2) Friedrich-Schiller-University, Jena; 3) Technical University of Denmark; 4) Aalborg University, Aalborg.

Filamentous fungi display a wide palette of colorful quinone pigments, which can provide protection against oxidative stress and act as antimicrobial agents. The biosynthetic pathways for quinone pigments in fungi are initiated by non-reducing polyketide synthases (NR-PKSs) to produce entry compounds that undergo various modifications (e.g. oxidation, methylation, ammonia incorporation and dimerization) resulting in huge structural variation. Besides their natural biological role, quinones are gaining increased interest as promising electrolytes in organic redox flow batteries (RFBs) that can be used to store energy from solar and wind power plants. However, so far the quinones used in RFBs have been chemically synthesized from crude oil, which is not aligned with the sustainable thinking behind renewable energy.
To determine the electrochemical potential of fungal quinones, we initially performed computational analyses of all known compounds and identified several promising candidates. In order to be able to identify the responsible gene clusters for biosynthesis of these candidate compounds, we have genome sequenced >150 fungal strains. Tapping into this dataset, we decided to develop a RFB based on the bibenzoquinone phoenicin, which is produced by several *Penicillium* species. Through bioinformatic analyses of known phoenicin producers in our sequence database, we identified a target NR-PKS in several strains. We then developed a CRISPR-Cas9 system to knockout the NR-PKS in *P. astrosanguineum*, which subsequently lost the ability to biosynthesize phoenicin. In addition to the NR-PKS, the gene cluster contains a hydroxylase, a laccase and a transcription factor. The following analyses showed that the entry compound orsellinic acid is hydroxylated to form the intermediate 6-methyl-1,2,4-benzenetriol, which is oxidized and dimerized to phoenicin. Through cultivation optimization, we were able to achieve a production of >3 g/L phoenicin in a week, which was extracted through liquid-liquid extraction to obtain a purity of >95%. The extracted phoenicin was then used as negolyte together with ferrocyanide as posolyte to generate a RFB with a cell voltage of 0.86 V and an initial capacity of 11.75 Ah/L. The electrochemical properties of phoenicin are similar to the published petro-quinones, which demonstrates that fungal biosynthesized quinones provide a sustainable solution for energy storage.

79 No genes left behind: Associating phenotypes with genes in *Neurospora crassa* *Scott Baker*[1], Kevin McCluskey[2] 1) Pacific Northwest National Laboratory, Richland, WA; 2) Bolt Threads, Emeryville, CA.

Despite its role as a premier model organism for fungal biology studies, many classically identified genes in *Neurospora crassa* are "anonymous." These genes have a phenotype that segregates in a cross and most have been mapped to a genetic region, but they have not been associated with physical location in the genome. As such there is no correlation between the genetic locus and any open reading frame in the genome. We have resequenced over 500 strains of *N. crassa* representing over 300 classically described but otherwise anonymous genes. Using an in silico subtraction approach we are able to identify and therefore present candidate loci for many of these genes.

80 Culturing *Aspergillus nidulans* in soil microcosm elucidates its ecological behavior and interaction with soil microbiota Marina Takata[1], Moriyuki Kawauchi[2], Kiminori Shimizu[3], Keishi Senoo[2,4], Yasuo Ohnishi[2,4], Syun-ichi Urayama[5,6], *Daisuke Hagiwara*[5,6] 1) Grad. Sch. Life and Env. Sci., Univ. of Tsukuba; 2) Grad. Sch. Agr. and Life Sci., The Univ. of Tokyo; 3) Fac. of Adv. Eng., Tokyo Univ. of Sci.; 4) CRIIM, The Univ. of Tokyo; 5) Fac. of Life and Env. Sci., Univ. of Tsukuba; 6) MiCS, University of Tsukuba.

Fungi profoundly inhabit in soil and play a key role in degrading biomass and consequent material cycle in soil biome. Despite their importance, fungal behavior in the community of soil microorganisms is poorly understood at a molecular level. To investigate fungal physiology and its effect on soil microbiome, we developed a soil microcosm where the model filamentous fungus *Aspergillus nidulans* was cultured. The germinated conidia of *A. nidulans* were inoculated in sterilized and unsterilized soils and incubated for 14 days. The DNA of *A. nidulans* was extracted from the soil and subjected to quantitative PCR to measure the biomass content. Maximum growth was shown on the first day in both sterilized and unsterilized soils, and then the growth declined. This result suggests that *A. nidulans* hyphae autolyzed as the culture progressed. The amounts of cells in unsterilized soil were apparently smaller than those in sterilized soil, suggesting a competition in the microbial community.
Transcriptome analysis by RNA-sequencing revealed a set of *A. nidulans* genes that were highly expressed in the soil but not in conventional media such as PDA and PDB. Chitinase and glucosidase genes, which are associated with autolysis, showed high expression levels in the soil. Several genes related to secondary metabolism were expressed in both sterilized and unsterilized soils, but not in the conventional media. To assess if fungal secondary metabolism affects the soil microbiome, the deletion and overexpression mutant strains of *laeA* encoding a master regulator for secondary metabolism were cultured in the soil microcosm. Growth level of the mutants was decreased compared with that of the WT strain in PDA and soil microcosm. Then, the effects of *A.*

nidulans on the soil microbiome and mycobiome were analyzed by 16S rDNA and ITS amplicon sequencing, respectively. A slight but significant difference in bacterial and fungal community structures was observed between the soil inoculated with and without *A. nidulans*. Notably, the fungal community structures were significantly different between the soil inoculated with *laeA* deletion mutant and that with the WT strain after 8 weeks incubation. These results suggest that *A. nidulans* growing in the soil affects the surrounding fungal community through LaeA-dependent secondary metabolism.

81 Deciphering lichen secondary metabolism by genetic dereplication, transcriptome analysis, and heterologous expression *Wonyong Kim*[1], Rundong Liu[1], Jaycee Paguirigan[1], Hyeonjae Kim[1], Jae-Seoun Hur[1] 1) Sunchon National University.

Lichens produce a plethora of secondary metabolites (SMs) that accumulate in the cortical or medullary layers of lichen thalli. In addition, axenic culture of lichen-forming fungi often contains unusual SMs, many of which are not seen in natural lichen thalli. Despite the taxonomic and ecological significance of lichen chemistry, there has been no single genetic evidence linking biosynthetic genes to lichen SMs. Here, we characterized several novel biosynthetic gene clusters (BGCs) responsible for production of lichen-specific SMs, such as atranorin, biruloquinone, cristazarin, and other depsidone class metabolites. Phylogenetic analysis of fungal non-reducing type polyketide synthases (NR-PKS) revealed the ninth clade that includes lichen NR-PKSs responsible for depside and depsidone biosynthesis. Also, we identified a BGC for the biosynthesis of usnic acid, one of the best known lichen SM, from a non-lichenized fungus. However, the BGC harbors additional biosynthetic genes that appear to be derived from another BGC found in *Penicillium* species, which led to structural variation of usnic acid produced by the non-lichenized fungus. Knockout studies and transcriptome analysis revealed dynamic interplay of three transcription factors controlling gene expression of the fusion BGC in the non-lichenized fungus. Finally, gene cluster variation and evolutionary insights on lichen BGCs will be discussed in relation to chemical diversity.

82 Novel secondary metabolites and their biosynthesis from new Aspergilli of Australia *Yit-Heng Chooi*[1], Hang Li[1], Cameron Gilchrist[1], Indra Roux[1], John Pitt[2], Ernest Lacey[2], Andrew Piggott[3] 1) University of Western Australia, Perth, Western Australia, Australia; 2) Microbial Screening Technologies, Sydney, New South Wales, Australia; 3) Macquarie University, Sydney, New South Wales, Australia.

The Australian continent's biodiversity has evolved in isolation over tens of millions of years since it was separated from Antarctica, which has allowed the evolution of species unique to the continent. Among the filamentous fungi, the genus *Aspergillus* is well-known to be endowed with biosynthetic capabilities to generate structurally diverse secondary metabolites, including several clinical drugs. The biosynthetic talent of Aspergilli is further demonstrated by genome sequencing of many species in the genus, which uncover a vast number of biosynthetic gene clusters. We have shown that systematic chemotaxonomic and genomic studies of rare or novel Aspergillus species isolated from various geographical locations in Australia have been a fruitful strategy for discovering novel bioactive secondary metabolites and their biosynthetic pathways. This taxonomy-guided biodiscovery approach has led to the discovery of numerous unprecedented bioactive metabolites, including antifungal burnettramic acids, phytotoxic hancockiamides, antibacterial nanangenines, cytotoxic burnettienes as well as cytotoxic nanangelenins, which harbour a novel benzazepine scaffold. Here, we will discuss our chemotaxonomic × genomics approach, the biosynthetic features of several unique metabolites, and how we can further unlock the hidden biosynthetic capabilities of the fungi via transcriptional activation and heterologous pathway expression, as exemplified by our recent discovery of hancockinone with a novel prenylated 6/6/6/5 carbocyclic scaffold.

83 Pathogenic fungi at the crossroads of metal starvation and oxidative stress *Valeria Culotta*[1], Francisco Hernandez[1], Yiran Wang[1] 1) Johns Hopkins University Bloomberg School of Public Health.

Upon entering an animal host, a microbial pathogen is immediately deprived of its essential micronutrient iron. Mammals respond to infection through a clinical condition known as anemia of inflammation, whereby available pools of iron are rapidly sequestered, starving the invading microbe of this micronutrient required for growth. Successful fungal pathogens adapt by activating innovative means for capturing host iron in spite of widespread iron limitation. We observe that a low iron environment can also signal "SOS" to fungal pathogens, alerting them to prepare for additional host inflammatory insults including chemical attacks of reactive oxygen species (ROS). When iron-starved, diverse *Candida* species including members of the CTG clade and *Candida auris* secrete an extracellular form of superoxide dismutase (SOD) enzyme that has no obvious role in iron homeostasis but is essential for guarding against host attack by ROS. These iron-regulated SODs represent members of a family of so-called Cu-only SODs that are unique to fungi and closely related oomycetes. These SODs are all extracellular and effectively combat the ROS attack of host phagocytes as well as participate in fungal signaling involving ROS. In numerous fungal pathogens for plants, animals and insects, the Cu-only SODs are important virulence factors. In addition to Cu-only SODs, iron-starved *Candida albicans* secretes small (≤1 kDa) soluble metal-reactive molecules into the extracellular environment. These include copious levels of riboflavin (vitamin B2) which can act in redox reactions to reduce extracellular Cu(II) and Fe(III) to the reduced Cu(I) and Fe(II) forms required for fungal uptake of the metal. Iron-starved *C. albicans* also secrete a small highly charged metal binding molecule that is not a metal reductant but binds extracellular Cu and Fe with high affinity. These multi-layer responses to iron starvation including secretion of anti-oxidant SODs and small molecules to modify extracellular metals, can help the fungal pathogen cope with the stressful environment imposed by its host.

84 ROS regulate mitochondrial dynamics in *Aspergillus nidulans* Veronica Garrido-Bazan[1], *Jesus Aguirre*[1] 1) Instituto de Fisiologia Celular-UNAM.

Our group contributed to establish the role of reactive oxygen species (ROS) as ubiquitous signals that regulate different aspects of development and cell physiology. In the fungus *Aspergillus nidulans* the dynamin-like protein DnmA and its receptor FisA are not only required for H_2O_2-induced mitochondrial fission but also for normal growth and development. In addition, Δ*dnmA* and Δ*fisA* mutants show decreased respiration and notably high levels of mitochondrial reactive oxygen species (ROS), which likely correspond to superoxide. We show that a close interaction between mitochondria and the endoplasmic reticulum is necessary for H_2O_2-induced mitochondrial remodeling and division. Our results indicate that H_2O_2 regulates mitochondrial division at different levels and that ROS production, mitochondrial division and development are critically interrelated processes.
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85 Phosphorylation / dephosphorylation of the *Cochliobolus heterostrophus* stress-activated MAPK Hog1 in response to plant phenolic acids *Rina Zuchman*[1], Roni Koren[1], Tamar Ziv[1], Benjamin A. Horwitz[1] 1) Faculty of Biology, Technion–Israel Institute of Technology, Haifa, Israel.

Protein phosphorylation cascades are universal in cell signaling. While kinome diversity allows specific phosphorylation events, relatively few phosphatases dephosphorylate key signaling proteins. Fungal MAP kinases, in contrast to their mammalian counterparts, often show detectable basal phosphorylation levels. Dephosphorylation, therefore, could act as a signal. In *Cochliobolus heterostrophus*, the Dothideomycete causing Southern corn leaf blight, ferulic acid (FA), an abundant phenolic found in plant host cell walls, acts as a signal to rapidly dephosphorylate the stress-activated MAP kinase Hog1. To identify the protein phosphatases responsible, we constructed mutants in Hog1 phosphatases predicted from the genome by homology to yeast and other species. We found that *Cochliobolus heterostrophus* mutants lacking PtcB, a member of the PP2C family, exhibited altered growth, sporulation and attenuated dephosphorylation in response to FA. Loss of the dual-specificity phosphatase CDC14 led to slow growth, decreased virulence, and attenuated dephosphorylation. Mutants in two predicted tyrosine phosphatase genes PTP1 and PTP2 showed normal development and virulence. A functional Hog1:Gfp fusion protein partitioned to the nucleus in response to osmotic stress, but upon exposure to FA, accumulated in cytoplasmic granules. Hog1 is well-studied in the context of high osmolarity stress, but the FA-induced pathway studied here led to new insights regarding the roles of Hog1 in virulence and development. The results indicate a complex relationship between Hog1 dephosphorylation and the response of the fungal cell to chemical stress by plant phenolics. Our results reveal multi-phosphatase regulation of Hog1 mediated by serine/threonine phosphatases rather than tyrosine phosphatases. The cytoplasmic granules where Hog1:Gfp accumulates following exposure to FA resemble stress granules. However, the phosphorylation state of this MAPK is decreased rather than increased, as currently known in stress conditions. These observations reinforce the view of dephosphorylation as a stress signal, together suggesting a novel MAPK stress response. By biochemical and proteomic methods, we are addressing (1) the sequence of phosphorylation and dephosphorylation events on Hog1 in response to FA and osmotic stress; (2) the subcellular localization of Hog1 upon exposure to FA.

86 Signaling Pathway Loss-of-Function Alleles and Evolutionary Hotspots in the Fungi *Paul Magwene*[1], Katherine Dura[1] 1) Duke University, Durham, NC.

A modest number of evolutionarily conserved signal transduction pathways are primary regulators of stress responses and morphogenetic processes across the fungal tree of life. Examples of these pathways include Ras-cAMP-PKA signaling, calcineurin signaling, TOR signaling, and a variety of MAPK signaling pathways. Because of the central role that these signaling networks play in regulating cellular physiology and differentiation, they have been intensively studied, both as tractable models for understanding the principles of signaling and as potential targets for strain improvement or antifungal drug design. From an evolutionary perspective, these pathways are expected to be under relatively strong stabilizing selection, as the phenotypic consequences of mutations in these pathways are pleiotropic and loss-of-function mutations (LoF) in these pathways typically lead to reduced growth rates and increased sensitivity to environmental stresses. We describe comparative population genomic analyses of signaling pathway LoF alleles for three model yeast genera, *Saccharomyces*, *Candida*, and *Cryptococcus.* We show that several pathways exhibit unusually high frequencies of naturally occurring putative LoF alleles and that specific genes in these pathways seem to be particularly tolerant to such mutations. We discuss the implications of this finding for the evolutionary lability of signaling pathways in the fungi, and combine information on loss-of-function alleles with related evidence from QTL mapping and experimental evolution studies to identify pathways that may act as «evolutionary hotspots» for adaptation to novel environments.

87 Regulation of cell shape and virulence factor expression in response to temperature in the fungal pathogen *Histoplasma capsulatum* *Anita Sil*[1] 1) University of California San Francisco.

The long-term goal of our research is to determine how environmental signals such as temperature regulate morphology and virulence in the fungal pathogen *Histoplasma capsulatum*. *H. capsulatum* grows in a multicellular filamentous form in the soil; once inhaled into a mammalian host, these cells switch their growth program to a unicellular parasitic yeast form that subverts the innate immune system to cause disease. Temperature is a key signal that regulates this morphogenetic switch, and we are intrigued by how this pathogenic microbe senses and responds to temperature as well as other stresses in the host. We identified the first transcriptional regulators required for growth in the yeast form in response to host temperature. These factors, named Ryp proteins, are orthologous to key developmental regulators in other fungi, and represent critical elements of the temperature-dependent regulatory circuit in *H. capsulatum*. We also discovered a cell surface protein (Msb2), a MAP kinase (Hog2), and a transcription factor (Stu1) that are required for filamentation at room temperature. Interestingly, our data indicate that the Ryp pathway and the Msb2 pathway antagonize each other, and that temperature determines which pathway dominates. The Ryp proteins also regulate the expression of effector proteins that influence the biology of macrophages during infection with the yeast form of *H. capsulatum*. We are interrogating these fungal effector proteins to identify those that are required for pathogenesis. Ultimately we hope to elucidate an integrated pathway of thermosensory and thermoresponsive proteins required for *H. capsulatum* to thrive either in the soil or in the mammalian host.

88 Circadian Clock-Controlled Translation of Specific *Neurospora crassa* mRNAs Requires Rhythmic eIF2α Activity and P-bodies *Kathrina Castillo*[1,2], Cheng Wu[2], Zhaolan Ding[1,2], Matthew Sachs[2], Deborah Bell-Pedersen[1,2] 1) Center for Biological Clocks Research, Texas A&M University; 2) Department of Biology, Texas A&M University.

At least half of proteins that accumulate with a circadian rhythm in *Neurospora crassa* are produced from mRNAs whose levels are not clock-controlled, indicating a prominent role for clock regulation of post-transcriptional processes. Phosphorylation of at least 30% of available *N. crassa* eIF2α, a conserved translation initiation factor, is clock-controlled, peaking during the subjective day. To determine the impact of rhythmic eIF2α phosphorylation on rhythmic translation, we carried out temporal ribosome profiling and RNA-seq in WT, clock mutant Δ*frq*, eIF2α kinase mutant Δ*cpc-3*, and constitutively active *cpc-3*^{c} cells. We discovered that ~14% of *N. crassa* mRNAs are rhythmically translated in WT cells, and translation rhythms for ~30% of these mRNAs were dependent on the clock and CPC-3. FunCat term analysis revealed that these cTICs primarily function either in anticipation of, or in direct response to various stresses experienced by the cells on a daily basis. Most circadian translation initiation-controlled genes (cTICs) are expressed from non-rhythmic

mRNAs, and contain a cytoplasmic P-body localization motif present in their 5' leader sequence. Deletion of the P-body component SNR-1, and deletion of the P-body motif in the 5' leader of one of these mRNAs *zip-1*, significantly altered rhythmic translation of *zip-1* mRNA. Furthermore, the deletion of P-body components SNR-1 and SNR-7 led to reduced linear growth rates in constant conditions, to a level comparable to cells with abolished rhythmic eIF2α phosphorylation. Together, these results revealed a mechanism by which the circadian clock regulates rhythmic translation of specific mRNAs, through rhythmic eIF2α activity and P-body metabolism.

89 Ccr4 and Gcn2 contribute differentially to stress-specific translational repression in *C. neoformans* *Corey Knowles*[1], John Panepinto[1] 1) Department of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY, USA.

Cryptococcus neoformans is a ubiquitous environmental fungus and opportunistic human pathogen, primarily impacting immunocompromised hosts such as those living with HIV/AIDS. One of *C. neoformans* key virulence traits is its ability to undergo the rapid transition from surviving in its environmental niche, to surviving the harsh environment inside of the human lung. Here, it is subject to the sudden temperature shift to the human core temperature of 37°C, and oxidative stress from resident lung macrophages, among other stressors. In a wild type (WT) strain, exposure to these stressors results in ribosome collision accompanied by a repression in translation, and rapid decay of abundant homeostatic mRNAs, many of which code for ribosomal proteins. This response results in a translatome reprogramming that promotes translation of mRNAs encoding stress response effectors. Our previous work has identified Ccr4-dependent mRNA decay to be a requirement for translatome reprogramming in response to stress in *C. neoformans,* and as a result, a *ccr4*Δ mutant is broadly stress sensitive. This observation has led us to investigate additional paths to translational repression, testing the hypothesis that stress responsive translatome reprogramming will require regulation of translation at the ribosome. Gcn2, the sole kinase of eIF2α in *C. neoformans*, is required for translational repression and subsequent RP mRNA decay during oxidative stress from H_2O_2, but is completely dispensable for adaptation to host temperature stress of 37°C. Additionally, a *gcn2*Δ mutant is sensitive to oxidative stress, and exhibits persistent disome accumulation. Interestingly, the defect in temperature-induced translational repression in a *ccr4*Δ mutant is rescued in minimal medium by robust phosphorylation of eIF2α. This rescue is dependent on Gcn2, and is abrogated in a *ccr4*Δ*gcn2*Δ mutant. These results point to deadenylation-dependent decay as a convergence point for translatome reprogramming in *C. neoformans*, and suggest that individual stressors and their magnitude contribute to the translational response to stress in this important pathogen through different ribosome associated pathways. Future work will determine which components of the ribosome quality control machinery are necessary for recognizing and resolving these changes in translation in response to environmental stressors relevant to host adaptation.

90 A Tor1 N-terminal region required for *Candida albicans* anabolic- and stress regulation *Wanjun Qi*[1], Maikel Acosta-Zaldívar[1], Peter Flanagan[2,3], Ning-Ning Liu[1,4], Niketa Jani[1,5], José Fierro[6], María Andrés[6], Gary Moran[2], Julia Köhler[1] 1) Division of Infectious Diseases, Boston Children's Hospital/Harvard Medical School, Boston, MA; 2) Division of Oral Biosciences, School of Dental Science, Trinity College Dublin, Ireland; 3) Department of Clinical Microbiology, St. James's Hospital, Dublin, Ireland; 4) School of Public Health, Shanghai Jiao Tong University School of Medicine, Shanghai, China; 5) BioAgilytix, Boston, MA; 6) Laboratory of Oral Microbiology, University Clinic of Dentistry (CLUO), and Department of Functional Biology (Microbiology), Faculty of Medicine, University of Oviedo, Oviedo, Asturias, Spain.

For eukaryotic cells, Target of Rapamycin Complex 1 (TORC1) makes an essential decision to either direct cellular resources toward growth and proliferation in favorable conditions, or toward stress responses in adverse environments. Loss of TORC1 function is lethal. A TORC1 inhibitor like rapamycin could be a potent antifungal against *Candida albicans*, but this agent that targets the highly conserved Tor kinase domain, is also severely toxic to human cells.
The least conserved region of fungal and human Tor kinases are the N-terminal HEAT domains. We here examined the role of the 8 most N-terminal HEAT repeats of *C. albicans* Tor1 during nutritional- and stress responses. Using cells expressing N-terminally truncated Tor1 from repressible *tetO* (*tetO-TOR1ΔHEAT*), full-length Tor1 from *tetO (tetO-TOR1*) or wild type Tor1 from the native promoter, we found specific stress responses to be significantly impaired by loss of Tor1 N-terminal HEAT repeats, including those to oxidative-, cell wall-, and heat stress. Specifically, during oxidative stress, translation was inappropriately upregulated in *tetO-TOR1ΔHEAT* cells, while activation of the oxidative stress response MAP kinase Hog1 was weak. In contrast, plasma membrane stress and antifungal agents that disrupt plasma membrane function were tolerated by *tetO-TOR1ΔHEAT* cells.
Cells lacking N-terminal HEAT repeats were unable to take advantage of favorable nutritional conditions by accelerating their growth. Genome-wide expression analysis showed simultaneous activation of both anabolic- and starvation responses in *tetO-TOR1ΔHEAT* cells in the absence of stress, accompanied with mis-regulation of carbon metabolism and translational machinery biosynthesis. Current work comparing the responses of cells lacking this region of Tor1 to cells with constitutively active Tor1 kinase will distinguish phenotypes due to inappropriate Tor1 activation during stress, from lack-of-function phenotypes in these cells.
Targeting fungal-specific Tor1 N-terminal HEAT repeats with small molecules might abrogate fungal viability, especially when during infection multiple stresses are imposed simultaneously by the host immune system.

91 Control and function of facultative heterochromatin in *Neurospora crassa* *Zachary Lewis*[1] 1) University of Georgia.

Polycomb Group (PcG) proteins are part of an epigenetic cell memory system critical for multicellular development and epigenetic processes including X-chromosome inactivation in animals and vernalization in plants. In animals, plants, and many fungi, Polycomb Repressive Complex 2 (PRC2) catalyzes methylation of histone H3 lysine 27 to assemble transcriptionally repressed facultative heterochromatin. Within fungi, PRC2 targets diverse genes for repression. For example, PRC2 represses secondary metabolism gene clusters in *Fusarium*, effector genes in *Magnaporthe*, and accessory chromosomes in *Zymoseptoria*. Despite insights into the control of PRC2 in *N. crassa,* the biological role of facultative heterochromatin is poorly understood in this fungus. The majority of PRC2 target genes in *N. crassa* encode hypothetical proteins that are unique to Ascomycetes, Sordariomycetes, or the *Neurospora* genus. To generate insights into the function of PRC2-dependent gene repression, we examined gene expression patterns of PRC2 target genes across a variety of environmental and developmental conditions. We found that most PRC2-target genes are strongly and uniquely induced during sexual development, suggesting PRC2 represses sexual development and reinforces somatic cell identify. Indeed, strains defec-

tive in PRC2 undergo precocious perithecial development in the absence of a partner. These data suggest that the commons ancestor of fungi, plants, and animals likely used PRC2 to control cell fate. We also sought to identify new components of the facultative heterochromatin pathway in *N. crassa.* We performed a targeted RNA-seq screen of *N. crassa* gene deletion strains. We identified several factors required for repression of PRC2 target genes, including the *Neurospora* homolog of IMITATION SWITCH (ISW). ISW is critical for normal transcriptional repression, nucleosome organization, and establishment of typical histone methylation patterns in facultative heterochromatin domains. Stable interaction between PRC2 and chromatin depends on ISW. Thus, ISW plays a critical role in assembling and maintaining transcriptionally silent facultative heterochromatin.

92 Heterochromatin marks perturb transcriptional robustness and underpin dispensability of genes across evolutionary timescales in fungi *Sabina Tralamazza*[1,2], Leen Abraham[1], Claudia Reyes[1], Benedito Correa[2], Daniel Croll[1] 1) Laboratory of Evolutionary Genetics, Institute of Biology, University of Neuchatel, Neuchatel, Switzerland ; 2) Department of Microbiology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil.

Epigenetic modifications are key regulators of gene expression and underpin genome integrity. Yet, how epigenetic changes affect the evolution and transcriptional robustness of genes remains largely unknown. Here, we show how repressive histone marks underpin the trajectory of genes across evolutionary timescales. To determine transcriptional robustness and protein sequence evolution, we focused on two parallel systems of major crop pathogens, the *Fusarium graminearum* species complex (FGSC) and *Zymoseptoria tritici*. We performed transcriptomic and methylome (H3K27me3, H3K4me2) profiling of by including multiple closely related species. Furthermore, we used a collection of a thousand isolates of *Z. tritici* spanning the world-wide distribution to define precise boundaries of epigenetic marks and regions of high variation in gene content. Expanding to closely related species, we found that gene expression variation is negatively correlated with gene conservation confirming that highly conserved genes show higher expression robustness. Interestingly, highly conserved genes with repressive histone marks show no clear association between protein conservation and transcriptional robustness compared to unmarked genes. Importantly, we show that genes marked by H3K27me3 result in low phenotypic response during host infection. Highly conserved marked genes show enrichment in environmental stress related functions, carry hallmarks of fast evolving genes, result in low phenotypic response during host infection and, hence, do not follow the housekeeping gene archetype. Hence, histone modifications provide a key association between protein evolvability and gene essentiality across evolutionary timescales.

93 Methylation of H4 controls gene expression in facultative heterochromatin *Mareike Moeller*[1], Devin Wright[1], Michael Freitag[1] 1) Department of Biochemistry and Biophysics, Oregon State University, Corvallis.

Facultative heterochromatin controls the development and differentiation in many eukaryotes. In metazoans, plants, and many filamentous fungi, facultative heterochromatin is characterized by transcriptional repression and enrichment with histones that are trimethylated at histone H3 (H3K27me3). While loss of H3K27me3 results in de-repression of transcriptional silencing in many species, additional up- and downstream layers of regulation are necessary to control transcription in these regions. Here, we investigated the effects of histone marks on histone H4 in the plant pathogen *Zymoseptoria tritici*. Deletion of the methyltransferase responsible for H4 methylation resulted in global gene activation, especially in facultative heterochromatin, and to a much greater extent than the loss of H3K27me3 we had previously observed. This gene activation is accompanied by chromatin reorganization affecting H3K27me3 distribution, H3K4me2 levels, and a complete loss of ASH1-mediated H3K36me in facultative heterochromatin regions. Strains with specific mutations in the single H4 gene of *Z. tritici* resemble these chromatin changes, underlining the importance of H4 methylation for overall chromatin structure. The mutants we obtained are more sensitive to genotoxic stressors and show a greatly increased rate of accessory chromosome loss. Using epifluorescence microscopy and immunoprecipitation, we are disentangling the interactions of three different histone methyltransferase complexes *in vivo*. Taken together, our results provide insights into a novel, and unsuspected, mechanism controlling the assembly and maintenance of facultative heterochromatin.

94 RNAi and heterochromatin independently control gene expression and transposable elements in Mucorales *María Isabel Navarro-Mendoza*[1], Carlos Pérez-Arques[1], Joseph Heitman[1] 1) Department of Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, NC, USA.

The RNA interference machinery silences expression and prevents movement of transposable elements in fungal species with active RNAi systems. The early-diverging fungus *Mucor lusitanicus* has an intricate RNAi system with two interacting pathways that control transposable elements and endogenous gene expression: one involves a canonical or Dicer-dependent pathway and the other an alternative Dicer-independent pathway. The pericentric regions flanking *Mucor* mosaic centromeres harbor numerous Mucoromycotina-specific L1-like retrotransposable elements known as Grem-LINE1s that are actively silenced by the canonical RNAi machinery. Inactivation of key components of the alternative RNAi pathway was found to increase silencing of these retrotransposons. We conducted ChIP-seq experiments to determine if this increase in post-transcriptional gene silencing was correlated with enhanced heterochromatin formation. Our results identified the presence of di- and trimethylated H3K9 (H3K9me2 and -me3) at Grem-LINE1 and other transposable elements genome-wide, often correlating with abundant antisense small RNAs. But surprisingly, H3K9me2 and -me3 levels were not significantly altered in mutants lacking key components of either the canonical (Dicer2, Ago1) or alternative RNAi pathways (Rdrp1, Rdrp3, R3B2), indicating that RNAi is dispensable for heterochromatin maintenance at transposable elements. RNAi also controls endogenous gene expression in the Mucorales. We identified protein-coding loci that harbor high levels of small RNAs displaying canonical siRNA features. In contrast to transposable elements, H3K9me2 or -me3 was not present in these genomic locations. The absence of heterochromatin marks at genes actively silenced by RNAi indicates that RNAi does not in and of itself recruit the machinery that forms heterochromatin. Taken together, our results suggest that RNAi and heterochromatin formation are independent genome defense and regulatory mechanisms in the Mucorales, contributing to a shift in paradigm from the co-transcriptional gene silencing observed in fission yeasts to models in which heterochromatin and RNAi operate independently in early-diverging fungi. This may also be the case in other ascomycetes and basidiomycetes such as *Neurospora crassa* and *Cryptococcus neoformans*, and the phylogenetic position of *Mucor* suggests that independence of function of RNAi and heterochromatin may represent an ancestral state in the fungal kingdom.

95 A prion accelerates proliferation at the expense of lifespan *David Garcia*[1,2], Edgar Campbell[2], Christopher Jakobson[2], Mitsuhiro Tsuchiya[3], Ethan Shaw[1], Acadia DiNardo[1], Matt Kaeberlein[3], Daniel Jarosz[2] 1) University of Oregon, Institute of Molecular Biology; 2) Stanford University, Department of Chemical and Systems Biology; 3) University of Washington, Department of Pathology.

In fluctuating environments, switching between different growth strategies, such as those affecting cell size and proliferation, can be advantageous to an organism. Trade-offs arise, however. Mechanisms that aberrantly increase cell size or proliferation—such as mutations or chemicals that interfere with growth regulatory pathways—can also shorten lifespan. Here we report a natural example of how the interplay between growth and lifespan can be epigenetically controlled. We find that a highly conserved RNA-modifying enzyme, the pseudouridine synthase Pus4/TruB, can act as a prion, endowing yeast with greater proliferation rates at the cost of a shortened lifespan. Cells harboring the prion grow larger and exhibit altered protein synthesis. This epigenetic state, [*BIG*$^{+}$] (*b*etter *i*n *g*rowth), allows cells to heritably yet reversibly alter their translational program, leading to the differential synthesis of dozens of proteins, including many that regulate proliferation and aging. Our data reveal a new role for prion-based control of an RNA-modifying enzyme in driving heritable epigenetic states that transform cell growth and survival.

96 Probing the role of N6-methyladenine DNA modification within the *Rhizopus microsporus* and *Mycetohabitans* symbiosis *Margaret Branine*[1], Imperio Real-Ramirez[1], Sue Hoseon Choi[2], Stephen Mondo[3], Teresa Pawlowska[2] 1) Graduate Field of Microbiology, Cornell University, Ithaca, NY; 2) School of Integrative Plant Science, Cornell University, Ithaca, NY; 3) US Department of Energy Joint Genome Institute, Berkeley, CA .

The early-diverging fungal phylum Mucoromycota displays a high degree of coevolution with bacteria as representatives from each of the three subphyla (Glomeromycotina, Mortierellomycotina, and Mucoromycotina) commonly harbor bacterial endosymbionts. It is not clear why members of Mucoromycota so commonly and intimately associate with bacteria relative to other fungal lineages. We hypothesize establishment and maintenance of the symbiosis is mediated by a shared epigenetic DNA modification of the two partners, N6-methyladenine (6mA). This hypothesis stems from the recent discovery that, unlike in most eukaryotes, 6mA is the predominant methylation mark in early-diverging fungi, a feature shared with bacteria. For this study, we focused on the symbiosis between *Rhizopus microsporus* (Mucorales) and its endosymbiont *Mycetohabitans* (Burkholderiales). The reproductive addiction (asexual and sexual) of host strains of *R. microsporus* to its endosymbiont along with the existence of strains naturally free of endosymbionts (i.e., non-hosts) permit comparative investigations into symbiosis factors. To this end, we assessed vegetative growth of two host and one non-host isolates when exposed to the small molecule DNA adenine methyltransferase inhibitor pyrimidinedione. While pyrimidinedione inhibited growth for each isolate, the effect of inhibition did not differ depending on host status. When we cured one host strain of its endosymbiont and repeated the inhibitor experiment, we observed growth of the cured strain was significantly inhibited by pyrimidinedione after 2 days. Furthermore, when we mated two compatible host strains in the presence of the inhibitor, we qualitatively observed little difference in sexual sporulation compared to control conditions. Taken together, our inhibitor experiments suggest endobacteria protect their hosts from the negative effects of the methylation inhibitor; however, we cannot exclude several confounding factors, namely rapid inhibitor degradation. To address these limitations, we are currently creating adenine methyltransferase mutants in *Mycetohabitans* to determine the role of bacterial 6mA modifications within the symbiosis. Additionally, we present our in-progress analysis of 6mA methylation from PacBio sequences of wildtype and cured host strains ATCC52813 and ATCC52814 and their endosymbionts, *Mycetohabitans* sp. B13 and B14, respectively.

97 Periodic DNA patterns associated with chromatin regulation in Fungi *Stephen Mondo*[1], Juna Lee[1], Guifen He[1], Hugh Salamon[1], Catherine Aime[2], Ronan O'Malley[1], Igor Grigoriev[1] 1) DOE Joint Genome Institute, Lawrence Berkeley National Lab, Berkeley, CA; 2) Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN.

Across Eukaryota, one of the most highly conserved and fundamental complexes is the nucleosome. Nucleosomes are the basic repeating units, each spanning ≈150bp, that structure DNA in the nucleus and precise positioning of these are critical for regulating gene activity. Consequently, a substantial number of epigenetic modifications in eukaryotes serve to move these complexes, making genes easier (or more difficult) to access by transcriptional machinery. However, previous studies in model eukaryotes have revealed the potential for DNA itself to play an important role in nucleosome organization, although its broader significance across taxa is currently poorly understood. Here, we discovered the presence of ≈150bp Periodic DNA Patterns (PDPs) across several diverse eukaryotes at key genomic positions, particularly surrounding transcriptional start sites and coding sequence start sites. PDPs were particularly abundant in the Basidiomycota and were enriched at highly conserved genes. Through *in vitro* and *in vivo* assays on multiple fungi we confirmed that the presence of these sequences is associated with increased nucleosome occupancy, particularly in the Basidiomycota, indicating an ancient contribution of DNA to organizing chromatin in this phylum. Occupied DNAs harbored AT-rich cores with peaks of GC ±37bp from nucleosome centers. This GC profile, coupled with local DNA structural features were the most important contributors to generation of nucleosome-favorable DNA landscapes. Using these features, we created a model for predicting *in vitro* nucleosome occupancy which showed agreement with both *in vitro* and *in vivo* occupied sites in the Basidiomycota phylum. Importantly, outside the Basidiomycota, lineages without PDPs showed little agreement between predicted and actual nucleosome-bound sites, indicating that the contribution of DNA to nucleosome organization can vary widely across taxa. This analysis brings to light the potentially substantial role DNA sequence might play in nucleosome organization in some organisms and allows us to more profoundly explore its relationship with epigenomic modifications and chromatin remodelers for final organization of eukaryotic chromatin.

98 Chromatin remodeling is required for the expression of small interfering RNAs from repetitive DNA loci in *Neurospora crassa* *Eugene Gladyshev*[1], Florian Carlier[1], Sebastian Castro Ramirez[1], Sara Chehboub[1] 1) Institut Pasteur (Paris, France).

We have discovered a new process that induces strong expression of small interfering RNAs from synthetic repetitive DNA loci in vegetative cells of *Neurospora crassa*. This process requires two canonical quelling factors QDE-1 (RNA polymerase) and QDE-3 (RecQ/SGS1 helicase) but can still occur in the absence of RAD51 and RAD52, suggesting its independence from homologous recombination. In this situation, the expression of small RNAs can be completely suppressed by removing one conserved SWI/SNF chromatin remodeler. The absence of this protein dramatically reduces nucleosome occupancy and increases nuclease sensitivity of the repetitive DNA locus. These and other current results suggest a model in which the expression of small RNAs is induced by an active mechanism that

restores (and thus generally maintains) nucleosomes on repetitive DNA.

99 The conidial coin toss: asymmetric spore adhesion in *Colletotrichum graminicola* *Brian Shaw*[1], Joseph Vasselli[1], Hope Hancock[1], Ellen Kainer[1], Thomas Chappell[1] 1) Texas A&M University.

Colletotrichum graminicola is an economically significant fungal pathogen of maize. The primary infective conidia of the fungus, the macroconidia (also called falcate conidia), are splash dispersed during rain events. The adhesion of the macroconidia is required for the development of infection structures. Macroconidia are capable of immediate adhesion due to hydrophobic interactions with the substrate. We report that rapid adhesion in *C. graminicola* is asymmetric, with a strip of adhesive material running the length of a single side of the conidium. This strip of adhesive is co-localized with dynamic transverse actin cables, and both the adhesive strip and actin cables are formed prior to adhesion to the infection court. These polarized adhesives determine early adhesion, and increases in adhesion rates can be induced by applying force to flip conidia onto their adhesive faces. We hypothesize that this selective adhesion helps to increase the dispersal of the spores beyond their initially site of deposition.

100 Differences in spore size and atmospheric survival shape stark contrasts in the dispersal dynamics of two closely related fungal pathogens *Anne Pringle*[1], Jacob Golan[1], Shunping Ding[1], Daniele Lagomarsino Oneto[2], Agnese Seminara[2], Amanda Gevens[1] 1) University of Wisconsin-Madison, USA; 2) Università di Genova, Italy.

A frequently ignored but critical aspect of microbial dispersal is survival in the atmosphere. Fungal dispersal is mediated by spores. While spores are reported to cross continents or oceans in air currents, whether spores remain viable after the crossings is unclear. We exposed spores of two closely related, morphologically dissimilar, and economically important fungal pathogens to typical atmospheric environments and modeled the distances travelled by viable propagules. We first tracked the mortality of *Alternaria solani* and *A. alternata* conidia in ranges of solar radiation, relative humidity, and temperature. We then measured survival in an ideal environment over 12 days. *A. solani* conidia are nearly 10 times longer than *A. alternata* conidia and most die after 24 hours. By contrast, over half of *A. alternata* spores remain viable at 12 days. HYSPLIT models of the movement of spores across North America predict 99% of the larger *A. solani* conidia will settle within 24 hours, with a maximum dispersal distance of up to100 km. But most *A. alternata* conidia remain airborne for more than 12 days and extremely long-distance dispersal is possible, even from Wisconsin to Greenland. The larger spores of *A. solani* survive less well than smaller spores of *A. alternata* but also disperse shorter distances. These data relating poorer survival in the atmosphere to shorter distances travelled highlight the radically different dispersal dynamics of even closely related fungi.

101 RNA Editing Controls Toxicity of a *Neurospora* Spore Killer *Nicholas Rhoades*[1], Thomas Hammond[1] 1) Illinois State University.

Sk-2 is a complex meiotic drive element found in *Neurospora* that is transmitted to offspring through sexual reproduction in a non-Mendelian manner. Typical Mendelian genetics dictates that each allele in a sexual cross should have an equal probability of being inherited by the proceeding generation; however, *Sk-2* is able to transmit itself through sexual reproduction in a biased manner by eliminating any meiotic product that does not inherit *Sk-2* via spore killing. In *Sk-2 ´ Sk*^{S} (*Spore killer*-sensitive) crosses, asci with four black, viable ascospores and four white, inviable ("killed") ascospores are produced. The four surviving ascospores almost always inherit the *Sk-2* element, resulting in a >99% biased transmission of *Sk-2* to the surviving population. Previous work has identified two genes that are crucial for successful meiotic drive by spore killing, *rsk* (*resistance to Spore killer),* and *rfk-1* (*required for killing-1*). These genes are located on opposite arms of chromosome III but are genetically linked due to several genomic rearrangements within *Sk-2*, suppressing recombination in this region and ensuring that both *rfk-1* and *rsk* are always inherited together. Here, we present evidence that the killing gene, *rfk-1,* encodes two protein variants: an innocuous 102 amino acid product (RFK-1^{A}) and a toxic 130 amino acid product (RFK-1^{B}). We also show that expression of RFK-1^{B} requires an early stop codon in *rfk-1* mRNA to undergo adenosine-to-inosine (A-to-I) mRNA editing, which occurs exclusively during the sexual cycle of *Neurospora*. In addition, we demonstrate that RFK-1^{B} is toxic when expressed within vegetative tissue of *Sk*^{S} strains, and that this vegetative toxicity can be suppressed by co-expressing the *Sk-2* allele of RSK, suggesting the resistance function of RSK is active in vegetative tissues. Currently, we are examining the possibility that the secondary structure of the *rfk-1* transcript is critical for RNA editing to occur.

102 The HMG Domain-Containing Transcription Factors Hgr1 and Hgr2 are Putative Dormancy Factors of *Cryptococcus* Spores *Megan McKeon*[1], Christina Hull[1,2] 1) Department of Biomolecular Chemistry, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI; 2) Medical Microbiology and Immunology, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI.

Spores are a dormant, stress-resistant cell type used by fungi to spread to new environments. To survive, these cells must remain dormant until they encounter favorable conditions for germination, an essential differentiation process in which dormant spores transition into vegetatively growing cells. Despite the importance of spore germination in the life cycles of the majority of fungi, the molecular networks governing this fundamental process remain relatively poorly understood. To determine the molecular mechanisms controlling germination, we carried out a time course transcriptomic analysis of *Cryptococcus* spores, assessing 11 time points over the 10 hour transition from spores to yeast. We discovered that the most dynamic differences in transcript levels occurred between dormant spores (time 0) and 20 minutes after initiation of germination. During this short time span, 2078 transcript levels increased and 863 transcript levels decreased, suggesting that spores are primed to respond quickly to both synthesize new transcripts and degrade existing spore transcripts upon initiation of germination. Through an analysis of known transcription factors in *Cryptococcus* we hypothesized that two high mobility group (HMG) domain-containing proteins would be regulators of germination initiation. To test this hypothesis, we evaluated strains with deletions of *HGR1* and *HGR2* for phenotypes during germination. Using a high-resolution, quantitative germination assay, we discovered that spores produced by both *hgr1Δ* and *hgr2Δ* strains germinate at a faster rate than wild type spores. This finding suggests that Hgr1 and Hgr2 play a regulatory role during germination in which they modulate the rate at which germination initiation occurs. Our working model is that Hgr1 and Hgr2 act as dormancy factors to maintain the ungerminated spore state by repressing transcriptional responses to germination signals. Future experiments will identify environmental signals that control Hgr1 and Hgr2, directly regulated targets of Hgr1 and Hgr2, and define the transcriptional regulatory network that coordinates spore

germination and subsequent vegetative growth.

103 *Aspergillus niger* conidial germination: 3D live cell exploration *Susanne Fritsche*[1,2], Matthias Steiger[1,2] 1) Austrian Centre of Industrial Biotechnology (ACIB GmbH), Vienna, Austria; 2) Technical University of Vienna, Vienna, Austria.

Conidial germination describes the transition of dormant spores to hyphal structures. Breaking dormancy is followed by isotropic and polarized growth with a cell wall constantly being remodeled.
To explore and understand the rearrangement and timing of key events in *A. niger*, we use the Nanolive stain-free live cell imaging system - a combination of holography and tomography. A temperature-controlled growth chamber is used for incubating the fungus on-stage of the microscope. With the 3D Nanoscopy technique, videos of the germination process can be recorded and morphological structures distinguished based on different refractive indices (RI). Images of germinating conidia and digital cell reconstruction in 3D based upon the cell's inherent physical properties revealed a ring formation that might direct germ tube formation.
This knowledge is critical to the development of future approaches to manipulate fungal growth for medical, agricultural or industrial purposes.

104 Developmental genetics of host invasion initiated by fungal conidia *Soumya Moonjely*[1], Wang Zheng[3], Jeffrey P Townsend[3, 4, 5], Frances Trail[1, 2] 1) Department of Plant Biology, Michigan State University, East Lansing, MI, USA; 2) Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI, USA; 3) Department of Biostatistics, Yale School of Public Health, New Haven, CT, USA; 4) Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT, USA; 5) Program in Computational Biology and Bioinformatics, Yale University, New Haven, CT, USA.

Conidial germination is a key step for initiating the colonization of fungi on any host or substrate. We are using an evolutionary approach to identify genes that have shifted in expression to acquire new roles in evolving lineages of fungi. We are examining seven ecologically and morphologically diverse lineages in the Sordariomycetes with a common ancestor. Comparative transcriptomics provides a powerful tool to identify the genes related to developmental differences between closely related organisms and as part of this project we are examining, *Fusarium graminearum* and *Metarhizium anisopliae*. *F. graminearum* is a plant pathogenic fungus and is a causal agent of Fusarium head blight in cereal crops, whereas *M. anisopliae* is an endophytic fungus that can form a mutualistic association with plant hosts. *M. anisopliae* is also well described as an insect pathogenic fungus, hence widely used as a biocontrol agent against agricultural pests. Four designated conidial germination stages were selected for transcriptome analysis: fresh conidia, polar growth, doubling of the long axis, and first hyphal branching. We examined the transcriptional differences between orthologous genes in these species across the four stages on a common growth medium and on the natural host, barley. Orthologs that have greatly increased in expression in one lineage when compared to another are hypothesized to have taken on new roles in that species. These orthologs, identified via ancestral character estimation, are then targeted for functional assays through gene knock-outs. Combining the transcriptome data with functional gene knock-out assays allows us to identify the network of genes necessary for conidial germination, pathogenesis/mutualism and the evolutionary path to new symbiotic relationships.

105 Sporulation environment drives variation in genetically-identical conidia *Michelle Momany*[1], S. Earl Kang[1], Brandi Celia[1], Douda Bensasson[1] 1) University of Georgia.

A single colony of *Aspergillus fumigatus* produces a huge number of genetically-identical uninucleate conidia via mitosis. *A. fumigatus* is ubiquitous, thriving across varied environments and its conidia are easily windborne. To investigate the effect of sporulation environment on the ability of conidia to germinate, we performed sporulation/germination condition swap experiments in complete medium, minimal medium, at high temperature, with excess copper, excess iron, limited iron, excess salt, excess reactive oxygen species, or limited zinc. Using flow cytometry we measured conidial swelling to monitor the germination and synchrony of hundreds of thousands of conidia. We also monitored conidial virulence in a *Galleria mellonella* host. We found that conidia produced in identical environments displayed heterogeneity in germination kinetics and synchrony and that the degree of heterogeneity was driven by the sporulation environment. We also found that sporulation environment had an impact on *G. mellonella* survival, with conidia produced at elevated temperature causing faster wax-moth death. Our results show that the environment of sporulation impacts germination and virulence potential and suggest that conidia employ a form of bet-hedging to allow progeny to survive in varied environments.

106 Transcription activity before dormancy in fungal conidia modulates phenotypic variation and affects the fitness and capabilities of fungal cells after germination Fang Wang[1], Winnie Weng In Chong[1], Zhiqiang Dong[1], Jeremiah Aquino Aslarona[1], Pooja Sethiya[1], Xiaohui Hu[1], Shuhui Guo[1], Yingying Chen[1], Ang Li[1], Kaeling Tan[1], *Koon Ho Wong*[1] 1) University of Macau.

Fungi produce large quantities of clonal asexual spores, which are highly resistant entities that can stay dormant for a long time until favorable conditions are encountered. How fungal spores prepare for dormancy is not known. Studies have shown that spores contain abundant stable messenger RNAs (mRNAs); however, their origin and purpose remain unclear. Our work showed that the so-called dormant conidia of three filamentous fungal species (*Aspergillus nidulans*, *Aspergillus fumigatus* and *Talaromyces marneffei*) have robust transcription activities to synthesize their own mRNAs. Conidia remain transcriptionally active and responsive to the changing environment (i.e., not dormant) until they leave the developmental structure. Environment-specific transcriptional responses can influence conidial content, expedite gene expression when dormancy is broken, and affect the subsequent fitness and capabilities of the fungal cells after germination, including drug and stress resistance, mycotoxin and secondary metabolite production, and virulence. Our findings uncover a mechanism for how genetically identical conidia achieve phenotypic variation and suggest that conidia prepare for the future by synthesizing and storing transcripts according to their experience before entering dormancy.

107 Two distinct lipid transporters together regulate invasive filamentous growth in *Candida albicans* Miguel Basante-Bedoya[1], Stephanie Bogliolo[1], Rocio Garcia-Rodas[1,2], Oscar Zaragoza[2], Robert Arkowitz[1], *Martine Bassilana*[1] 1) University Cote d›Azur/CNRS/INSERM, Nice, France; 2) Mycology Reference Laboratory, National Centre for Microbiology, Health Institute Carlos III, Madrid, Spain.

Flippases transport lipids across the membrane bilayer to generate and maintain asymmetry. The human fungal pathogen *Candida al-*

bicans has 5 flippases, including Drs2, which is critical for filamentous growth and phosphatidylserine (PS) distribution (1). Furthermore, a *drs2* deletion mutant is hypersensitive to the antifungal drug fluconazole and to copper (1,2). We now show that such a mutant also has an altered distribution of phosphatidylinositol 4-phosphate, PI(4)P, and ergosterol, as well as reduced virulence in a murine model of systemic candidiasis. Analyses of additional lipid transporters, *i.e.* the flippases Dnf1-3, and oxysterol binding protein (Osh) family lipid transfer proteins, *i.e.* Osh2-4 and Osh7, indicate that they are not critical for filamentous growth. However, deletion of *OSH4*, which encodes a lipid transporter that exchanges PI(4)P for sterol, in a *drs2* mutant specifically bypass the requirement for Drs2 in invasive filamentous growth. In addition, removal of the lipid phosphatase Sac1, which dephosphorylates PI(4)P, in a *drs2* mutant results in a synthetic growth defect, suggesting that Sac1 and Drs2 function in parallel pathways. Together these data indicate that a balance between the activities of two different classes of lipid transporters regulates invasive filamentous growth, *via* PI(4)P. In contrast, deletion of *OSH4* in the *drs2* mutant does not restore growth on fluconazole, copper or papuamide A, a toxin that binds PS in the outer leaflet of the plasma membrane, suggesting that Drs2 has additional role(s) in plasma membrane organization, independent of Osh4.

(1) Labbaoui *et al.,* PLoS Pathog., 2017, 13(2):e1006205.
(2) Douglas & Konopka, PLoS Genet., 2019, 15(1):e1007911.

108 Lipid flippase mediated Cryptococcus-host interaction during pulmonary cryptococcosis *Siddhi Pawar*[1,2], Yina Wang[1], Orchi Dutta[2], Varsha Gadiyar[2], Raymond Birge[1,2], Chaoyang Xue[1,2] 1) Public Health Research Institute, New Jersey Medical School, Rutgers University, Newark, NJ ; 2) Department of Microbiology, Biochemistry, and Molecular Genetics, New Jersey Medical School, Rutgers University, Newark, NJ .

Cryptococcus neoformans is a facultative intracellular pathogen that infects the lung and disseminates to the central nervous system in immunocompromised patients. Alveolar macrophages are the first line of defense against *C. neoformans* infection. However, the molecular basis of macrophage recognition and interaction with this yeast pathogen remains incompletely understood. Our previous studies on the mechanism of antifungal drug resistance identified Cdc50, a regulatory subunit of lipid translocase (flippase), not only mediates drug resistance, but also is essential for virulence in *C. neoformans*. We found that loss of Cdc50 increases exocytoplasmic phosphatidylserine (PS) accumulation, making it susceptible to phagocytosis and rendering an avirulent phenotype in a murine model of cryptococcosis. Mice infected with *cdc50Δ* mutant cells induced high Th1 and Th17 cytokine production, increased fungal clearance in the lungs and prevented dissemination to secondary organs. We hypothesize that the accumulation of PS on *cdc50Δ* cell surface induces macrophage recognition and phagocytosis, which helps clear the infection in the lung. We are testing this hypothesis using other fungal strains with altered PS exposure to study their interaction with macrophages in both cell line J774 and murine primary macrophages. In collaboration with Dr. Raymond Birge at Rutgers, we are also evaluating the activation of PS receptors in IFN-γ chimeric reporter cell lines. If our hypothesis is confirmed, we will block the PS receptor activation using PS inhibitors to study PS mediated cryptococcus-host recognition. Overall, our study may lead to a novel target for antifungal development.

109 Role of Arv1 protein in sterol metabolism and pathogenicity of the chestnut blight fungus *Cryphonectria parasitica* *Soumyadip Kundu*[1], Chathuri Mohottige[1], Todd Mlsna[1], Angus Dawe[1] 1) Mississippi State University.

Intracellular sterol redistribution is a very important step in the lipid homeostasis of organisms. Lipid homeostasis is also directly linked to the organizational arrangement in the plasma membrane (PM) of the cells. Previous studies in the budding yeast *Saccharomyces cerevisiae* have demonstrated that the ARV1 (ACAT-related enzyme-2 required for viability 1) protein is a major regulator of the sterol transport mechanism from the endoplasmic reticulum to the plasma membrane, thus contributing to the structural organization of the PM, rendering it resistant to anti-fungal compounds as well as maintaining the integrity of the ER. This study is aimed to assess the significance of ARV1 in the plant pathogenic fungus *Cryphonectria parasitica* (*CpARV1)* and investigate its role in the pathogenesis and virulence of the fungus. *C. parasitica* is the major causative agent of Chestnut blight, which has wreaked havoc on the American chestnut species. Genomic analysis has revealed that the *CpARV1* gene is very closely linked to another gene that putatively encodes a cynamide hydratase (*CpCAH1)*. An initial gene deletion event using a prior gene prediction for unrelated work resulted in the elimination of both genes and a highly deformed phenotype in *C. parasitica* that was fully recoverable by complementing with the deleted region. Expression analysis through both qPCR and endpoint PCR has determined that the specific lack of *CpARV1* was primarily responsible for the debilitated phenotype of the double mutant, with no transcript detectable from the putative hydratase gene. Subsequent complementation of the *CpARV1* gene was also observed to restore the wild-type phenotype. Mass spectrometry-based methods including single ion monitoring were employed to analyze the sterol content of the wild type strain, *CpARV1* deletion strain and the *CpARV1* complemented strain. The Single Ion Monitoring results clearly indicated a substantial decrease in sterol content of the *CpARV1* mutant strain compared to wild type EP155 (approximately half the peak height and area in the raw chromatogram) thus confirming a role for *CpARV1* in sterol homeostasis. It has been previously shown that infection of *C. parasitica* with virulence-attenuating hypoviruses altered intracellular lipid content and protein secretion. We have also explored sterol content in *C. parasitica* infected with CHV1-EP713 and found decreased sterol content (peak heights and areas approximately similar to that of the mutant strains in the raw chromatogram) suggesting a potential connection between the hypovirus-infected phenotype and *CpARV1*. Current ongoing mass-spectrometric analyses are targeted at ascertaining wider metabolomic differences between the strains. Transmission electron microscopic study is also being performed to analyze the ER integrity of the different strains.

110 Sterol homeostasis is critical for surface structure organization and virulence in *Cryptococcus neoformans* *Hau Lam Choy*[1], Tamara Doering[1] 1) Washington University in St. Louis, St. Louis MO, USA.

Cryptococcus neoformans is an opportunistic fungal pathogen that causes pulmonary and meningeal infections in immunocompromised hosts and is responsible for 15% of AIDS-related deaths. The standard treatment for Cryptococcosis comprises three antifungals, two of which, Fluconazole and Amphotericin B, target ergosterols. These lipids have essential cellular and biophysical functions in fungal cells. Ergosterol biosynthesis has been well-defined in *Saccharomyces cerevisiae.* However, our understanding of the relationship between sterol homeostasis and cryptococcal pathogenesis is lacking.

Our objective is to determine the function of the lipid transfer protein Ysp2 and its effect on cryptococcal pathogenesis. Ysp2 is homologous to an *S. cerevisiae* retrograde sterol transporter that moves sterols from the plasma membrane to the endoplasmic reticulum. There have been no previous reports about lipid transfer proteins in *C. neoformans*, making this protein and its roles in pathogenesis of great interest. We found that when *YSP2 (CNAG_00650)* was deleted, the mutant strain had reduced ability to survive within phagocytes and significantly attenuated virulence in a mouse infection model. Based on the known Ysp2 function, we hypothesized that the accumulation of sterols at the plasma membrane is detrimental to cryptococcal survival in host-like environments. Consistent with this hypothesis, we observed that when grown in host-like conditions, *ysp2Δ* cells had a significantly reduced growth rate and increased sterol levels at the surface. They also displayed invaginations of both the plasma membrane and cell wall. Strikingly, when ergosterol synthesis was reduced by fluconazole addition, all of these defects were rescued. To further test our hypothesis, we are currently isolating plasma membrane fractions to analyze sterol composition using GC-MS.

We are also interested in potential novel roles of the cryptococcal Ysp2. In *S. cerevisiae*, Ysp2 is one of six lipid transfer proteins with different functions. In *C. neoformans*, however, we find only one homolog, suggesting that cryptococcal Ysp2 acts at multiple sites.

Overall, we have shown that a lipid transfer protein is critical for the virulence of *C. neoformans*. By determining how Ysp2 affects sterol organization, we will elucidate the mechanisms of cryptococcal cellular distribution of ergosterol, an important drug target during human infection.

111 Oxylipin Signals Affecting Host and Pathogen Interactions *Nancy Keller*[1], Breanne Steffan[1], Taylor Schoen[1], Dante Calise[1], Anna Huttenlocher[1] 1) Univ Wisconsin, Madison.

Oxylipins, or oxygenated lipids, are universal signaling molecules across all kingdoms of life. In the filamentous fungal pathogen *Aspergillus fumigatus,* oxylipins – both fungal and host derived – mediate developmental switches in development such as hyphal branching and spore production. In vertebrate hosts, oxylipins activate either pro- and anti-inflammatory pathways that can exacerbate or resolve microbial disease. The secreted *A. fumigatus* oxylipin 5,8-diHODE induces hyperbranching via activation of the fungal transcription factor ZfpA (1). Here we explore virulence attributes of ZfpA deletion and overexpression mutants in the zebrafish model of invasive aspergillosis, assess the impact of fungal oxylipins in microbial development and immune cell activation and address the hypothesis that the vertebrate oxylipin receptor G2A may recognize 5,8-diHODE and play a role in host response to *A. fumigatus* infections.
1. Fungal oxylipins direct programmed developmental switches in filamentous fungi. Niu M, et al. Nat Commun. 2020 Oct 14;11(1):5158.

112 Eisosomes mediate a novel pathway for regulating PI(4,5)P_2 in *Candida albicans* that is critical for cell wall morphogenesis and virulence *Carla Lanze*[1], James Konopka[1] 1) Stony Brook University.

The ability of *Candida albicans* to resist host-mediated stress allows it to infect numerous sites in the human body. The plasma membrane plays a central role in defense by acting as a barrier and by mediating dynamic processes including cell wall synthesis, morphogenesis, and membrane transport. Specialized subdomains of the fungal plasma membrane known as eisosomes are important mediators of stress resistance. Mutants lacking the eisosome protein Sur7 display sites of aberrant cell wall formation and are more sensitive to agents that exacerbate membrane or cell wall stress. Microscopic analyses revealed that *sur7Δ* mutant cells display mislocalized patches of phosphatidylinositol 4,5 bisphosphate (PI(4,5)P_2) that correspond to large invaginations of plasma membrane and cell wall. Mutagenesis studies revealed that the cytoplasmic C-terminus of Sur7 is needed for proper regulation of PI(4,5)P_2 and cell wall morphogenesis. We hypothesize that Sur7 regulates PI(4,5)P_2 levels in *C. albicans* by recruiting phosphatidylinositol phosphatases to eisosomes. To test this hypothesis, we deleted all of *C. albicans'* phosphatidylinositol 5' phosphatase genes (*INP51*, *INP52*, and *INP54*) and found that mutants lacking the phosphatase genes phenocopy features of *sur7Δ*, such as increased sensitivity to cell wall stress, misregulated PI(4,5)P_2, and abnormal cell wall growth, and were defective in invasive hyphal growth. While all three of the *inp* mutants showed defects similar to *sur7Δ*, the phenotypes of the individual mutants were weaker than the *sur7Δ* mutant, indicating that Sur7 appears to regulate more than one phosphatase. These studies have shown that one critical role of eisosomes in promoting resistance to stress is to promote the ability of Sur7 to regulate PI(4,5)P_2. Further work will determine the nature of Sur7's interaction with the PI(4,5)P_2 phosphatases. Delineating the mechanisms by which eisosomes regulate PI(4,5)P_2 will help define how this lipid controls cell signaling, morphogenesis, and virulence in *C. albicans* and in other eukaryotic organisms.

113 Role of the *Malassezia* lipidome in human skin health *Thomas Dawson*[1] 1) Agency for Science, Technology and Research
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The gut microbiome has achieved near celebrity status but the skin microbiome, particularly the skin mycobiome, remains elusive and poorly investigated. Even today, most skin biome investigations focus on bacteria via 16S sequencing, with few studies inclusive of shotgun metagenomic or ITS data sets which move beyond bacteria. This is despite the fact that many recent clinical publications propose a causative role for fungi in common skin disorders such as dandruff / seborrheic dermatitis and a role in exacerbating many others including wounds, atopic dermatitis, and eczema. To date, *Malassezia* lipid metabolism is implicated in seborrheic dermatitis pathogenesis, via release of irritating free fatty acids by a secreted lipase. However, as *Malassezia* can be commensal, pathogenic, and likely mutualistic, we hypothesized that their lipid metabolism may be involved in more complex communication between the mycobiome and their human host. Common language mediators across multiple kingdoms are oxygenated polyunsaturated fatty acids. Interestingly, these mediators in fungi and plants are referred to as "oxylipins", while in human and animal biology they are referred to as "eicosanoids". This has created research silos and led to poor inter-field communication and a lack of collaborative research on this crucial aspect of host/microbe interaction. By leveraging a novel mass-spectroscopy based lipidomic method we have identified a series of signaling molecules produced by *Malassezia* and found on human skin, identifying the human skin "core lipidome". We have quantitatively assessed oxylipins from *in vitro Malassezia* cultures and from the surface of human skin, detected temporally stable inter- and intra-individual lipid mediator profile differences, and demonstrated direct effects of specific *Malassezia*-secreted lipids on human skin cells in culture. This work should provide a toolbox for further investigation of the role of the human microbiome in health and

disease.

114 Lipid peroxidation and mitochondrial metabolism enable regulated cell death in Rice Blast *Qing Shen*[1], Naweed Naqvi[1] 1) Temasek Life Sciences Laboratory.

Magnaporthe oryzae, which causes the destructive blast disease in rice, undergoes highly regulated cell death in the conidium during its pathogenic development. We found that Ferroptosis, a cell-death program driven by iron-dependent peroxidation of membrane lipids to lethal levels, enables such precise conidial demise essential for proper appressorium development, and pathogenicity in *M. oryzae.* We optimized ratiometric-fluorescence imaging to precisely detect the subcellular levels and locales of lipid hydroperoxides. Increased accumulation of such oxidized lipids was found to be produced via iron-dependent NADPH oxidases anchored along the plasma membrane in the particular conidial cell(s) undergoing ferroptosis. Interestingly, mitochondria in such ferroptotic conidial cells underwent fission and subsequent degradation via mitophagy. Conversely, the mitophagy-defective *atg24*Δ conidia showed increased mitochondrial fusion (or filamentous mitochondria) and abnormal distribution of lipid peroxides; and a consequent decrease in ferroptosis. Such association between mitochondrial metabolism and ferroptosis was also evident when mitochondrial membrane potential was chemically abolished. Rather surprisingly, loss of mitochondrial β-oxidation, which breaks down fatty acids for acetyl-CoA generation, had no effect on ferroptotic cell death in conidia. Therefore, functional mitochondria capable of undergoing precise mitophagy or fission in a regulated manner are required for fungal ferroptosis executed by lipid peroxides.

115 Metal tolerance in the mycorrhizal fungus *Suillus luteus* *Sara Branco*[1], Anna Bazzicalupo[2], Kaile Zhang[3,4], Joske Ruytinx[5], Hui-Ling Liao[3,4] 1) Department of Integrative Biology, University of Colorado Denver, Denver, CO; 2) 2- Department of Zoology, University of British Columbia, Vancouver, BC, Canada; 3) North Florida Research and Education Center, University of Florida, Quincy, FL; 4) Soil and Water Sciences Department, University of Florida, Gainesville, FL; 5) Vrije Universiteit Brussel, Brussels, Belgium.

Exposure to toxic levels of metals disrupts homeostasis and leads to cell damage, decreased fitness, and death. Some species develop strategies to withstand metal rich environments, however how metal tolerance evolves, is maintained, and affects mycorrhizal fungi and their plant partners remains elusive. Here, we use the ectomycorrhizal fungus *Suillus luteus* and one of its pine partners as models to unveil the patterns and mechanisms of metal tolerance in mycorrhizal mutualisms. This fungus is known for inhabiting metal contaminated soils and has been found to include both metal sensitive and tolerant individuals, with the latter thriving in high metal concentrations. We used a combination of genomic and imaging approaches to assess the genetic basis of metal tolerance in *S. luteus*, as well as its role in pine metal uptake. Our work showed metal tolerance in *S. luteus* is a polygenic trait, with genomic differentiation including candidate genes involved in metal exclusion, immobilization, and detoxification. In addition, we found *S. luteus* alleviates pine metal uptake by preferentially accumulating metals in the cells surrounding the root tip (mantle). Future work will use functional and experimental approaches to validate candidate genes and further assess both how *S. luteus* tolerates high metal levels and the role of fungal metal tolerance on the biology of the plant partner.

116 Using machine learning to gain insight on how environment and diet influence the evolution of galactose metabolism across the budding yeast subphylum *Marie-Claire Harrison*[1], Abigail LaBella[1], Dana Opulente[2], Chris Hittinger[3], Antonis Rokas[1] 1) Vanderbilt University; 2) Villanova University; 3) University of Wisconsin-Madison.

The metabolisms, isolation environments, and genomes of all ~1,200 known species of budding yeasts are now characterized by the Y1000+ project, led by the Hittinger & Rokas labs. The dataset's broad evolutionary scope, coupled with its inclusion of ecological data and of quantitative and qualitative growth rate data on dozens of substrates for nearly all ~1,200 species, makes it uniquely powerful for understanding the evolution of metabolic pathways. Galactose is a monosaccharide that is abundant in nature and is found in many forms: for example, in lactose, in glycoproteins and glycolipids, or in raffinose and melibiose, common polysaccharides in grains and other plants. Even though genes from the *GAL* pathway are present in most species, there is substantial variation in the strength of growth on galactose across budding yeasts, suggesting that the *GAL* pathway varies in its genomic structure, function, and regulation across the subphylum. In my presentation, I will show that growth on galactose can be predicted with a high degree of accuracy from either qualitative and quantitative growth data on diverse substrates or from data on the environments where species are found as input, using a supervised machine learning approach and data from nearly all known budding yeast species. These findings suggest that both isolation environment and diet influence the evolution of galactose metabolism in budding yeasts, and that there is substantial variation that is yet to be studied in the *GAL* pathway. More broadly, my results raise the hypothesis that ecology is a reliable predictor of metabolic specialization in microbial eukaryotes.

117 The extrachromosomal circular DNAs of the rice blast pathogen *Magnaporthe oryzae* contain a wide variety of LTR retrotransposons, genes, and effectors *Pierre M Joubert*[1], Ksenia V Krasileva[1] 1) University of California, Berkeley.

One of the ways genomes respond to stress is by shedding extrachromosomal circular DNAs (eccDNAs). EccDNAs can contain genes and dramatically increase their copy number. They can also reinsert into the genome, generating structural variation. They have been shown to provide a source of phenotypic and genotypic plasticity in several species. However, whole circularome studies have so far been limited to a few model organisms. Fungal plant pathogens are a serious threat to global food security in part because of their rapid adaptation to disease prevention strategies. Understanding the mechanisms fungal pathogens use to escape disease control is paramount to curbing their threat. We present a whole circularome sequencing study of the rice blast pathogen *Magnaporthe oryzae*. We find that *M. oryzae* has a highly diverse circularome containing many genes and showing evidence of large LTR retrotransposon activity. We find that genes enriched on eccDNAs in *M. oryzae* occur in genomic regions prone to presence-absence variation and that disease associated genes are frequently on eccDNAs. Finally, we find that a subset of genes is never present on eccDNAs, which indicates that the presence of these genes on eccDNAs is selected against.

118 Clonality and recombination in natural populations of *Candida auris* Yue Wang[1], *Jianping Xu*[1] 1) McMaster University.

Candida auris is a recently emerged pathogenic yeast capable of causing a diversity of human infections worldwide.
Sequence analyses have clustered strains of this species into five divergent clades, with each clade containing a single mating

type, *MTL***a** and *MTL***a**. While significant divergence was observed among the five clades, there is limited genetic variation within individual clades, consistent with multiple recent emergence and clonal spread of this pathogen across the world. However, most genes involved in mating and meiosis in *Candida* species are also found in the *C. auris* genomes, suggesting its potential for sexual reproduction in nature. In the present study, we analyzed the patterns of associations among single nucleotide polymorphisms (SNPs) in both the nuclear and the mitochondrial genomes of 1,031 strains to investigate potential evidence for recombination in natural *C. auris* populations. Overall, we found that at the clade level, polymorphisms in the nuclear and mitochondrial genomes clustered the strains similarly into the five clades, consistent with limited evidence for hybridization among clades. However, variable numbers of nuclear loci within each of four clades (clades I-IV where multiple sequenced genomes are available for analyses) showed evidence of linkage equilibrium and phylogenetic incompatibility, consistent with recombination during the evolution of *C. auris*. Interestingly, the nuclear SNPs that are shared among the four clades showed greater evidence for recombination than those found only within individual clades, suggesting that there was more frequent recombination before the divergence of the clades than afterwards. Though very limited, our observed evidence for recombination within individual clades suggests the potential presence of strains with alternative mating types in nature within each clade.

119 Genomic variation across a clinical *Cryptococcus* population linked to disease outcome *Poppy Sephton-Clark*[1], Jennifer Tenor[2], Dena Toffaletti[2], Nancy Meyers[2], Charles Giamberardino[2], Sile Molloy[3], Adrienne Chan[4], Tarsizio Chikaonda[4], Robert Heyderman[4], Mina Hosseinipour[4], Newton Kalata[4], Cecilia Kanyama[4], Christopher Kukacha[4], Duncan Lupiya[4], Henry Mwandumba[4], Thomas Harrison[3], Tihana Bicanic[3], John Perfect[2], Christina Cuomo[1] 1) Broad Institute of MIT and Harvard; 2) Duke University School of Medicine; 3) St George›s University of London; 4) ACTA Study Group.

Cryptococcus neoformans is the causative agent of cryptococcosis, a disease with poor patient outcomes, accounting for approximately 180,000 deaths each year. Patient outcomes may be impacted by the underlying genetics of the infecting isolate, however, our current understanding of how genetic diversity contributes to clinical outcomes is limited. Here, we leverage clinical, in vitro growth and genomic data for 284 *C. neoformans* isolates to identify clinically relevant pathogen variants within a population of clinical isolates from patients with HIV-associated cryptococcosis in Malawi. Through a genome-wide association study (GWAS) approach, we identify variants associated with fungal burden and growth rate. We also find both small and large-scale variation, including aneuploidy, associated with alternate growth phenotypes, which may impact the course of infection. Genes impacted by these variants are involved in transcriptional regulation, signal transduction, glycolysis, sugar transport, and glycosylation. When combined with clinical data, we show that growth within the CNS is reliant upon glycolysis in an animal model, and likely impacts patient mortality, as CNS burden modulates patient outcome. Additionally, we find genes with roles in sugar transport are under selection in the majority of these clinical isolates. Further, we demonstrate that two hypothetical proteins identified by GWAS impact virulence in animal models. Our approach illustrates links between genetic variation and clinically relevant phenotypes, shedding light on survival mechanisms within the CNS and pathways involved in this persistence.

120 Genetic and epigenetic variants underpinning within-species transcriptional polymorphism in a major fungal pathogen *Leen Abraham*[1], Ursula Oggenfuss[1], Daniel Croll[1] 1) Laboratory of Evolutionary Genetics, Institute of Biology, University of Neuchâtel, Neuchâtel.

In agricultural ecosystems, outbreaks of diseases are frequent and pose a significant threat to food security. A successful fungal pathogen undergoes a complex and well-timed sequence of regulatory changes to avoid detection by the host immune system, hence well-tuned gene regulation is essential for survival. However, how regulatory adaptation enables pathogens to overcome host resistance and cause damage is poorly understood. Here, we used *Zymoseptoria tritici*, one of the most important pathogens of wheat, to generate a genome-wide map of genetic and epigenetic regulatory polymorphism governing gene expression. For this, we performed expression quantitative trait loci (eQTL) mapping on 146 con-specific strains. We identified cis-eQTLs for 65.3% of all genes and the majority of all eQTL were within 2kb of the transcription start site. Core genes were more likely to segregate eQTLs compared to accessory genes. We also found that insertion-deletion polymorphisms are more likely to act as a cis-eQTL and had a higher effect size than SNPs. Next, we contrasted the amount of cis-eQTL mapped across categories of pathogenicity-related genes. Effector genes were less likely to present cis-eQTLs compared to other genes including genes encoding CAZymes. This suggests that regulatory variation in effector genes is governed rather by epigenetic factors than by genetic polymorphism. This is consistent with pathogenicity genes tending to overlap regions of heterochromatin compared to other gene categories. To better understand epigenetic variation in the genome, we analyzed the transcriptional activity of individual copies of transposable elements (TEs) across isolates. We found 23 TE insertion loci with regulatory variation explained by cis-eQTLs. Furthermore, TE insertion polymorphism was associated with variation in pathogenicity traits among isolates. Our study establishes the first genome-wide map of genetic and epigenetic variation underpinning transcriptional plasticity and trait variation in a fungal pathogen. The extensive regulatory polymorphism is likely to fuel rapid adaptation to resistant hosts and environmental changes.

121 Giant *Starship* elements mobilize accessory genes in fungal genomes *Emile Gluck-Thaler*[1,2,3], Timothy Ralston[3], Zachary Konkel[3], Cristhian Grabowski Ocampos[4], Veena Devi Ganeshan[3], Anne E. Dorrance[3], Terry L. Niblack[3], Corlett W. Wood[2], Jason C. Slot[3], Horacio D. Lopez-Nicora[3,5], Aaron A. Vogan[6] 1) University of Neuchâtel, Switzerland; 2) University of Pennsylvania, USA; 3) The Ohio State University, USA; 4) Universidad Nacional de Asunción, Paraguay; 5) Universidad San Carlos, Paraguay; 6) University of Uppsala, Sweden.

Accessory genes are variably present among members of a species and are a reservoir of adaptive functions. In bacteria, differences in gene distributions among individuals largely result from mobile elements that acquire and disperse accessory genes as cargo. In contrast, the impact of cargo-carrying mobile elements on eukaryotic evolution remains largely unknown. Here, we show that variation in genome content within multiple fungal species is facilitated by *Starships*, a novel group of massive mobile elements that are 110 kb long on average, share conserved components, and carry diverse arrays of accessory genes. We found hundreds of *Starship*-like regions across every extant class of filamentous Ascomycetes, including 32 unique Starships that range from 27-393 kb and last shared a common ancestor ca. 400 mya. Using new long-read assemblies of the plant pathogen *Macrophomina phaseolina*, we characterize

4 distinct *Starships* whose past and ongoing activities contribute to standing variation in genome structure and content. One of these elements, *Voyager*, inserts into 5S rDNA and contains a candidate virulence factor. Phenotypic assays revealed that *Voyager* copy number has contrasting associations with pathogenic and saprophytic growth, suggesting its activity underlies an ecological trade-off. We propose that *Starships* are eukaryotic analogs of bacterial integrative and conjugative elements based on parallels between their conserved components, and may therefore represent the first known agents of active gene transfer in a eukaryote. Together, our results suggest that *Starships* have shaped the content and structure of fungal genomes for millions of years, revealing a new concerted route for evolution across an entire eukaryotic kingdom.

123 Evolution repeats itself in fungal morphogenetic transitions - in search of mechanisms of convergent evolution *Laszlo Nagy*[1] 1) Biological Research Center.

Fungi are a hyperdiverse kingdom that evolved a stunning variety of lifestyles, resulting in omnipresence across all habitats on Earth and include a wide spectrum of relationships with other organisms, ranging from mutualisms to pathogenicity. One of the means of adaptation to various habitats is morphological innovation. Transitions between cellularity levels (unicellular, multicellular, complex multicellular) happened several times in the fungal kingdom, as opposed to animals and plants, where such transitions are singular events. This includes multiple origins of complex multicellular fruiting bodies as well as repeated transitions from multicellular hyphae to unicellular yeasts and dimorphic fungi. The repeated nature of these morphogenetic transitions suggests that the underlying genetics has to involve simple and/or high-probability changes. We investigate the genetic bases of the evolution of fruiting bodies and that of the origins of dimorphic fungi, in search of genetic mechanisms that systematically increase the likelihood of repeated evolution. In this talk I provide an overview of the evolution of fungal multicellularity, that of fruiting bodies and dimorphic fungi and outline potential homologies across and driving forces of disparate morphogenetic transitions. Fungi have a significant potential to highlight the general principles of the evolution of multicellularity and mechanisms of convergent evolution.

124 Rise and fate of mutations in the fairy ring mushroom Marasmius oreades *Hanna Johannesson*[1], Markus Hiltunen[1], Martin Ryberg[1], Lorena Ament[1,2], Aleksandar Stanojković[3], Nahid Heidari[1] 1) Uppsala University; 2) Stockholm University; 3) Palacký University.

In fungi, where periodic sexual reproduction may be interleaved with extended vegetative phases, generators of variability are not restricted to act only during the sexual cycle. Such generators may be mutations affecting single base pairs up to largescale rearrangements, movement of transposable elements, or non-meiotic shuffling of genetic variants by mitotic recombination or parasexuality. Particularly in mushroom-forming fungi, where mycelia may become large and old, the evolutionary potential of variation acquired over vegetative growth is expected to be large. In this talk, I will present our recent studies of the rise and fate of variation gained during vegetative growth in the mushroom-forming fungus Marasmius oreades: a non-model bipolar species known for growing in 'fairy rings'. By taking advantage of state-of-the-art genome sequencing technology, and using resulting data during development of new bioinformatics methods, we successfully reconstructed the genome sequence of M. oreades. This resource was combined with genome re-sequencing to identify different types of mutations in M. oreades fairy rings, and to investigate the transmission of such mutations into the next generation through sexual spores. The results reveal that the M. oreades genome is extremely stable at all levels during vegetative, dikaryotic, growth in its natural environment. A significant amount of transposon movement was however revealed in monokaryotic strains during laboratory work, both in monokaryons retrieved from protoplasting and from cultures obtained by germination of basidiospores. Furthermore, we have preliminary data suggesting that the few mutations that arise do not seem to be transferred to the sexual spores. The combination of these results suggests that fungi may possess an unknown system to suppress the accumulation of mutations during dikaryotic growth in nature, while the monokaryotic stage is highly prone to mutate. Thus, contrary to expectations, the vegetative life stage in long-lived mushroom-forming fungi does not contribute much genetic variation. The findings add to what is known about how genetic variation is introduced into natural populations, how fungi deal with mutations, and highlight the complexity of genetic systems in mushroom-forming fungi.

125 The genetics and genome biology of multinucleate arbuscular mycorrhizal fungi *Nicolas Corradi*[1] 1) University of Ottawa.

The genetics of arbuscular mycorrhizal fungi (AMF) have been notoriously difficult to assess due to their perpetual multinucleated state, obligate plant biotrophy, and lack of observable sexual reproduction. Here, I will present recent collaborative work that combines AMF genomics, single nucleus analysis and chromatin conformation capture, and discuss how this work has reshaped our understanding of AMF genetics and (para)sexual potential, and revealed intricate genetic interactions between these fungi and their plant hosts.

126 Genomics, species limits, and evolution of the shiitake genus *Lentinula* *David Hibbett*[1] 1) Clark University.

Lentinula is a broadly distributed group of mushroom-forming fungi that contains the cultivated shiitake, *L. edodes*. To assess the evolutionary history and genetic diversity of *Lentinula*, we analyzed 24 genomes representing eight of the eleven accepted species in the genus and several unnamed lineages. We also assembled and annotated 60 genomes of *L. edodes* from China that were previously published as raw Illumina reads. Four major lineages of *Lentinula* arose in the Oligocene, of which three are restricted to the Americas and one is in Asia-Australasia. Bayesian species delimitation analyses, corroborated by prior taxonomy, resolve at least 11 species of *Lentinula* in our dataset, but genomes from several lineages, including those in Africa, remain unsampled. Mating compatibility tests and the standard 97% sequence identity "barcode gap" in ITS both dramatically underestimate species in *Lentinula*. *Lentinula edodes* sensu lato, comprises at least two lineages that may warrant recognition as species. One lineage is represented in our dataset by a single isolate from Nepal, while the other is broadly distributed throughout Asia. The latter group contains two main populations, one of which contains the majority of cultivated strains. Two populations of hybrid origin are also resolved using population structure and species network analyses. The pangenome of *L. edodes* contains 20,308 groups of orthologous genes, but only 6438 orthogroups (32%) are shared among all strains. Wild populations of *L. edodes* sensu lato contain a high proportion of the accessory genome and should be targeted for conservation.

127 Deep tissue infection by an invasive human fungal pathogen requires novel lipid-based suppression of the IL-17 response Pauline Basso[1], Eric Dang[1], Anatoly Urisman[1], Leah Cowen[2], Hiten Madhani[1], *Suzanne Noble*[1] 1) UCSF School of Medicine, San Francisco, CA, USA; 2) University of Toronto, Toronto, ON, Canada.

Candida albicans is the most common cause of human fungal infection, but the mechanisms of invasive pathogenesis remain poorly defined. Here we identify an unexpected mechanism: lipid-mediated immunosuppression. Through forward genetics, we found that *C. albicans* secretes a lipase, Lip2, that is critical for invasive disease. Murine infection with *C. albicans* strains that lack Lip2 display an exaggerated host IL-17 response that leads to fungal clearance from solid organs and host survival. IL-17 signaling is required for Lip2 action. The lipase activity of Lip2 inhibits IL-17 production indirectly through suppression of IL-23 production by tissue resident dendritic cells. We conclude that *C. albicans* suppresses antifungal IL-17 defense in solid organs by altering the tissue lipid milieu.

128 Talking to your inner self – on the interaction between *Trichoderma reesei* QM6a and its endohyphal *Methylobacterium* *Monika Schmoll*[1,2], Miriam Schalamun[1], Sabrina Beier[1], Ida Scalmani[1], Stephane Compant[1], Wolfgang Hinterdobler[1] 1) University of Vienna, Austria; 2) AIT Austrian Institute of Technology GmbH, Tulln, Austria.

In nature, complex organismic communities have evolved for optimal colonization of habitats. Interkingdom interactions between fungi and bacteria can be mutualistic, but also parasitic. In *Trichoderma reesei,* we detected potential endohyphal bacteria by confocal microscopy and specific staining. We could confirm the presence of a *Methylobacterium* species in the hyphae, cured QM6a and sequenced the isolated bacterium. Thereby, the association with *Methylobacterium* is rather strain specific than species specific. Isolation of the bacterium from *T. reesei* QM6a showed that it is not obligate biotroph and both the bacterium and the fungus are viable individually.
In order to evaluate the interrelationship of *Methylobacterium* and *T. reesei*, we use RNAseq from wildtype and cured QM6a as well as several mutants in the signal transduction pathway, which should also reveal stages of interaction and the relevance of the assumed symbiosis. Additionally, we applied phenotype microarrays to assess metabolic contributions of the bacterium and performed functional assays. Omics analysis indicates an influence of different growth conditions of *T. reesei* on sulphur/amino acid metabolism of the bacterium.
In summary, we discovered an intriguing new physiological aspect of *T. reesei,* which opens up a new field of research with high potential for gaining an in depth understanding of interkingdom interaction of fungi with their prokaryotic inhabitants.

129 Human mediated contact between amphibian-killing chytrid variants results in repeated recombination *Thomas Jenkinson*[1], Timothy James[2], Erica Rosenblum[3] 1) California State University, East Bay; 2) University of Michigan; 3) University of California, Berkeley.

Global biodiversity is under threat from introductions of non-native fungal disease. The pathogenic chytrid Batrachochytrium dendrobatidis (Bd) causes chytridiomycosis – the infectious disease implicated in frog, toad, and salamander population declines and extinctions worldwide. Where this fungus has been introduced, a single hypervirulent strain (Bd-GPL) proliferates through host populations. In the southern Atlantic Forest of Brazil, recent human introduction brought the globally invasive Bd-GPL strain into secondary contact with a distantly related, endemic strain, Bd-Brazil. In most anthropogenically mediated secondary contact scenarios such as this one, the epidemiological and evolutionary consequences of strain interaction remain unknown. We show that Bd, long considered obligately asexual, is capable of second-generation introgression following the human induced contact of divergent lineages. Using whole-genome sequencing of fungal isolates cultured from wild-infected Brazilian frogs, we characterize the hereditary relationships among disease populations in this strain invasion zone. Our analyses reveal regions of the Bd genome that are potentially driving adaptive variation among invasive and endemic strains. The patterns of hybrid inheritance we observe offer new insights into the genetic underpinnings of fungal reproductive isolation, the process which ultimately results in speciation of emerging fungal diseases. These new southern Brazil hybrid strains we describe are of particular ecological and evolutionary concern because they demonstrate the ability of anthropogenic change to drive novel recombinant genetic variation in a deadly pathogen. These findings show how humans are actively creating new evolutionary trajectories for emerging diseases, such as chytridiomycosis, by creating novel mating opportunities between previously allopatric strains.

130 Characterizing variation within the European *Batrachochytrium salamandrivorans* epidemic *Moira Kelly*[1], Frank Pasmans[1], Jose Muñoz[2], Terrance Shea[2], Matthew Gray[3], Christina Cuomo[2], An Martel[1] 1) Ghent University; 2) Broad Institute of MIT and Harvard; 3) University of Tennessee Institute of Agriculture, Knoxville, Tennessee.

Pathogens rarely drive their hosts to extinction. The chytrid fungus *Batrachochytrium dendrobatidis (Bd)*, however, is frequently associated with population extinctions in amphibians, resulting in the most biodiversity-devastating epidemic in recorded history. In 2013, a closely related chytrid, *Batrachochytrium salamandrivorans* (*Bsal*), was discovered in association with the collapse of salamander populations in northern Europe. Analyses of host-pathogen dynamics suggest Bsal poses a similar extinction threat as its sister fungus *Bd*. However, all studies of Bsal to date have focussed on a single isolate, assuming the European epidemic to be homogenous. Through genomic and phenotypic analyses of *Bsal* strains isolated from across the European *Bsal* epidemic, we identified highly divergent genomic landscapes, rapid evolutionary rates and isolate-specific gene family expansions and acquisitions. Phenotypic analyses found this genomic variation to be associated with surprising levels of phenotypic variation, including isolate-specific metabolic capacities, a saprotrophic lifecycle, and highly variable thermal ranges, which have important implications for developing effective mitigation strategies. We employed comparative genomic analyses of a high passage isolate displaying species-specific reduction in virulence, compared to the index site isolate, to characterise active mechanisms of genomic evolution, and biological processes and molecular functions that may be important in determining pathogenicity.

131 Deciphering the molecular mechanisms involved with plant-insect-fungal interactions *Marcio Silva-Filho*[1], Flavia Franco[1], Amanda Túler[1], Diego Gallán[1], Felipe Gonçalves[1], Arodí Favaris[1], Maria Fernanda Penaflor[2], Walter Leal[3], Daniel Moura[1], José Mauricio Bento[1] 1) University of São Paulo; 2) Federal University of Lavras; 3) University of California, Davis.

Pathogens can manipulate their host plants and insects to optimize their fitness, increasing the attraction of insects to the infected plant, in ways that facilitate the pathogen acquisition. In sugarcane crops, *Colletotrichum f*alcatum and *Fusarium verticillioides* usually occurs in association with *Diatraea saccharalis*resulting in large losses to the crop. Considering this association, we aimed to identify the effects of both fungi in *D. saccharalis* host preference, performance, and effect of fungal infection. Here, we show that both fungi modulate *D. saccharalis* behaviour to its own benefit. More specifically, sugarcane plants infected either with *C. falcatum* either with *F. verticillioides* showed a dramatic increase in volatile compounds, which in turn attract *D. saccharalis* caterpillars and adults to feed and to lay eggs, respectively, on the infected plants. Our data demonstrate that the fungus manipulates both the host plant and insect herbivore across life cycle to promote its infection and dissemination.

132 Genes for an extended phenotype: Biosynthesis of volatile sesquiterpenes in a pathogenic fungus is used to entice male flies into fatal mating's with infected female cadavers Andreas Naundrup[1], Björn Bohman[2], Charles A. Kwadha[2], Annette B. Jensen[1], Paul G. Becher[2], *Henrik De Fine Licht*[1] 1) University of Copenhagen, Denmark; 2) Swedish University of Agricultural Sciences, Alnarp, Sweden.

To ensure dispersal, many parasites and pathogens behaviourally manipulate infected hosts. Other pathogens and certain insect-pollinated flowers use sexual mimicry and release deceptive mating signals. However, it is unusual for pathogens to rely on both behavioural host manipulation and sexual mimicry. Here, we show that the host-specific and behaviourally manipulating pathogenic fungus, *Entomophthora muscae*, generates a chemical blend of volatile sesquiterpenes and alters the level of natural host cuticular hydrocarbons in dead infected female house fly (*Musca domestica*) cadavers. We used three different approaches unravel chemical attraction pathways in *E. muscae*. First, we quantified male sexual attraction to fungus-killed cadavers and fungal conidia using behavioural assays. Second, we identified the chemical cues eliciting male mating attraction using chemical analyses (GC-MS) and physiological mechanisms enabling males to detect these cues using electroantennography (GC-EAD). Third, we verified the fungus *E. muscae* as source of the behaviourally active volatile compounds in fungus-killed cadavers using transcriptional profiling (RNAseq) of expressed genes in volatile chemical biosynthesis pathways. We show that healthy male house flies respond to the fungal compounds and are enticed into mating with dead female cadavers. This is advantageous for the fungus as close proximity between host individuals leads to an increased probability of infection. The fungus-emitted volatiles thus represent the evolution of an extended phenotypic trait that exploit male flies' willingness to mate and benefit the fungus by altering the behavioural phenotype of uninfected healthy male host flies.

133 Repeat-driven genome expansion and two-speed genome architecture of amphibian-infecting chytrids *Theresa Wacker*[1], Helmstetter Nicolas[1], Wilson Duncan[1], Fisher Matthew C.[2], Studholme David J.[3], Farrer Rhys A.[1] 1) Medical Research Council Centre for Medical Mycology at the University of Exeter, Exeter, United Kingdom; 2) MRC Centre for Global Infectious Disease Analysis, Imperial College London, London, United Kingdom; 3) Biosciences, University of Exeter, Exeter, United Kingdom.

Over the past half century, the chytridiomycosis panzootic has led to the decline of over 500 amphibian species with 90 attributed extinctions. Chytridiomycosis of amphibians is caused by two fungal species *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*). The genetic mechanisms underlying host-specificity and pathology in the *Batrachochytrium* genus remain elusive and their evolution and origins of virulence are largely unknown. Using deep nanopore sequencing, we found that *Bsal* is extremely repeat-rich with high numbers of long terminal repeats, long interspersed nuclear elements and transposable elements. This repeat-driven genome expansion in *Bsal* has resulted in a tripling of its length compared with *Bd*. Key pathogenicity genes including M36 metalloproteases have expanded compared with *Bd*, and are enriched for flanking transposable elements, suggesting its genome expansion is connected to selective evolutionary processes. Both batrachochytrids have evidence of a two speed genome architecture, including an enrichment of functional categories in compartments of repeat richness or sparsity. Furthermore, among *Bd* lineages, M36 metalloproteases with signatures of positive selection and, both in *Bsal* and *Bd*, genes upregulated during infection *in vivo* are enriched in repeat-rich and gene-sparse compartment of the genome. This is the first evidence for a two-speed genome in an animal pathogen, shedding new light on the role of repetitive sequences on the evolution of fungal pathogens driving global declines and extinctions of amphibians.

134 Regulation of infection of insects by the fungus *Metarhizium robertsii* *Weiguo Fang*[1] 1) Zhejiang University.

The insect pathogenic fungus *Metarhizium robertsii* is a representative fungus in which to study broad themes of fungal pathogenicity as it resembles some major plant and mammalian pathogenic fungi in its pathogenesis. In its pathogenesis progression, *M. robertsii* encounters two different microenvironments: the insect cuticle and the insect hemocoel. A complex regulatory network has been revealed to control the response of *M. robertsii* to different microenvironments. The Fus3-MAPK cascade is indispensable for cuticle penetration. On the cuticle, the Fus3-MAPK directly phosphorylates the transcription factor RNS1, which facilitates the entry of RNS1 into nuclei. The phosphorylated RNS1 binds to its own promoter to self-induce expression, which then activates the expression of genes for degrading cuticular proteins, chitin, and lipids. Fus3-MAPK also phosphorylates the transcription factor MrSt12 that induces the transcription factor AFTF1 by physically interacting with the promoter of *Aftf1*, which is essential for formation of the infection structure appressorium. On the other hand, the AFTF1 is negatively regulated by the membrane protein Mr-OPY2, which expression is upregulated during cuticle penetration. Increased production of Mr-OPY2 protein on the cuticle is achieved by expression of a transcript variant lacking a small upstream open reading frame that would otherwise inhibit translation of Mr-OPY2. On the insect cuticle, the transcription factor COH2 also activates expression of cuticle penetration genes. In the hemocoel, the protein COH1 is expressed due to the reduction in epigenetic repression conferred by the histone deacetylase HDAC1 and the histone 3 acetyltransferase HAT1. COH1 interacts with COH2 to reduce COH2 stability, and this down-regulates cuticle penetration genes and up-regulates genes for hemocoel colonization.

135 Unraveling the biology of Nematophagy During a Fungal-Nematode Predator-Prey Interaction Using Time-Course Transcriptomic analysis *Hung-Che Lin*[1], Guillermo Vidal-Diez de Ulzurrun[1], Sheng-An Chen [1], Ching-Ting Yang[1], Pedro Gonçalves [1], Chih-Yen Kuo [1], Tsung-Yu Huang[1], Erich Schwarz [2], Yen-Ping Hsueh [1] 1) Institute of Molecular Biology, Academia Sinica,

Taipei, Taiwan; 2) Cornell University, Ithaca, NY.

Nutritional deprivation triggers a saprotrophic to predatory lifestyle switch in soil dwelling nematode-trapping fungi (NTF). In particular, *Arthrobotrys oligospora* has evolved to secrete food and sex cues to lure their prey – Nematoda animals – into an adhesive network of traps, specialized structures that originate from the vegetative mycelium. Upon capture, the nematodes are invaded and digested by the fungus, thus serving as a food source. We employed RNA-sequencing to examine the response of *A. oligospora* upon exposure to the model nematode *Caenorhabditis elegans*. A dynamic transcriptomic reaction that indicated a strong reliance on protein secretion was observed. A comprehensive prediction of the secretome of *A. oligospora* resulted in 1084 transcripts, 64% of which are upregulated in the presence of *C. elegans* at all tested time points. We found a large number of genes related to ribosome biogenesis induced at early time point, 2hr-post *C. elegans* exposure, suggesting that the TOR signaling pathway might be critical for sensing the presence of nematodes. Rapamycin treatment inhibited both trap development and function. Moreover, a plasma membrane t-SNARE protein, SSO2, involved in membrane fusion of secretory vesicles, plays a major role in nematode-adhesion. We subsequently predicted the putative effectors of *A. oligospora* and found that they represent approximately 19% of the secretome and that their expression peaked after 10 hours of introduction of nematodes. Specifically, we found that genes of the Egh16 family were highly upregulated upon nematode exposure. *In situ* hybridization reveals the accumulation of the top three highly expressed *Egh16* transcripts in the traps cell. Thus, we named these gene family as Trap-enriched Secreted Protein (TSP). Gene deletion of the highest expressed gene *TSP1* impairs the function of trap. Lastly, Egh16 gene family is highly expanded in the genomes of several nematode-trapping fungi, suggesting that this gene family may have a critical role for the evolution of the predatory life style in Ascomycetes.

136 Integrating multifaceted genetic tools to gear up the discovery of fungal mechanisms of wood decay *Jiwei Zhang*[1], Weiran Li[1], Jonathan Schilling[1], Hugh D Mitchell[2], Lye Meng Markillie[2] 1) University of Minnesota; 2) Pacific Northwest National Laboratory.

Fungi evolved efficient ways to degrade and recycle carbons sequestered in woody biomass. Their degradative mechanism offers industrially-relevant toolkits for developing green technologies for plant biomass conversions. Among these, brown- and white-rot fungi are two distinctive classes of wood decomposers that dominate the carbon degradation in the forest system. During wood decomposition, white-rot fungi can completely degrade and consume all formats of carbons in lignocellulose, while brown-rot shifted its strategy to first depolymerize the lignocellulose structures and then selectively utilize carbohydrates for fungal metabolism, but leave lignins as residues. We have known that the brown-rot strategy causes a faster depolymerizing rate than white-rot, and the biochemical analysis indicated that this is largely due to the use of reactive oxygen radicals (ROS) for intensive polymer deconstruction. However, we haven't known the genetic bases driving the ROS mechanism for this brown-rot efficacy. Genomic analyses implied that brown-rot fungi have adapted special genetic inventories, at both gene and gene regulation levels, to implement and manage ROS attacks during wood decay, but their genetic functions haven't been validated. Also, the regulatory systems controlling brown-rot genes expression remain uncharacterized. One of the main obstacles to this research is the lack of available genetic tools in those multiploidy, basidiomycete brown-rot variants that are difficult to genetically manipulate. To fill these gaps to facilitate the validation/investigation of the distinctive brown-rot mechanisms, our recent work has been focusing on combining systems biology and genome-editing for large-scale brown-rot phenotypic screening. In this talk, we will report our recent progress towards building the brown-rot genetic platform and then using it to dissect the fungal mechanisms involved in fast wood biomass decomposition.

137 Characterization and engineering of non-model microorganisms for biotechnological applications *Hugh Purdy*[1], Michelle O'Malley[1] 1) University of California, Santa Barbara, Santa Barbara, CA.

Microorganisms hold great promise for applications in biotechnology, including the renewable production of fuels and chemicals, the biosynthesis of therapeutics, and even the creation of novel materials. However, it is well known that only a relatively small percentage of microbial species are isolated and characterized sufficiently to be amenable for engineering purposes, thereby limiting our repertoire of available biological tools. This issue is particularly true for hard-to-culture organisms. One such group of organisms, the anaerobic gut fungi, hold significant, largely untapped biotechnological potential. These fungi, found predominantly in the digestive tracts of herbivores, possess an expansive array of uncharacterized carbohydrate-active enzymes, indicating a high-degree of potential for applications involving the processing and conversion of lignocellulosic material. Furthermore, as these fungi natively exist in a competitive, crowded microbial environment, they are believed to possess diverse secondary metabolites with potential for therapeutic applications. We are working to isolate and characterize these anaerobic gut fungi in order to leverage their unique catabolic and biosynthetic capabilities. In addition to the isolated characterization of these fungi, we are studying the behavior of broader anaerobic consortia derived from herbivore digestive systems and other relevant sites (e.g. landfills and commercial digesters). An improved understanding of the biological principles underpinning these consortia will allow us to develop biotechnologies that take advantage of the enhanced metabolic capabilities exclusive to natural microbial communities. In the same vein of searching for untapped biological potential, we have recently expanded our work into the study and engineering of diatoms for the production of silica-based materials. This group of microalgae is relatively understudied from an engineering perspective considering their biosynthetic capabilities as the primary producers of biogenic silica on Earth. We are beginning investigations into the genetic mechanisms underpinning diatom silicification with the ultimate goal of developing novel siliceous materials. Overall, the O'Malley Lab at UC Santa Barbara is working to expand the range of useful biological tools by studying and engineering these and other non-model microbial systems.

138 Chance favours the prepared spore – how to jumpstart cellulase production *Wolfgang Hinterdobler*[1], Miriam Schalamun[1], Monika Schmoll[1,2] 1) AIT Austrian Institute Of Technology, Tulln, Austria; 2) University of Vienna, Vienna, Austria.

Asexual propagation in filamentous fungi is tightly regulated as many resources are needed for spore production. In *T. reesei*, the cellobiohydrolase CBH2/Cel6a is deposited on the spore surface and sporulation correlates with CAZyme expression. Recently, it was shown that the conditions of spore production are relevant for physiology and stress resistance of the fungi growing from these spores. These findings suggest that the protein composition on the spore surface may be important for the ability of fungi to efficiently colonize a habitat or to initiate growth in a fermenter.

We investigated the surface proteome isolated from spores grown under different light regimes (constant light, constant darkness) and on different carbon sources (cellulose, glucose, malt extract). Indeed, we detected significant differences in these proteomes, indicating an altered preparation for germination conditions. Using these conditions in liquid cultivations on cellulose showed considerable differences in secreted cellulase activity early after germination (48 hours). Transcriptome analysis of these conditions was performed to evaluate cellulose sensing and regulation mechanisms differentially modulated by those spore proteins. Among others, *cbh1*, *cre1* and *xyr1* are characteristically regulated depending on the conditions of inoculum production.

Consequently, *T. reesei* deposits a protein mixture adjusted to the conditions to be expected for germination on its spores. These proteins are involved in sensing and cellulase regulation after germination, reflecting a potential to improve fermentations.

139 Lignocelluloses and solid waste substrates transformed by wood-decay fungi for production of natural compounds *Taina Lundell*[1], Tuulia Mali[1], Eero Kiviniemi[1], Hans Mattila[1], Janina Österman-Udd[1] 1) University of Helsinki, Helsinki, Finland.

Wood-decay fungi of Basidiomycota demonstrate applicability for bioconversion and biological treatment of plant biomass and lignocelluloses. The fungi adapt to changes in environmental and laboratory conditions and may be cultivated even on mixtures of solid plant-based and waste substrates. Fungal metabolic potentiality is also seen in their ability to produce diverse bioactive compounds and secondary metabolites. We recently opened the transcriptome and proteome of the white rot fungus *Phlebia radiata* on spruce wood during decomposition (1, 2) and fungal metabolic and gene expression response under anaerobic, ethanol-producing fermentative conditions (3).
One aim is to adopt the fungus and other isolates in bioreactor conversions of waste lignocelluloses and plant-based industrial sidestreams for production of biofuels like bioethanol, and added-value natural compounds, organic acids and aromatic compounds. Specific interest is in metabolic profiles of wood-decay fungi with different decomposition traits, and efficiency in degradation of plant biomasses.
For these purposes, we have tested various agricultural, municipal, and wood-based wastes like straw, core board, sawdust, and disposed construction wood (4). Culture atmosphere was the major regulator for primary metabolism together with influencing on differential expression of the wood-decaying, carbohydrate-active enzyme encoding genes (3). Ethanol production was prominent in phlebioid fungi on solid lignocellulose cultures under oxygen depleted conditions (4).
Results on our recent experiments on the effect of reactive oxygen species on overall gene expression and accumulation of natural products by the fungi will be presented. Experimental findings of fungal single species (5) and co-cultures on production of enzyme activities and metabolites indicate that both the lignocellulose substrate and fungal interactions are major effectors guiding the decomposition events. The functional resilience of these fungi on varying wood and waste substrates will aid us in designing more sustainable solutions for production of biofuels, enzymes, and natural compounds.
References: [1]Mäkinen M (2019) BMC Genomics 20:430, [2]Kuuskeri J (2016) Biotechnol Biofuels 9:192, [3]Mattila H (2020) Biotechnol Biofuels 13:26, [4]Mattila H (2017) Bioresource Technol 225:254-261, [5]Veloz Villavicencio E (2020) Microorganisms 8:73

140 The transcription factor Roc1 is a regulator of cellulose degradation in the wood-decaying mushroom *Schizophyllum commune* *Peter Jan Vonk*[1], Ioana M. Marian[1], Ivan D. Valdes[1], Kerrie Barry[1], Benedict Bostock[1], Akiko Carver[2], Chris Daum[2], Harry Lerner[1], Anna Lipzen[2], Hongjae Park[2,3], Margo B.P. Schuller[1], Martin Tegelaar[1], Andrew Tritt[2], Jeremy Schmutz[2,4], Jane Grimwood[2,4], Luis G. Lugones[1], In-Geol Choi[2,3], Han A.B. Wösten[1], Igor V. Grigoriev[2,5], Robin A. Ohm[1,2] 1) Microbiology, Utrecht University, Utrecht, The Netherlands; 2) U.S. department of Energy Joint Genome Institute, Berkeley, CA, USA; 3) Department of Biotechnology, College of Life Sciences and Biotechnology and Graduate School, Korea University, Seoul, South Korea; 4) HudsonAlpha Institute for Biotechnology, Huntsville, AL, USA; 5) Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA.

Wood-decaying fungi of the class Agaricomycetes (phylum Basidiomycota) are saprotrophs that break down lignocellulose and play an important role in the nutrient recycling. They secrete a wide range of extracellular plant cell wall degrading enzymes that break down cellulose, hemicellulose and lignin, the main building blocks of plant biomass. Although the production of these enzymes is regulated mainly at the transcriptional level, no activating regulators have been identified in any wood-decaying fungus in the class Agaricomycetes. We studied the regulation of cellulase expression in the wood-decaying fungus *Schizophyllum commune*. Comparative genomics and transcriptomics on two wild isolates revealed a Zn2Cys6-type transcription factor gene (*roc1*) that was highly up-regulated during growth on cellulose, when compared to glucose. It is only conserved in the class Agaricomycetes. A *roc1* knockout strain showed an inability to grow on medium with cellulose as sole carbon source, and growth on cellobiose and xylan (other components of wood) was inhibited. Growth on non-wood-related carbon sources was not inhibited. Cellulase activity was reduced in the growth medium of the *Δroc1* strain. ChIP-Seq identified 1474 binding sites of the Roc1 transcription factor. Promoters of genes involved in lignocellulose degradation were enriched with these binding sites, especially those of LPMO (lytic polysaccharide monooxygenase) CAZymes, indicating that Roc1 directly regulates these genes. A GC-rich motif was identified as the binding site of Roc1, which was confirmed by a functional promoter analysis. Together, Roc1 is a key regulator of cellulose degradation and the first identified in wood decaying fungi in the phylum Basidiomycota.

141 Degradation strategy of wood extractives by conifer-degrading wood decay fungus *Chiaki Hori*[1] 1) Hokkaido University.

Wood extractives, solvent-soluble fractions of woody biomass, are considered to be a factor impeding or excluding fungal colonization on the freshly harvested conifers. Among wood decay fungi, the basidiomycete *Phlebiopsis gigantea* has evolved a unique enzyme system to efficiently transform or degrade conifer extractives but little is known about the mechanism(s). In this study, to clarify the mechanism(s) of softwood degradation, we examined the transcriptome, proteome, and metabolome of *P. gigantea* when grown on defined media containing microcrystalline cellulose and pine sapwood extractives. Beyond the conventional enzymes often associated with cellulose, hemicellulose and lignin degradation, an array of enzymes implicated in the metabolism of softwood lipophilic extractives such as fatty and resin acids, steroids and glycerides was significantly up-regulated. Among these, a highly expressed and inducible lipase is likely responsible for lipophilic extractive degradation, based on its extracellular location and our characterization of the recombinant enzyme. Our results provide insight into physiological roles of extractives in the interaction between wood and fungi[1)].

[1)]Iwata, M., *et al*. Scientific Reports 11:12528 (2021)

144 Human skin microbiome: trans-kingdom characterization and investigating an emerging fungal pathogen *Julia Segre*[1], Sara Saheb Kashaf[1,2], Diana Proctor[1], Alexandre Almeida[2], Robert Finn[2], Mary Hayden[3], Heidi Kong[4] 1) National Human Genome Research Institute, NIH; 2) European Bioinformatics Institute (EMBL-EBI), Wellcome Genome Campus; 3) Rush University Medical Center, Chicago,IL; 4) National Institute of Arthritis, Musculoskeletal and Skin Diseases, NIH.

The varied topography of human skin offers a unique opportunity to study how the body's microenvironments influence the functional and taxonomic composition of microbial communities. However, the paucity of commensal microbial genomes has limited our ability to comprehensively interpret the structure and function of these communities. Innovative metagenomic methods have unlocked some of this potential for gut microbiomes, but low-biomass samples possess additional challenges. We combined extensive culturing and co-assembly of shotgun metagenomic datasets spanning multiple body sites of multiple individuals to elucidate novel constituents, structure and functions of the human skin microbiome. We further validated the quality of many of our metagenome-assembled genomes using isolates obtained from the same body site and/or same healthy volunteer. These genomes expand the known species catalogue of the human skin and characterization of the spatial patterning of associated microbes.
Candida auris is an emerging multi-drug resistant fungal pathogen. *C. auris* skin colonization results in environmental shedding, which underlies hospital transmissions, and predisposes patients to subsequent infections. Combining culturing and skin microbiome sequencing of an outbreak at a high-acuity long term care facility provided novel insight into prevalence and site tropism for C. auris colonization. Modeling human mycobiome dynamics suggested skin fungal community structure as a risk factor, and possible point of intervention. We developed a murine skin topical exposure model for *C. auris* to dissect risk factors predisposing patients for colonization and to test interventions that might protect patients.

145 Intestinal mycobiome in allogeneic hematopoietic cell transplantation *Bing Zhai*[1,2], Thierry Rolling[2,3], Chen Liao[2], Siddharth Jaggavarapu [4], Mergim Gjonbalaj[2], Jonathan Peled[2], Joao Xavier[2], David Weiss[4], Ying Taur[2], Tobias Hohl[2] 1) Shenzhen Institute of Advanced Technology, Shenzhen, China; 2) Memorial Sloan Kettering Cancer Center, New York, NY; 3) University Medical Center Hamburg-Eppendorf, Hamburg, Germany ; 4) Emory University, Atlanta, GA.

Allogeneic haematopoietic cell transplantation (allo-HCT) induces profound shifts in the intestinal microbiome, including the fungal compartment (i.e. the mycobiome). To characterize the dynamics of intestinal mycobiome during allo-HCT, we integrated an optimized analytical pipeline with high-throughput fungal ITS1 amplicon sequencing and fungal culturomics assays. We identified a subset of patients with fungal dysbiosis defined by culture positivity and stable expansion of *Candida parapsilosis* complex species. Interestingly, these patients had worse overall survival and higher transplant-related mortality. We further examined the relationship between *Candida parapsilosis* domination and the prophylactic usage of micafungin. 47% patients with intestinal *Candida parapsilosis* domination had been colonized with micafungin heteroresistant strains, and had significantly higher risker to develop breakthrough fungal bloodstream infections. Taken together, our study suggested that targeting fungal dysbiosis may help to improve long-term patient survival and identify patients at risk of fungal bloodstream infections.

146 Adaptive immunity induces mutually beneficial interactions with gut fungi *Kyla Ost*[1], Teresa O' Meara[2], W. Zac Stephens[1], Tyson Chiaro[1], Haoyang Zhou[1], Jourdan Penman[1], Rickesha Bell[1], Jason Catanzaro[5], Deguang Song[3], Shakti Singh[4], Daniel Call[9], Elizabeth Hwang-Wong[6], Kimberly Hanson[7], John Valentine[8], Kenneth Christensen[9], Brendan Cormack[6], Ashraf Ibrahim[4, 10], Noah Palm[3], Suzanne Noble[11], June Round[1] 1) Department of Pathology, University of Utah School of Medicine, Division of Microbiology and Immunology, Huntsman Cancer Institute, Salt Lake City, UT; 2) Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI; 3) Department of Immunobiology, Yale University School of Medicine, New Haven, CT; 4) The Lundquist Institute of Biomedical Innovation, Harbor-University of California Los Angeles Medical Center, Torrance, CA; 5) Section of Pulmonology, Allergy, Immunology and Sleep Medicine, Department of Pediatrics, Yale University School of Medicine, New Haven, CT; 6) Department of Molecular Biology and Genetics John's Hopkins School of Medicine, Baltimore, MD; 7) Department of Pathology, Clinical Microbiology Division, University of Utah, Salt Lake City, UT; 8) 8 Department of Internal Medicine, Division of Gastroenterology, University of Utah School of Medicine, Salt Lake City, UT; 9) Department of Chemistry and Biochemistry Department, Brigham Young University, Provo, UT; 10) David Geffen School of Medicine at University of California, Los Angeles, CA; 11) Department of Microbiology and Immunology, UCSF School of Medicine, San Francisco, CA .

Pathogenic fungi reside in the intestinal microbiota though rarely cause disease. Little is known regarding immune-fungal interactions that promote commensalism. Here, we explored the role of adaptive immune responses in promoting fungal commensalism. We found that potentially pathogenic *Candida* species induce and are targeted by intestinal IgA responses. Focused studies on *Candida albicans* revealed that the pathogenic hyphal morphotype, specialized for adhesion and invasion, is preferentially targeted and suppressed by intestinal IgA responses. IgA from mice and humans directly targeted hyphal-enriched cell surface adhesins. While typically required for pathogenesis, *C. albicans* hyphae are less fit for gut colonization and we show that immune selection against hyphae improves the competitive fitness of *C. albicans*. *C. albicans* exacerbates intestinal colitis and we demonstrate that hyphae and an IgA-targeted adhesin exacerbate intestinal damage. Finally, induction of an adhesin-specific immune response, using a clinically relevant vaccine, protects mice from *C. albicans*-associated damage during colitis. Together, adaptive immunity suppresses harmful fungal effectors, benefiting both *C. albicans* and its host. Thus, IgA uniquely uncouples colonization from pathogenesis in commensal fungi to promote homeostasis.

147 Characterizing the role of anaerobic fungi in lignocellulolytic microbial communities and the gut mycobiome of herbivorous non-human primates *Katharine Dickson*[1], Michelle O'Malley[1] 1) University of California - Santa Barbara, Santa Barbara, CA.

The gut microbiome plays a critical role in the health of herbivorous non-human primates (NHPs), and its composition is linked to features of their ecology, behavior, and responses to habitat destruction. Little is understood about the plant-degrading activities of fungi in the gut microbiomes of herbivorous NHPs. Historically, research on the gut microbiomes of herbivorous NHPs has focused on their prokaryotic members, or employed methods of surveying their eukaryotic members that do not fully resolve the fungal popu-

lation of the microbial community (the mycobiome). In particular, these methods often fail to identify anaerobic gut fungi of the phylum Neocallimastigomycota, which are widely recognized to constitute the majority of the gut fungal population in large herbivores, where they secrete an arsenal of carbohydrate active enzymes (CAZymes) to degrade ingested plant biomass. Anaerobic gut fungi have been identified in fecal samples of the Western lowland gorilla (*Gorilla gorilla gorilla*), and are likely to be distributed more broadly across highly herbivorous primates, particularly the foregut-fermenting members of subfamily Colobinae, who possess chambered stomachs and whose gut microbiota exhibit characteristics convergent with those of ruminants. To characterize the mycobiome of these primates, fecal samples have been obtained from wild mantled howler monkey (*Alouatta palliata*) and black howler monkey (*Alouatta pigra*), and will be collected from captive *G. gorilla gorilla*, black-and-white colobus (*Colobus guereza*), and Francois' langur (*Trachypithecus francoisi*). Our work seeks to test the hypothesis that anaerobic fungi can be cultivated from *G. gorilla gorilla*, *C. guereza*, and *T. francoisi* fecal samples, which would suggest a functional role for these fungi in the primate digestive tract. Shotgun sequencing of genomic DNA in samples from all species will be employed along with ITS and LSU amplicon sequencing to probe fungal metagenomes and characterize their CAZyme repertoire. Full characterization of the community membership, population dynamics, and lignocellulolytic capacities of anaerobic fungi in herbivorous NHP mycobiota will generate critical insights about the microbial ecology of herbivory in primates that are likely to not only play a central role in conserving these vulnerable species, but also lead to a fuller understanding of the evolutionary trajectory of herbivory among all primates, including humans.

148 When and how do fungi impact the evolution of bacteria? *Benjamin Wolfe*[1], Emily Putnam[1], Brittany Niccum[1], Casey Cosetta[1], Ruby Ye[1] 1) Tufts University - Biology.

The functional consequences of fungal-bacterial interactions have often been studied at short ecological timescales, but how fungi impact the evolution of neighboring microbes over longer timescales is less clear. Fungi may promote or inhibit the evolution of bacteria by providing (e.g. metabolite secretion, decomposition) or removing (e.g. resource competition) ecological opportunities within microbial communities. Using experimental evolution with a range of fungi (*Penicillium*, *Debaryomyces*, and *Galactomyces* species) and bacteria (*Staphylococcus* and *Pseudomonas* species) from the cheese rind model system, we have been determining when and how fungi impact rates and modes of bacterial evolution. While many fungi do not alter phenotypic or genotypic diversity of co-cultured bacteria, some can dramatically promote diversification of bacterial populations. For example, the yeast *Debaryomyces hansenii* strongly promoted diversification of the bacterium *Staphylococcus xylosus*; nearly all replicate populations of *S. xylosus* co-cultured with *D. hansenii* were dominated by mutants with loss-of-function mutations in genes associated with the sigma factor B (SigB) and accessory gene regulator (agr) systems. Surprisingly, the long-term impact of fungi on bacterial evolution cannot be clearly predicted by their short-term ecological impacts on bacterial growth. Ongoing work in the lab is trying to identify the chemical and genetic mechanisms by which fungi promote or inhibit bacterial diversification. Our results demonstrate that fungal-bacterial interactions have the potential to shape evolutionary processes within microbial communities and may be important to consider when designing and managing fungal-dominated ecosystems.

149 Interrogating the poplar fungal microbiome interactions using meta-transcriptomics and constructed communities *Jake Nash*[1], Keaton Tremble[3], Brian Looney[1], Corbin Bryan[1], Khalid Hameed[1], Yi-Hong Ke[1], Melissa Cregger[2], Nicholas Dove[2], Christopher Schadt[2], Rytas Vilgalys[2] 1) Duke University, Durham, NC; 2) Oak Ridge National Laboratory, Oak Ridge, TN; 3) The University of Utah, Salt Lake City, UT.

Poplar trees (genus *Populus*) are host to a diverse root fungal microbiome including ectomycorrhizal, arbuscular mycorrhizal, and endophytic fungi. These fungi perform services for the plant host including growth promotion, nutrient acquisition, protection from pathogens, and conferral of abiotic stress tolerance. Meta-transcriptomics can provide large amounts of data on the function and taxonomic composition of the poplar root fungal microbiome. We developed an RNA-seq method using a synthetic spike-in standard curve that allows for the calculation of absolute abundances of fungal transcripts on poplar roots. We implemented a bioinformatics workflow that provides taxonomic and functional annotations of assembled fungal contigs from meta-transcriptomic data. These methods were applied to an ecosystem-scale time-series field experiment to document taxonomic and functional shifts of the poplar fungal microbiome in response to a historic drought in the semi-arid American West during the summer of 2021. We identified transcripts from a previously isolated dark septate endophyte in the genus *Hyaloscypha* as a highly active root colonizer across our field sites. Dark septate endophytes are a functionally diverse group of root associates that have been described as either mutualists, commensalists, or latent pathogens. We conducted further work to understand the characteristics of the *Populus-Hyaloscypha* association. *In* vitro inoculations with this fungus demonstrated compatibility with both *Pinus* and *Populus*, and suggested that it engages in antagonistic interactions with arbuscular mycorrhizal fungi during plant host colonization. We were also able to establish simplified constructed communities with this fungus and three common ectomycorrhizal fungi, ranging in diversity from one to four species. These constructed communities will allow us to identify interactions between fungi during root colonization and evaluate the effects of fungal diversity on plant performance and nutrient uptake. Future work will also 1) dissect the molecular mechanisms of the antagonistic interaction with arbuscular mycorrhizal fungi, 2) evaluate the ability of this fungus to confer drought tolerance to *Populus*, and 3) identify common and unique symbiosis-induced genes when colonizing different plant hosts.

150 Metabarcoding as a tool for investigating the influence of endosymbiotic bacteria on Mucoromycota fungal host community structure in the Sonoran Desert *Nicole Reynolds*[1], Kevin Amses[2], Jessie Uehling[2], Rasheed Adeleke[3] 1) Cornell University, School of Integrative Plant Science, Ithaca, NY, USA; 2) Oregon State University, Department of Botany and Plant Pathology, Corvallis, OR, USA; 3) North-West University, School of Biological Sciences, Potchefstroom, North West Province, SA.

The Mucoromycota comprises a diverse group of filamentous fungi including mycorrhizal symbionts (Glomeromycetes, Endogonomycetes) and rhizosphere-associated and soil saprotrophs (Mortierellomycetes, Mucoromycetes). Several species within these groups may also be opportunistic human pathogens, post-harvest pathogens, or used in industrial applications for food or biofuel production. Despite the importance of these fungi, many questions remain regarding the patterns and drivers of their diversity, ecology, and distribution. To answer these questions, we are generating comprehensive Mucoromycota culture and sequence based genomic libraries. Furthermore, recent and ongoing discoveries about the endosymbiotic bacteria (EB) that many Mucoromycota species harbor have

generated new questions. EB have different effects on the host fungi depending on the species, influencing asexual and sexual reproduction and metabolic functioning. To investigate the potential role of EB in the structuring of Mucoromycota communities, we are using metabarcoding to analyze soils collected from the rhizosphere of two different shrubs (*Larrea tridentata*, Zygophyllaceae and *Ambrosia dumosa*, Asteraceae) in the Sonoran Desert (California). Using both bacterial (16S) and fungal (28S) primers, we are investigating the co-occurrence of potential EB species and fungal hosts. Sequences are generated on the Illumina MiSeq platform, and bioinformatic analyses performed using the AMPtk pipeline with customized reference databases. One essential aspect to further understanding EB and their functional effects on their host fungi is quantifying how technical experimental biases such as primer and sequencing bias influence our interpretation of community-based sequence data. To evaluate the effect of methodological biases, we generated a biological mock community including genomic DNAs from a diversity of Mucoromycota fungi (with or without EB) and are processing it alongside the environmental samples. Our results show not only the utility of metabarcoding for understanding communities of Mucoromycota and their putative EB, but also the importance of accounting for methodological biases that can impact the results. Additionally, we explore the effects of biotic filtering influenced by host plant and dispersal filtering based on geographic distance. This work is the first step in a larger project to compare two biomes (deserts, xeric shrublands and Mediterranean scrub) across two disjunct geographic areas (California, USA and Western Cape, SA).

151 Global evolutionary patterns and drug resistance acquisition in the human pathogen *Aspergillus fumigatus* *Johanna Rhodes*[1], Alireza Abdolrasouli[2,3], Katie Dunne[4], Thomas Sewell[1], Yuyi Shang[1], Eloise Ballard[5], Amelie Brackin[1], Norman van Rhijn[6], Harry Chown[6], Paul Dyer[7], Paul Bowyer[6], Michael Bromley[6], Elizabeth Johnson[8,9], P. Lewis White[10], Adilia Warris[5,9], Richard Barton[11], Silke Schelenz[12], Thomas Rogers[4], Darius Armstrong-James[2], Matthew Fisher[1] 1) MRC Centre for Global Disease Analysis, Imperial College London, London; 2) Department of Infectious Diseases, Imperial College London, London ; 3) Department of Medical Microbiology, King's College University Hospital, London; 4) Department of Clinical Microbiology, Trinity College Dublin, Dublin; 5) Aberdeen Fungal Group, Institute of Medical Sciences, University of Aberdeen; 6) Manchester Fungal Infection Group, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester; 7) School of Life Sciences, University of Nottingham, Nottingham; 8) National Mycology Reference Laboratory, Public Health England, Bristol; 9) MRC Centre for Medical Mycology, University of Exeter, Exeter; 10) Public Health Wales Microbiology, Cardiff; 11) Mycology Reference Centre, Leeds Teaching Hospitals National Health Service Trust, Leeds; 12) Infection Sciences, Kings College University Hospital, London.

Aspergillus fumigatus is a globally ubiquitous environmental mould capable of causing opportunistic lung disease. Invasive aspergillosis (IA) occurs in at-risk populations, such as neutropenic patients, those receiving immunosuppressive therapy or stem cell and solid organs transplants, and CF patients. It is also emerging as an important pathogen as an influenza and COVID-19 associated infection. Here, we use whole-genome sequencing of over one thousand globally sourced isolates sampled over 102 years to solve the spatiotemporal origins of *A. fumigatus*. We describe the polymorphisms associated with drug resistance, including novel drug resistance polymorphisms, and the spatiotemporal origins of the most prominent polymorphism TR_{34}/L98H. Our findings also indicate a two clade structure, with the majority of drug resistance polymorphisms assigned to Clade A; we will present data on crosses aimed at exploring whether there is a reproductive barrier between Clades A and B, and whether these polymorphisms are capable of being transferred onto new genetic backgrounds *via* recombination.

152 Identifying novel sexual reproduction defects by TN-seq in *Schizosaccharomyces pombe* *Caroline Craig*[1], Blake Billmyre[1], Michael Eickbush[1], Jeffrey Lange[1], Christopher Wood[1], Rachel Helston[1], Sarah Zanders[1,2] 1) Stowers Institute for Medical Research, Kansas City, MO; 2) University of Kansas Medical Center, Kansas City, KS.

Traditional genetic analyses examine single isolated mutants. However, pooled analysis can reveal types of phenotypes not observed in single mutants. We have developed a genome-wide transposon insertion (TN-seq) screen to identify genes important for sexual reproduction. Using this approach, we found over 500 genes involved in sexual reproduction. Some of the mutants, such as those in *ifs1* (Important for Sex), are unable to produce viable spores. Two other mutants, *plb1*Δ and *alg9*Δ have a previously undescribed phenotype, where mutant cells are capable of undergoing meiosis and sex but the spores produced are sick and delay germination. To analyze this phenotype, we used a competitive growth assay and found that *plb1*Δ and *alg9*Δ spores but not vegetative cells were outcompeted by wildtype. Furthermore, we live imaged mutant spores using a microfluidic device and used deep learning to analyze the size, rate of growth, and aspect ratio of the spores from these timelapse videos. These data revealed that *plb1*Δ and *alg9*Δ spores are smaller and have a delay in germination. Pooled analyses using TN-seq are an invaluable tool for studying sex and can reveal phenotypes that are difficult to detect using traditional approaches.

153 Obligate sexual reproduction of a homothallic fungus closely related to the *Cryptococcus* pathogenic species complex *Marco A. Coelho*[1,6], Shelly Applen Clancey[1,6], Andrew R. Passer[1], Terrance Shea[2], Márcia David-Palma[1], Anna Floyd Averette[1], Teun Boekhout[3], Betina Porcel[4], Minou Nowrousian[5], Christina A. Cuomo[2], Sheng Sun[1], Joseph Heitman[1] 1) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina, USA; 2) Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA; 3) Westerkdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; 4) Commissariat à l'Energie Atomique, Institut de Génomique, Genoscope, Evry, France; 5) Lehrstuhl für Molekulare und Zelluläre Botanik, Ruhr-Universität Bochum, Bochum, Germany; 6) contributed equally.

Sexual reproduction is an ancient trait of eukaryotic life on earth and the fact that most eukaryotes reproduce sexually is evidence of its evolutionary success. However, we now appreciate that the mechanisms of sex-determination and mate compatibility are quite diverse and evolve rapidly in many taxa. While sexual organisms are usually faced with the challenge of finding a compatible mating partner, species as diverse as animals, plants, and fungi have repeatedly evolved the ability to reproduce sexually without an obligate requirement for another individual. In this study, we uncovered the underlying mechanism of self-compatibility (homothallism) in *Cryptococcus depauperatus*, a fungal species sister to the clinically relevant human fungal pathogens *Cryptococcus neoformans* and *Cryptococcus gattii* species complexes. In contrast to *C. neoformans* or *C. gattii*, which grow as a yeast in the asexual stage, and produce hyphae, basidia, and infectious spores during the sexual stage, *C. depauperatus* grows exclusively as hyphae decorated with basidia and abundant spores and appears to be continuously engaged in sexual reproduction. By combining whole-genome

sequencing, comparative genomics, and genetic analyses of mutants defective in key mating and meiosis genes, we uncovered that self-compatibility in *C. depauperatus* is orchestrated by the expression, in the same cell, of an unlinked mating receptor (Ste3**a**) and pheromone ligand (MFa) pair derived from opposite mating types of a heterothallic (self-sterile) ancestor. We isolated the first genetic mutants in this species and show the sexual cycle involves meiosis and can produce recombinant progeny during outcrossing. Comparisons with closely related *Cryptococcus* species revealed a putative mating-type (*MAT*) determining region containing a few genes phylogenetically aligned with *MAT***a** alleles of other species, as well as *MAT*α alleles scattered throughout the genome, but no homologs of the mating-type determining homeodomain proteins *SXI1* (HD1) and *SXI2* (HD2). Synteny analyses reveal a dramatic remodeling of the *MAT* locus in *C. depauperatus,* possibly owing to reduced selective constraints to maintain mating-type genes in tight linkage, associated with a transition to self-fertility. These findings support *C. depauperatus* as an obligately sexual, homothallic fungal species and provide additional insight into the repeated transitions between modes of sexual reproduction that have occurred throughout the fungal kingdom.

154 Agaricomycete multicellular development and biomolecule formation with focus on the model system *Cyclocybe aegerita* and its relatives Axel Orban[1], Hannah Elders[2], Roman A. Frings[2], Jose G. Maciá-Vicente[3], Sandra Bube[2], Adéla Čmoková[4], Annsophie Weber[1], Robert Herzog[5], Harald Kellner[5], Martin Hofrichter[5], Martin Rühl[1], *Florian Hennicke*[2] 1) Justus-Liebig-Universität Giessen, Institute of Food Chemistry and Food Biotechnology, Giessen, Germany; 2) Ruhr-Universität Bochum (RUB), Department Evolution of Plants and Fungi, Project Group Genetics and Genomics of Fungi, Bochum, Germany; 3) Wageningen University & Research (WUR), Plant Ecology and Nature Conservation, Wageningen, The Netherlands; 4) Charles University Prague, Department of Botany, Prague, Czech Republic; 5) Department of Environmental Biotechnology, Technische Universität Dresden, Zittau, Germany.

Cyclocybe aegerita (syn. *Agrocybe aegerita*) is a widely cultivated reportedly almost cosmopolitan edible mushroom species that has been re-popularized as a model system, e.g., for studying complex multicellular development (fruiting body formation) and biomolecule formation of Agaricomycetes. Focusing on strains from different continents, and related species, via multilocus phylogenetic reconstruction and analysis of fruiting patterns, we have resolved two monophyletic geographic lineages of "*C. aegerita sensu lato*" delimiting a new Asian species complex from European *C. aegerita*. Also, we have revealed the Pacific parasitic species *Cyclocybe parasitica* to be capable of monokaryotic fruiting *sensu stricto* (fruiting without mating) displaying phenotypes known from bracket fungi and from *C. aegerita* which could possibly dissemble some clever genetic trick(s) permitting a non-deleterious bypassing of meiotic reproduction. Applying a comprehensive transcriptomic approach to fruiting body and volatile formation by *C. aegerita*, we have uncovered that fruiting body formation by this species seems to be regulated in a more complex manner by fruiting-related genes (FRGs) than in other model agarics. In addition, the combination of transcriptome and volatilome data has led us to identify genes coding for enzymes that may trigger the biosynthesis of C8 oxylipins: lipoxygenases (LOXs), dioxygenases (DOXs), hydroperoxidelyases (HPLs), alcohol dehydrogenases (ADHs) and ene-reductases. Furthermore, by this, the mycelium has been identified as the main source of sesquiterpenes that are predominant during sporulation in the headspace of *C. aegerita* cultures. Vice versa, C8 profile changes detected during late stages of complex multicellular development are probably contingent on the activity of enzymes located in fruiting body tissue. Since molecular tools for functional gene characterization in *C. aegerita* have been established recently, our work has provided us with a big variety of most interesting candidate genes that may now be analyzed by functional genetics-based approaches in order to get to a more definitive molecular understanding of complex multicellular development in this species. Likewise, further characterization of multicellular development-associated biomolecule formation and their role(s) as defense effectors or ‹infochemicals› as well as their biotechnological application appear to be of great prospective interest.

155 Role of A-to-I RNA editing in *Sordaria macrospora* sexual development Kathrin Zilske[1], Metaxenia Skendrou[1], Jana Grygosch[1], Hendrik Strotmeier[1], Bernhard Blank-Landeshammer[2], Minou Nowrousian[3], Albert Sickmann[2], *Ines Teichert*[1] 1) Allgemeine und Molekulare Botanik, Ruhr-University Bochum, Germany; 2) Leibniz-Institut für Analytische Wissenschaften – ISAS e.V., Dortmund, Germany; 3) Molekulare und Zellulaere Botanik, Ruhr-University Bochum, Germany .

RNA editing is the selective insertion, deletion, or substitution of nucleotides and is conserved in all domains of life. RNA editing of protein-coding transcripts leads to sequence changes in the transcript as well as the protein that could alternatively be directly encoded in the DNA. In filamentous ascomycetes, adenosine (A) to inosine (I) RNA editing was recently detected to occur in protein-coding transcripts during sexual reproduction. Interestingly, in fungi, amino acid codons, but also stop codons tend to be affected by editing, the latter leading to a change of *TAG* or *TGA* codons to *TGG* tryptophan codons. Why editing occurs during sexual development, how it is mediated and why the induced protein sequence changes are not directly DNA-encoded, is still under investigation.
Our model system is the ascomycete *Sordaria macrospora*, which has been studied genetically since the 1950s. It generates perithecia and ascospores within seven days on solid medium without a mating partner and is thus well-suited for studying the effects of RNA editing. We detected evidence for RNA editing at the transcript level by RNA-seq and at the protein level by mass spectrometry with artificial peptide libraries as well as Proteogenomics. We named the genes whose transcripts undergo editing *edited in fungal development* (*efd*) genes. Further functional analysis focused on transcripts that undergo stop-loss editing, because here we proposed to find a stronger effect on protein function then by a single amino acid variation. Deletion of several *efd* genes indeed revealed a function of these genes during ascospore formation. Complementation studies with mutations of the native stop codon to a *TGG* (always long protein) or a *TAA* (always short protein) revealed possible functions for the editing sites. We show results on several *efd* mutants as well as on mutagenesis approaches to identify the fungal editing enzyme and regulatory factors, which so far remain elusive.

156 Systematic deletions of histone methyltransferase and demethylase genes reveal their role in RIP and sexual development. *Pierre Grognet*[1], Mengyuan Li[1], Fabienne Malagnac[1] 1) I2BC, Universite Paris-Saclay, CNRS, Gif-sur-Yvette.

The model fungus *Podospora anserina* is widely used to study a great variety of processes. Its life cycle is short and relies on sexual reproduction only. In addition to the well-described stages of the sexual development, a key event is the occurrence of a silencing phenomenon called RIP (Repeat-Induced Point mutation) after fecundation, during the dikaryotic stage, prior to karyogamy. First described in *N. crassa*, RIP can target sequences that occur at least in two copies and introduces CpA to TpA mutations in repeated

sequences and lead to DNA methylation and heterochromatin formation on such sequences regardless of their coding capacity and relative position in the genome. Beside RIP, epigenetic regulation has been shown to be crucial for proper development in many organisms including fungi. We focused our study on histone lysine methylation, a post-translationnal modification that is deposited by enzyme containing a SET domain (histone methyltransferase, KMT) and removed by enzyme containing a Jmj domain (histone demethylase, KDM). Searching the genome of *P. anserina* for putative protein with SET domain and JmJ domain, we were able to predict 32 and 12 genes respectively.
Thanks to the ease of gene knockout in *P. anserina*, we generated a collection of KO strains for KMT and KDM genes. One of the first candidates, namely *PaKmt6*, have been shown to be involved in all stage of sexual development including RIP.
Here we show that among the other genes we inactivated, several of them are required for normal development and more interestingly, we found a new gene involved in RIP.
In addition, an experimental evolution approach using the PaKmt6 mutant showed that after only a few generations, genome instability may appear.

157 Live-Cell Imaging of Sexual Reproduction in *Podospora anserina*: the foreplay *Sylvain Brun*[1] 1) Institut Jacques Monod - UMR 7592, Universite de Paris, CNRS, Paris, France.

Sexual reproduction in fungi and fruiting body development have attracted interest of researchers for centuries [1]. Imaging these structures under non-live-cell imaging conditions, such as electronic microscopy, have highlighted the extraordinary complexity of fruiting body development [2–5]. However, lack of live-cell biology has been an obstacle to better understand sexual reproduction. Using live-cell imaging, for the first time we have observed live male and female nuclei during sexual reproduction in the model fungus *Neurospora crassa*. This study has revealed the specific behaviour of resident female nuclei within the trichogyne (the female organ) after fertilization and the extraordinary manner with which male nuclei migrate across the trichogyne [6]. To test whether this process is conserved in ascomycetes, I have started the live-cell imaging of fertilization in the model fungus *Podospora anserina* and first observations suggest overall conservation of the process between both species. These studies started in *P. anserina* will aim at answering important questions such as, which elements of cytoskeleton control nuclear movements during sexual reproduction in filamentous fungi, is there a system avoiding polyspermy in fungi, which determinants control male *vs.* female identity of nuclei, *etc*. These studies will also aim at investigating male and female nuclei in the next steps of sexual development by imaging nuclei in the core of the perithecium where they proliferate before entry into the ascogenous cell and karyogamy. These cell biology approaches will be expanded by the functional study of the genes involved through forward and reverse genetics strategies easily undertaken in this amenable model fungus.

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158 Diverse sexual strategies underpinned by the mating-type locus in the non-model fungal family *Ceratocystidaceae* *Markus Wilken*[1], Emma Steenkamp[1], Michael Wingfield[1], Brenda Wingfield[1] 1) Forestry and Agricultural Biotechnology Institute (FABI), Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria, South Africa.

The *Ceratocystidaceae* (*Microascales*) accommodates more than 120 species that include plant pathogens and insect-associated fungi. For many years, these species were grouped in the single genus *Ceratocystis*, even though species complexes defined by morphological and ecological differences were apparent. Subsequent taxonomic revisions, mainly based on molecular phylogeny, allowed resolution of this taxon and recognition of the *Ceratocystidaceae* that currently contains 15 well-defined genera, with a unique biology that often extends to idiosyncrasies regarding their sexual strategies. The latter includes heterothallism, both primary and secondary homothallism, as well as unisexual reproduction. Some species are also considered putatively asexual due to the lack of an observed sexual cycle. Using a large-scale targeted genome sequencing approach, we have systematically analysed the mating-type loci of selected species representing 14 genera in the *Ceratocystidaceae*. This has revealed conservation in the mating-type locus linked to sexual reproduction. This pattern is sufficiently robust to enable prediction of the reproductive mode in the apparently asexual species, based on the structure of the *MAT* locus alone. Some variation in the structure of the mating-type locus among species in a single genus was found, highlighting a complex evolutionary history for sexual reproduction in the group. This research has also led to the identification of *MAT1-2-7*, a lineage-specific mating-type gene unique to the *Microascales*. Functional studies of *MAT1-2-7* have also provided insights into its role in sexual development in these species. The foundation provided by these studies continues to drive ongoing functional studies aimed at understanding the molecular intricacies of atypical and unexplored sexual processes in the fungi.

159 Functional analyses of putative target genes of Argonaute-like protein (FgAGO2) required for sexual development in *Fusarium graminearum* Da-Woon Kim[1], Sang-Won Lee[1], *Sung-Hwan Yun*[1] 1) Soonchunhyang Univ.

Fusarium graminearum, the causal agent of Fusarium head blight in cereal crops, produces fruiting bodies called perithecia containing sexual progeny (ascospores) on plant debris as overwintering propagules. Previous studies have suggested that the Argonaute-like protein designated FgAGO2, which is a part of the RNA-induced silencing complex (RISC) for specific cleavage of target mRNAs in a RNA interference (RNAi) pathway, is essential for sexual development in *F. graminearum*. Here, we focus on the investigation of putative target genes of FgAGO2-RISC by employing a RNA immunoprecipitation sequencing (RIP-seq) assay. We used a GFP antibody to pull down bound RNAs to the FgAGO2-RISC in the transgenic *F. graminearum* strain (OE::FgAGO2-GFP) overexpressing a *GFP*-tagged *FgAGO2*, which was grown on carrot agar 7 days after sexual induction. A high throughput sequencing of the pulled

down RNAs identified a total of 8,922 small RNAs (sRNAs) ranging from 17 to 32-nucleotides, a half of which were mapped to exon regions of the fungal genome. Twenty two out of 33 fungal genes, which were selected based on genomic mapping of sRNAs, were up-regulated during the sexual developmental stage in *F. graminearum*. Targeted deletions of 7 out of the selected 19 genes led to reduced or abolished capability to produce perithecia or asci/ascospores in the corresponding transgenic strains. To validate the genomic regions mapped to the sRNA reads are the targets of FgAGO2-RISC-mediated RNAi pathway, we introduced a plasmid DNA into the *F. graminearum* genome, which contains a firefly luciferase-coding region fused with a ~300 bp partial fragment derived from the gene carrying sRNA-reads. Fungal transformants each generated by introducing individual DNA fragments from five different genes showed much reduced luciferase activities compared to those carrying only luciferase gene. Moreover, when each of two DNA fragments was reduced in size to ~170bp, the resulting fungal transformants showed similar levels of reduced luciferase activities, indicating these DNA fragments were responsible for reducing luciferase activities probably due to the degradation of their transcripts by FgAGO2-mediated RNAi process. Taken all together, it is clear that most target genes of FgAGO2 examined are transcriptionally specific to and/or functionally required for sexual development, and their expressions are properly regulated probably by a RNAi pathway in *F. graminearum*.

160 A common FOLD among plant symbiotic and pathogenic fungi Albin Teulet[1], Clement Quan[1], Weibing Yang[1], Edouard Evangelisti[1], *Sebastian Schornack*[1] 1) University of Cambridge, Sainsbury Laboratory (SLCU).

Effectors are key to microbial colonisation success of plant tissues, often via suppression of plant immune responses. Our understanding of effector function predominantly originates from plant pathogens but much less is known about effector families of fungi that for a symbiotic mycorrhiza with plant roots. Recent structural studies have identified several structural groups of microbe-secreted effector proteins. Examples are oomycete RXLR-WY effectors, the *Magnaporthe* MAX effectors and Fusarium FOLD effectors.
Using sequence based motif searches and AlphaFold protein structure prediction we identified candidate effectors resembling effectors from fungal pathogens in the growing list of Arbuscular Mycorrhiza (AM) fungal genomes from the Glomeromycotina clade. Interestingly, we found families of structurally related effectors resembling *Fusarium oxysporum* SIX6 and related FOLD effectors. Similar to SIX6, these AM fungal effector candidates are able to suppress effector-triggered immunity in plants and maybe expressed at arbuscule interfaces. Interestingly, members of this family are differentially expressed in different host plant species.Collectively, our findings indicate that AM fungal genomes carry a significantly enlarged but diversified group of effectors, structurally related to plant pathogen effectors. Our findings enable future studies into the functional diversity and biological relevance of the class of FOLD effector candidates from symbiotic fungi.

161 Conserved secreted effectors determine endophytic growth and multi-host plant compatibility in a vascular wilt fungus Amey Redkar[1], Mugdha Sabale[1], Christian Schudoma[2], Bernd Zechmann[3], Yogesh K. Gupta[4], Manuel S. López-Berges[1], Giovanni Venturini[7], Selena Gimenez-Ibanez[5], David Turrà[6], Roberto Solano[5], *Antonio Di Pietro*[1] 1) Universidad de Cordoba, Cordoba, Spain; 2) Earlham Institute, Norwich Research Park, Norwich, United Kingdom; 3) Baylor University, Waco, Texas; 4) The Sainsbury Laboratory, Norwich, United Kingdom; 5) Centro Nacional de Biotecnologıa, CSIC, Madrid, Spain; 6) Università di Napoli Federico II, Portici, Italy; 7) Isagro S.p.A., Novara, Italy.

Fungal interactions with plant roots, either beneficial or detrimental, have a crucial impact on agriculture and ecosystems. The cosmopolitan plant pathogen *Fusarium oxysporum* (Fo) provokes vascular wilts in more than a hundred different crops. Isolates of this fungus exhibit host-specific pathogenicity, which is conferred by effectors secreted in xylem (SIX) and encoded on lineage-specific genomic regions. However, such isolates also can colonize the roots of other plants asymptomatically as endophytes or even protect them against pathogenic strains. The molecular determinants of multi-host colonization are largely unknown. Here we characterized a set of Fo effectors from root apoplastic fluid, all of which are secreted during early biotrophic growth on both host and non-host plants. In contrast to SIX effectors, these Early Root Compatibility effectors (ERCs) have homologs across the entire Fo species complex as well as in other plant-interacting fungi, suggesting a conserved role in fungus-plant associations. Targeted deletion of ERC genes in a pathogenic Fo isolate resulted in reduced virulence and rapid activation of plant immune responses, while its deletion in a non-pathogenic isolate led to impaired root colonization and biocontrol ability. Strikingly, some ERCs also contribute to Fo infection on the non-vascular land plant *Marchantia polymorpha*, revealing an evolutionarily conserved mechanism for multi-host colonization by root infecting fungi.

162 *Blumeria graminis* effector proteins target a conserved host cell polarity pathway for establishment of biotrophic infection structures *Ralph Hückelhoven*[1], Lukas Weiss[1], Adriana Trutzenberg[1], Stefan Engelhardt[1] 1) Technical University of Munich.

Many obligate biotrophic fungal parasites, such as *Blumeria graminis* f.sp. *hordei* (*Bgh*), establish biotrophy by producing haustoria as feeding structures that invade intact host cells. It is discussed that host susceptibility factors may support the accommodation of haustoria in intact plant cells. However, little is known about the nature and mechanism of the associated host support and how parasitic fungi take over control of host susceptibility factors. RHO of plant proteins (ROPs or RACs) are common signaling hubs in plant polar cell development. The barley RHO protein RACB is a susceptibility factor in interaction with *Bgh* and is controlled by plant proteins that influence its GTPase activity, GTP loading and protein abundance. Here, we present data that *Bgh* targets RACB by two different effector proteins. One of them, ROPIP1, appears to affect RACB´s function in host microtubule organization, whereas the other, 9o9, may interfere with phospholipid signaling function of RACB. RACB has further function in cell polarity during epidermal cell development and RACB and its downstream protein interactors accumulate at the site of fungal accommodation in barley epidermal cells. Together, this suggests that *Bgh* targets a conserved host cell polarity pathway for establishment of biotrophic infection structures in intact epidermal cells.

163 Co-repressor Topless, a central effector hub for the *Ustilago maydis* / maize interaction *Armin Djamei*[1], Martin Darino[2], Janos Bindics[2], Fernando Navarrete[2], Mamoona Khan[1] 1) University of Bonn, INRES; 2) Gregor Mendel Institute, Vienna.

The biotrophic fungus *Ustilago maydis* causes smut disease in maize (*Zea mays*) and teosinte *(Zea mays ssp. parviglumis)*. Upon establishment of biotrophy, *U. maydis* secretes manipulative molecules, called effectors, to shape this interaction, to suppress immune

responses and to redirect host metabolism and development in favor of the pathogen. Transcriptomic analysis of *U. maydis*-infected maize show changes in several phytohormone signaling pathways, among others an upregulation of auxin and jasmonate signaling during establishment of biotrophy, but the molecular basis of this signaling manipulation was long time unknown.
Here we report our recent findings on several translocated effectors that all target maize Topless (TPL) co-repressor family members and lead upon *in planta* expression to specific de-repression of either Ethylene/Jasmonate signaling or auxin signaling. We demonstrate a direct link between TPL and PAMP-triggered Immunity responses, highlightening the role of TPLs as molecular players in plant defense / growth antagonisms in plants. Furthermore, the sheer number of TPL manipulating effectors implicate an outstanding importance of these effectors to smut fungi, giving many interesting insights both, in plant and pathogen biology.

164 Cytoplasmic effector translocation during early biotrophic invasion by the rice blast fungus *Ely Oliveira-Garcia*[1,2], Jungeun Park[3], Melinda Dalby[1], Magdalena Martin-Urdiroz[4], Clara Rodriguez Herrero[4], Sunghun Park[3], Nicholas J. Talbot[4,5], Barbara Valent[1] 1) Department of Plant Pathology, Kansas State University, Manhattan, KS, USA; 2) Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA, USA; 3) Department of Horticulture and Natural Resources, Kansas State University, Manhattan, KS, USA; 4) School of Biosciences, University of Exeter, Exeter, UK ; 5) The Sainsbury Laboratory, Norwich Research Park, Norwich, UK.

Host-adapted lineages of *Magnaporthe oryzae* (synonym of *Pyricularia oryzae*) cause blast diseases on rice, on millets and, most recently, on wheat. We previously presented evidence that the fungus co-opts the host's clathrin-mediated endocytosis (CME) system for internalization of secreted cytoplasmic effectors inside living host cells. Details of when and how this happens remain unclear. We have shown that the specialized blast biotrophic interfacial complex (BIC) is highly enriched in plant clathrin, based on analysis of transgenic rice lines expressing a translational fusion protein with rice clathrin light chain-1 (CLC1) and green fluorescent protein (GFP), infected by *M. oryzae*. Cytoplasmic effectors in BICs are packaged in effector vesicles bounded by GFP-labeled plant plasma membrane. Additionally, effector vesicles can be visualized in the host cytoplasm surrounding BICs at later stages of BIC development. Live cell imaging with various fluorescently-labeled effectors indicate that effector translocation is highly active before and during growth of the tubular primary hyphae (PH) that first colonize host cells. We analyzed a novel effector, Bas170, which accumulates in effector vesicles beneath the appressorium and in the host nucleus before visible growth of the PH with its associated BIC. Early effector translocation is supported by results in which CME was inhibited using both virus-induced gene silencing (VIGS) and pharmacological approaches. Silencing of two rice CME genes, Adaptor protein complex-2 (AP2) and clathrin heavy chain-1 (CHC1) as well as treatment with CME inhibitors caused a 'swollen BIC phenotype' in BICs associated with PH at about 20% of infection sites. However, at the remaining sites, penetration failed to occur and fluorescent cytoplasmic effectors, such as red fluorescent Pwl2, abnormally accumulate in a cloud of fluorescence under the appressorium. Taken together, our results suggest that extensive cytoplasmic effector translocation happens before and during growth of PH, with some residual translocation activity after hyphal differentiation and BIC relocation beside the first bulbous invasive hyphal cells. The challenge now is to find and characterize blast effectors that are involved in co-opting host endocytosis and achieving effector translocation and host cell targeting.

165 Deletion of the killer kinase *KIL1* abolishes penetration peg formation in the predator yeast *Saccharomycopsis schoenii* *Mareike Rij*[1], Yeseren Kayacan[2], Juergen Juergen[1,2] 1) Hochschule Geisenheim University; 2) Vrije Universiteit Brussel.

Predator yeasts are either homothallic or heterothallic ascomycetes of the genus *Saccharomycopsis*. These yeasts represent a unique genus of necrotrophic mycoparasites that infect a wide range of yeasts and filamentous fungi. Infection can be divided into recognition, adhesion, penetration and killing/nutrient uptake phases. For the penetration of a prey cell a dedicated penetration peg is formed. Penetration may be promoted by multigene families of cell wall degrading enzymes, including chitinases, glucosidases and proteases, which are specifically upregulated during predation. Penetration pegs grow in a polarized manner into the prey cell and are highly enriched in chitin. They do not contain a nucleus and do not grow further or develop into daughter cells. Each penetration peg is thus a one-time investment. We have recently sequenced the genomes of several *Saccharomycopsis* species and determined that in this genus the CTG codon is translated into serine instead of leucine. Predation is promoted by starvation as *Saccharomycopsis* yeasts are natural organic sulphur auxotrophs (i.e. methionine auxotrophs). We also developed a toolbox to initiate the molecular characterization of genes potentially involved in this predacious behavior. Deletion of a map kinase gene, *KIL1*, homologous to the *Magnaporthe grisea PMK1* and the *Saccharomyces cerevisiae KSS1* genes results in avirulent strains as determined by dilution series predation spot assays. *KIL1* is specifically required for penetration peg formation as *kil1* cells are unable to differentiate these structures. We will present *in vivo* time lapse data studying the predation process using GFP-tagged strains and *S. cerevisiae* as model prey cells.

166 Conditional role of a signal peptidase component in the establishment of biotrophy by the maize anthracnose pathogen *Colletotrichum graminicola* *Renata Belisario*[1], Lisa Vaillancourt[1] 1) University of Kentucky.

Colletotrichum graminicola infects maize during several phases of plant growth, resulting in anthracnose leaf blight, stalk rot, and top die-back diseases. This ubiquitous fungus has a significant destructive potential and causes millions of dollars of losses annually in North America. *Colletotrichum graminicola* is hemibiotrophic and initially invades living host cells via biotrophic infection hyphae before switching to necrotrophy and inducing host cell death, cell-wall degradation, and lesion development. An insertion mutation in the 3'UTR region of a gene (*Cpr1*), encoding a homolog of the noncatalytic glycosylated SPC22/23 subunit of the signal peptidase complex, impaired pathogenicity to maize leaves and stalks. The mutation is conditional, in that it has little or no effect on growth and development of the fungus in culture, or in dead maize tissues. The *Cpr1* mutant (MT) is interrupted early in infection, and never shifts to necrotrophy, produces lesions, or sporulates. Although it makes appressoria, penetrates epidermal cells, and produces infection hyphae normally, it fails to move biotrophically beyond the first invaded cell. Interestingly, when the MT is inoculated adjacent to the wild type (WT) strain, it can establish a successful biotrophic infection. This suggests that the WT may be secreting one or more factors that induce susceptibility in surrounding host cells. To test the hypothesis that the MT is deficient in the secretion of proteins that are necessary for pathogenicity, we are visualizing individual effector proteins and cell wall degrading enzymes (CWDE) as fluorescent fusions in the WT and MT strains, and in the complemented MT strain, in maize leaf sheaths. Additionally, we are quantifying secretion of CWDE *in vitro* for all three strains. Since all fungi have a homolog of the *Cpr1* gene and share the conserved secretion pathway, this work may

reveal a novel target for broad-spectrum control of fungal pathogens, including the important *Colletotrichum* species. Furthermore, it may uncover a previously unsuspected role for the SPC22/23 subunit in the regulation of secretory activity.

167 Impairment of the cellulose degradation machinery enhances *Fusarium oxysporum* virulence but limits its reproductive fitness Francisco M Gamez-Arjona[1], Stefania Vitale[2,3], Antonio Di Pietro[2], *Clara Sanchez-Rodriguez*[1] 1) Department of Biology, ETH Zurich; 2) Departamento de Genética, Universidad de Córdoba; 3) IPSP-Instituto per la Protezione Sostenibile delle Piante, CNR.

Fungal pathogens grow in the apoplastic space, in constant contact with the plant cell wall (CW) that hinders microbe progression, while representing a source of nutrients. Although numerous fungal CW modifying proteins have been identified, their role during host colonization remains underexplored. Here we show that the root-infecting plant pathogen *Fusarium oxysporum* (Fo) does not require its complete arsenal of cellulases to infect the host plant. Quite the opposite, Fo mutants impaired in cellulose degradation become hypervirulent by enhancing the secretion of virulence factors. On the other hand, the reduction on cellulase activity had a severe negative effect on saprophytic growth and microconidia production during the final stages of the Fo infection cycle. These findings enhance our understanding on the function of plant CW degradation on the outcome of host-microbe interactions and reveal an unexpected role of cellulose degradation in a pathogen›s reproductive success.

168 Ecological generalism drives hyperdiversity of secondary metabolite gene clusters in xylarialean endophytes *Jana M. U'Ren*[1], Mario Emilio Ernesto Franco[1], Jennifer H. Wisecaver[2], A. Elizabeth Arnold[1], Jason C. Slot[3], Steven Ahrendt[4], Igor V. Grigoriev[4,5], Roxanne Bantay[1] 1) University of Arizona, Tucson, AZ, USA; 2) Purdue University, West Lafayette, IN, USA; 3) The Ohio State University, Columbus, OH, USA; 4) Department of Energy The Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA, USA; 5) University of California, Berkeley, CA, USA.

The plant microbiome, including diverse fungi and bacteria living on and within roots, stems, and leaves, is increasingly recognized for its critical role in plant health and productivity in both natural and agricultural ecosystems. Yet despite the tremendous surge of interest in the plant microbiome, there is a significant gap in our knowledge of the molecular interactions between plants and the most diverse group of plant-associated symbionts: foliar fungal endophytes. This is due in large part to high phylogenetic diversity, cryptic infections, and low *in planta* biomass of endophytes in photosynthetic tissues, which together limit the transcriptomic studies typically used to identify genes and pathways involved in establishing symbioses. Compounding the challenge is that only a handful of foliar endophyte genomes from a limited number of hosts have been sequenced to date. We performed comparative genomic analyses of 96 genomes of endophytes and closely related saprotrophs and pathogens in two clades of a species-rich and ecologically diverse order, Xylariales (Xylariaceae s.l. and Hypoxylaceae). Fungi selected for genome sequencing were isolated from a diversity of plant and lichen hosts, biomes, and biogeographic regions worldwide. We used sister taxa representing different ecological modes (endophytic and non-endophytic) to perform phylogenetically-controlled genomic comparisons of trophic modes. We observed no significant differences in genome size or content between endophytic and non-endophytic fungi in either clade. However, pairwise comparisons showed that Hypoxylaceae endophyte genomes contained significantly fewer genes with signaling peptides, protein-coding genes, transporters, peptidases, PCWDEs (especially those involved in the decomposition of cellulose and lignin), secondary metabolite gene clusters (SMGCs) and catabolic gene clusters (CGCs) compared with non-endophytes. These differences were not observed between trophic modes in the Xylariaceae, consistent with the observation that endophytes in that family display ecological generalism in both substrate use and the phylogenetic breadth of symbiotic associations compared with members of the Hypoxylaceae. Overall, our analyses show that gene duplication, gene family expansion, and horizontal gene transfer (HGT) of SMGCs, effectors, and peptidases from putative bacterial and fungal donors drive metabolic versatility in the Xylariaceae. Ongoing transcriptomics and metabolomics experiments will further examine the functional roles of secondary metabolites in this ecologically important and dynamic clade.

169 Fungal digestive enzyme profile: Essential for fitness and integrated part of speciation and evolution *Lene Lange*[1], Anne S. Meyer[2], Kristian Barrett[2] 1) BioEconomy, Research & Advisory; 2) Bioengineering, Technical University of Denmark, Lyngby, Denmark .

Fungal growth impacts the environment significantly by its invasive power; made possible by the hyphal enzyme secretome. Therefore, analysis of evolution, function and composition of secretome gives valuable insight into fungal biology *per se*. Robust prediction of enzyme function, using the CUPP method, (Conserved Unique Peptide Pattern), opened for developing and testing a hypothesis for how the fungal digestive enzyme secretome evolved during evolution. Hypothesis: Increased fitness of fungal species is achieved by the fungi having *the right type of protein* (stability, pH and temperature optimum steric substrate accessibility etc) with *the right functions* for efficient degradation of available and accessible substrate. An evolutionary, fitness-relevant genome annotation can be achieved by annotating not to protein family and to function separately, but by using "EC-Function;Protein-Family" as one integrated observation, distinguishing and counting the type of specific type of protein with a specific function. Hereby also capturing the frequent occurrence in strong biomass degrading fungi, having several different types of enzyme protein with the same function. The hypothesis was tested by a Yule calculation for relatedness of "F;F"-observation profiles of all *Penicillium* and *Aspergillus* species. (Notably, Yule gives equal weight to "shared-presence" and "shared absence" of CAZyme "F;F" observations. The hypothesis was confirmed for these two genera, as the resulting "F;F"-relatedness dendrogram was stunningly similar to the organismal phylogenetic tree. This indicates that fungal digestive enzyme profile is an integrated part of speciation and evolution. *Global Hotspots of fungal CAZymes.* Summing up "F;F"-observations for 1.932 genomes demonstrated that fungal enzyme hotspots are found in species of very different taxonomy, lifestyle, ecology, physiology and substrate/host affinity. Surprisingly, most CAZyme hotspots are found in enzymatically understudied and unexploited species and the most well-known fungal enzyme producers, industrially exploited are not found to be among the top-ranking. The results contribute to elucidating the evolution of fungal substrate-digestive CAZyme profiles, ecophysiology, and habitat adaptations, and expand the knowledge base for novel and improved biomass resource utilization

170 Genomic diversity across 17 clinical isolates of *Candida auris* shapes *in vitro* evolution and rapid development of fluconazole resistance *Laura Burrack*[1,2], Robert Todd[2], Natthapon Soisangwan[2], Anna Selmecki[2] 1) Department of Biology, Gustavus

Adolphus College, St. Peter, MN; 2) Department of Microbiology and Immunology, University of Minnesota, Minneapolis, MN.

Antifungal drug resistance and tolerance poses a serious threat to global public health. In the newly emerged human fungal pathogen, *Candida auris,* resistance to azoles, including fluconazole, and amphotericin B is frequent, and resistance to echinocandin antifungals is rising, resulting in multidrug resistant isolates. Here, we use *in vitro* evolution of a set of seventeen new clinical isolates of *C. auris* from Clades I and IV to determine how quickly resistance mutations can arise, the stability of the resistance in the absence of drug, and the influence of genetic background on antifungal drug resistance. We evolved each isolate in the absence of drug as well as in low (1μg/ml) and high (256μg/ml) concentrations of fluconazole. In just three passages (~30 generations), we observed genomic and phenotypic changes including karyotype alterations, aneuploidy, acquisition of point mutations and increases in MIC values within the populations. Fluconazole resistance in the clinical isolates was stable in the absence of drug, indicating little to no fitness cost associated with resistance. Importantly, two isolates substantially increased fluconazole resistance to ≥256μg/ml fluconazole. Multiple evolutionary pathways and mechanisms to increase fluconazole resistance occurred simultaneously within the same population, including missense alleles in transcriptional regulators of azole resistance and acquisition of different aneuploidies. Strikingly, the sub-telomeric regions of *C. auris* were highly dynamic during the evolution experiment as deletion of multiple genes near the sub-telomeres occurred during the three passages in several populations. Finally, we identified the first example of a mutator phenotype in a clinical isolates of *C. auris*. This isolate acquired substantial resistance during the evolution experiments and had mutation rates approximately ten-fold higher than other strains, supporting that the genetic background of clinical isolates can have a significant effect on evolutionary potential.

171 Genome-scale phylogeny of the fungal order Sordariales *Noah Lisa Hensen*[1], Lucas Bonometti[2], Markus Hiltunen[1], Anna Lipzen[5], Chris Daum[5], Fabien Burki[1], Francis Martin[3], Igor Grigoriev[5], Jasmyn Pangilinan[5], Philippe Silar[4], Robert Riley[5], Vivian Ng[5], Pierre Gladieux[2], Hanna Johannesson[1] 1) Uppsala University, UU, Uppsala, Sweden ; 2) PHIM Plant Health Institute, Univ Montpellier, INRAE, CIRAD, Institut Agro, IRD, Montpellier, France; 3) Université de Lorraine, INRAE, UMR Interactions Arbres/Microorganismes, Centre INRAE Grand Est-Nancy, Champenoux, 54 280, France; 4) UMR8236 Laboratoire Interdisciplinaire des Energies de Demain, Université de Paris, France; 5) Joint Genome Institute, Lawrence Berkeley National Laboratory, UC Berkeley, USA.

Mutations are the main source of genetic variation, both within and between organisms in a population. As a result, knowledge about the rate of mutations and about the factors determining mutation rate in nature are central to our understanding of numerous processes, including evolution and ecological niche divergence. Mutation rate is expected to vary with organismal and ecological traits, but as of yet, the mutational processes and factors driving mutation rate variation are poorly understood.
The order Sordariales (Ascomycotina) is taxonomically diverse, with closely related species inhabiting a wide variety of natural habitats. The order therefore provides a unique opportunity to study the connection between mutation rates and life history traits (such as longevity), as well as between mutation rates and ecological traits (such as thermophili). In order to study these connections, a robust framework of phylogenetic relationships within the order is essential.
Previous molecular phylogenetic analysis of Sordariales has relied on a few genes-many taxa approach. In our project, we use whole genome data from 106 genomes publicly available and/or sequenced as part of JGI Community science Programs (proposals 504394 and 662/300789). This dataset includes representatives from different families within the order, creating a more representative source of genomic data across the order than previously available.
We compiled a genome-wide phylogenomic data matrix using the BUSCO genes present in the majority of the 106 species. Analysis of the data matrix using concatenation- and coalescence-based approaches is used to infer a robust genome-wide phylogenetic framework of the order. This phylogeny is used as a basis for the comparisons of genomic properties and inference of the direction of evolutionary change amongst Sordariales fungi. The created phylogeny will furthermore be used as a basis for ongoing studies on the influence of species-specific traits on the rate of molecular evolution. Additionally, the phylogeny will be helpful for researchers studying other biochemical, ecological, genetic and evolutionary questions in this group.

172 Allele specific expression during fruiting body formation in *Pleurotus ostreatus* *Zsolt Merényi*[1], Mate Viragh[1], Emile Gluck-Thaler[2], Jason C. Slot[3], Brigitta Kis[1], Torda Varga[1], Andras Geosel[4], Botond Hegedus[1], Balazs Balint[1], Laszlo G. Nagy[1,5] 1) Synthetic and Systems Biology Unit, Biological Research Center, Szeged, 6726, Hungary; 2) Department of Biology, University of Pennsylvania, 433 S University Ave, Philadelphia, PA 19104-4544, USA; 3) College of Food, Agricultural, and Environmental Sciences, Department of Plant Pathology, The Ohio State University, Columbus, USA; 4) Institute of Horticultural Science, Department of Vegetable and Mushroom Growing, Hungarian University of Agriculture and Life Sciences, Budapest, 1118, Hungary; 5) Department of Plant Anatomy, Institute of Biology, Eötvös Loránd University, Budapest, 1117, Hungary.

The mycelia in the fruiting body of Basidiomycota fungi are composed of dikaryotic cells, containing mostly two nuclei, originating from two parental strains. The heterokaryotic nature of hyphal cells opens the possibility for the two nuclei to contribute differently to gene expression, with one transcribing the given gene more actively than the other. The only previous study in this field demonstrated that most of the genes show equal expression from the two nuclei, while those genes which show allele specific expression (ASE) were regulated at the gene level. However, the exact genetic mechanism that generates ASE and its significance in the fungus' life remained elusive. The availability of the genomes of both parental monokaryons of *Pleurotus ostreatus* (PC15 and PC9) as well as new strand-specific RNA-Seq data across fruiting body development, allowed us to analyse the ASE during fruiting body morphogenesis of *P. ostreatus*. We found that 2,626 genes (25.2% of expressed genes) show ASE in *P. ostreatus*, the vast majority of them being expressed predominantly from one of the parental alleles in every developmental stage. In addition, the significantly higher difference in upstream regions of ASE genes implied that promoter divergence could cause these different expression patterns. Gene age enrichment and trend analyses proved that ASE was characteristic of young or recently duplicated genes. Since, young genes are known to be under weaker evolutionary constraint than conserved ones, it is possible that ASE arise neutrally in the transcriptome. Therefore, ASE may be a tolerated, rather than an adaptive phenomenon in fungi. However, even if neutral at the level of the individual, ASE may generate useful gene expression variation —e.g. developmental expression— that can serve as substrate for adaptive evolution. ASE may have important implications in mushroom breeding, where intraspecific hybrids harbouring different cis-regulatory alleles may result strains

with industrially relevant phenotypes.

173 Understanding the nature of the reproductive barriers within the wood decay species *Trichaptum abietinum* *Dabao Sun Lu*[1], David Peris[1], Jørn Henrik Sønstebø[2], Sundy Maurice[1], Håvard Kauserud[1], Mark Ravinet[3], Inger Skrede[1] 1) Department of biosciences, University of Oslo, Norway, Oslo Norway; 2) Department of Natural Sciences and Environmental Health, University of South-Eastern Norway, Bø Norway; 3) School of Life Sciences, University of Nottingham, Nottingham UK.

In basidiomycetes, an extreme diversity of alleles at the mating loci promote outcrossing within a species, but the genetic mechanisms maintaining reproductive barriers between species and how these barriers arise are largely unknown. Reproductive barriers within a single morphospecies have been reported for numerous fungi, and in several wood decay fungi these have been shown to be stronger between sympatric lineages. In the wood decay fungus *Trichaptum abietinum,* crossing experiments from the 1960´s revealed two sympatric intersterility groups in North America. The monokaryons from these two groups are unable to mate with each other and form dikaryons, but both groups are in part able to mate with European isolates. In this study, we use population genomic analyses together with *in vitro* crosses to investigate the genetic basis and infer the evolutionary origin of the pre-mating barriers in *T. abietinum*. To this end, we have whole genome sequenced 350 *T. abietinum* samples from Asia, Europe and North America. Our phylogeographic analyses show four major lineages, one in Asia and one in Europe, and two in North America corresponding to the known intersterility groups. Coalescence analyses identify one of the North American lineages as early diverging, whereas the other lineages coalesce more recently, but the splits among the lineages are hard to date and link to biogeographic events due to unknown generation time of wood decay fungi. Our *in vitro* crosses reveal additional partial intersterility groups between sub-lineages within Asia which also differ in their ability to mate with the other major lineages in Europe and North America. Since reproductive isolation in *T. abietinum* is not correlated with overall genomic divergence and appears in sympatry and parapatry, we propose that reinforcement could have been involved in the development of these barriers in North America and Asia. We use demographic modelling to test if secondary contact is more likely to have occurred between lineages where reproductive barriers exist. The complex and nested pattern of incompatibility between lineages indicate that it may be governed by several loci, and we use genome scans and association studies to identify regions correlated with the ability to mate across lineages.

174 Host specificity determines a new fungal plant pathogen population *Wagner Calegari Fagundes*[1], Janine Haueisen[1], Idalia Rojas[1], Alice Feurtey[2,3], Fatemeh Salimi[4], Alireza Alizadeh[5], Eva H. Stukenbrock[1] 1) Environmental Genomics group, Max Planck Institute for Evolutionary Biology, Plön & Christian-Albrechts University Kiel, Kiel, Germany; 2) Laboratory of Evolutionary Genetics, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland; 3) Plant Pathology, Institute of Integrative Biology, ETH Zürich, Zürich, Switzerland; 4) Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran; 5) Department of Plant Protection, Faculty of Agriculture, Azarbaijan Shahid Madani University, Tabriz, Iran.

Host specialization is considered the strongest driver of pathogen evolution. To successfully infect, colonize and complete the life cycle, plant pathogens are under constant selective pressures imposed by hosts, leading to adaptative genomic evolution and possibly new pathogen species or lineages radiation. Implementing population and comparative genomics approaches, we aim to identify evolutionary and molecular patterns of host specialization in fungal plant pathogens using the fungal pathogen *Zymoseptoria tritici* (causal agent of Septoria tritici blotch in wheat) as a model of study. Unique collections of *Z. tritici* were isolated from wild (*Aegilops* spp.) and domesticated (*Triticum aestivum*) host grasses in the Middle East, and whole-genome sequencing was performed in a subset of isolates from each collection. We observed distinct population structure between the two host-diverging collections and particular genomic features in the *Aegilops*-infecting isolates that may have shaped their evolutionary history. Phylogenetic analyses indicated that the *Aegilops*-infecting population forms a separate *Z. tritici* cluster when compared to worldwide collections of *Z. tritici* and to closely-related species. Population genomic analyses and demographic inference allow us to characterize the distinct populations and to detect signatures of recent selection. Moreover we find evidence that divergence of the *Z. tritici* populations on wild and domesticated hosts likely coincided with wheat domestication. Using infection experiments we show that *Z. tritici* isolates collected from *Aegilops* spp. only infect their respective host species and not *T. aestivum.* Together with other aspects, our findings highlight the interplay between agricultural and wild hosts on the evolution of fungal plant pathogens and illustrate a possible route of crop disease emergence.

175 Three-dimensional chromatin organization determines the evolution of adaptive genomic regions in the plant pathogen *Verticillium dahliae* *David E Torres*[1,2], Martin H Kramer[1], Vittorio Traccana[3], Gabriel L Fiorin[1], David E Cook[1,4], Michael F Seidl[2], Bart PHJ Thomma[1,3] 1) Laboratory of Phytopathology, Wageningen University and Research, The Netherlands; 2) Theoretical Biology & Bioinformatics Group, Department of Biology, Utrecht University, The Netherlands; 3) Institute for Plant Sciences, Cluster of Excellence on Plant Sciences (CEPLAS), University of Cologne, Germany; 4) Department of Plant Pathology, Kansas State University, USA.

The spatial organization of the eukaryotic nuclear genome is intimately linked to its biological functions. Beyond the linear organization of genetic elements in the DNA, chromatin folding and organization play an important role in the regulation of gene expression and genome evolution. In fungi, the 3D organization of the genome is still under-investigated. Therefore, the role of 3D organization in transcriptional regulation, genome organization, and evolution remains unclear, particularly in fungal plant pathogens that are constantly challenged by the immune systems of their hosts. Such challenges necessitate genomic responses on short and long evolutionary time scales. *Verticillium dahliae* is a filamentous fungal plant pathogen that causes disease on hundreds of plant hosts. The *V. dahliae* genome contains designated plastic regions, known as adaptive genomic regions (AGRs), that are enriched in transposable elements and in *in planta*-induced genes that mediate pathogen aggressiveness during host infection. Here, we explore the *V. dahliae* chromatin conformation with DNA proximity ligation followed by sequencing (Hi-C) to uncover the spatial organization of the core genome and the AGRs. Our analysis reveals the presence of topologically associating domains (TADs) in *V. dahliae.* Interestingly, we observe that TAD boundaries are gene-rich regions that display quantitatively lower gene expression than the genome-wide average. Moreover, we observe enrichment of facultative heterochromatin in weak TAD boundaries within AGRs. Interestingly, TADs in AGRs cluster physically within the nucleus, suggesting a common 3D organization of AGRs. By comparing different *V. dahliae* strains and other *Verticillium* species, we show that TAD boundaries are depleted in genomic variation. Thus, our analysis demonstrates that the 3D organization

is conserved within the *Verticillium* genus and indicates that this organization contributes to the evolution of AGRs in *V. dahliae.*

176 The diversity in fungal volatile organic compound profiles Yuan Guo[1], Werner Jud[1], Fabian Weikl[2], Andrea Ghirardo[1], Robert Junker[3], Andrea Polle[4], Philipp Benz[5], Karin Pritsch[2], Jörg-Peter Schnitzler[1], *Maaria Rosenkranz*[1] 1) Research Unit Environmental Simulation, Institute of Biochemical Plant Pathology, Helmholtz Zentrum München, Neuherberg, Germany; 2) Institute of Biochemical Plant Pathology, Helmholtz Zentrum München, Neuherberg, Germany; 3) Evolutionary Ecology of Plants, Department of Biology, Philipps-University Marburg, Marburg, Germany; 4) Forest Botany and Tree Physiology, University of Göttingen, Göttingen, Germany; 5) Holzforschung München, TUM School of Life Sciences Weihenstephan, Technical University of Munich, Freising, Germany.

Fungi produce a wide variety of volatile organic compounds (VOCs), which play central roles in the initiation and regulation of fungal interactions with other organisms. A systematic research on the global fungal VOC profiles is a prerequisite for elucidating the mechanisms of VOCs-mediated interactions. Here we present a versatile system enabling a high-throughput screening of fungal VOCs under controlled temperature. The developed platform allows automated and fast detection of VOCs from up to fourteen simultaneously growing fungal cultures in real-time. The comprehensive analysis of fungal scents is achieved by employing proton transfer reaction - time of flight - mass spectrometry and gas chromatography-mass spectrometry. Using this system, we characterized the volatile metabolic fingerprints of forty-three fungal species. Our data reveal dynamic, development-dependent and extremely species-specific VOC profiles. The VOC patterns were used to describe the phyla and the trophic mode of the fungi. Overall the analyses suggest that, depending on trophic mode, either individual VOCs or more complex VOC patterns (i.e. chemical communication displays) can be ecologically important. Present results stress the ecological significance of VOCs and serve as prerequisite for more comprehensive VOCs-involving ecological studies.

177 Using random barcoded transposon-site sequencing (Rb-TnSeq) bacterial libraries to explore the effects of volatiles from *Trichoderma atroviride* *Catharine Adams*[1, 2], Jose Manuel Villalobos Escobedi[1, 2], Mitchell Thompson[1,2], Robin Herbert[1,2], Adam Deutschbauer[1,2], Louise Glass[1,2] 1) UC Berkeley; 2) Lawrence Berkeley National Laboratory.

Plant associated fungi provide their hosts with a number of important health related benefits, and can even protect the plant from invading microbial pathogens. *Trichoderma atroviride* IMI is a plant root-associated filamentous fungus with potent antimicrobial effects, and volatile organic compounds (VOCs) from *T. atroviride* have been shown to discourage growth of a range of pathogenic microbes. However, few studies have explored how these VOCs may impact beneficial root-associated microbes. Here, we used Random Barcode Transposon-site Sequencing (Rb-TnSeq) to investigate the mechanisms of how VOCs from T. atroviride affect the physiology of six beneficial bacterial species selected from across the proteobacteria: *Azospirillum brasilense* Sp245 and *Sinorhizobium meliloti* 1021 (alpha-proteobacteria), *Burkholderia phytofirmans* PsJN and *Herbaspirillum seropedicae* SmR1 (beta), and *Klebsiella michiganensis* M5al and *Pseudomonas simiae* WCS417(gamma). We identified 32 genes across these bacteria that may have fungal volatile-induced phenotypes. In *P. simiae*, we see a physiologically consistent set of genes related to cell division and cell wall modification that show lower fitness when disrupted. Similarly, in *B. phytofirmans*, we see a coherent set of genes related to motility. In *S. meliloti*, we found the bacteria had increased fitness when a gene encoding an outer-membrane lipoprotein was disrupted, and this gene product may therefore be a receptor for one or more fungal VOCs. Furthermore, the overall effect of fungal derived VOCs on *S. meliloti* were similar to that of low pH. Follow up analysis revealed that when *S. meliloti* was grown in the presence of *T. atroviride*, the pH of the bacterial growth environment was lowered, suggesting that fungal VOCs have a role in altering host metabolism. On the plant host, low pH is a critical environmental signal for many plant-associated bacteria. Ongoing work with Gas Chromatography-Mass Spectroscopy (GC-MS) will endeavor to identify the precise fungal VOCs involved in these interactions. By elucidating the system wide effects of fungal derived VOCs in the rhizosphere, we can begin to design microbially driven strategies to enhance beneficial plant relationships, and improve overall plant health.

178 MERLIN unlocks the secrets to chitin signaling: Using gene-network inference to predict mediators of fungal response to lipo-chitooligosaccharides *Cristobal Carrera Carriel*[1], Spencer Halberg-Spencer[5], Saptarshi Pyne[5,6], Jean-Michel Ané[3,4], Nancy P. Keller[2,3], Sushmita Roy[5,6] 1) Department of Genetics, University of Wisconsin, Madison, WI, USA; 2) Department of Medical Microbiology and Immunology, University of Wisconsin, Madison,WI, USA; 3) Department of Bacteriology, University of Wisconsin, Madison, WI, USA; 4) Department of Agronomy, University of Wisconsin, Madison, WI, USA; 5) Biostatistics and Medical Informatics, Madison, WI, USA; 6) Wisconsin Institute for Discovery, Madison, WI, USA.

Chitin is a naturally occurring polymer composed of *N*-acetylglucosamine and is synthesized by many organisms, including fungi. Although chitin is mainly considered a structural component, derivatives can also serve as signaling molecules. A 2020 study found that all fungi produce a lipid-containing derivative of chitin called a lipo-chitooligosaccharide (LCO), and that treatment of the filamentous fungus *Aspergillus fumigatus* with LCOs increases germination and reduces hyphal branching. To investigate the gene networks important for LCO response, we used the MERLIN algorithm to infer regulatory gene networks for *A. fumigatus* using publicly available RNA-seq datasets. MERLIN implicated transcription factor AtfA as an important regulator of many genes that were differentially regulated in response to LCOs response, and we hypothesized that *atfA* is important for mediating *Aspergillus'* phenotypic response to LCOs. Studies of *aftA* deletion and overexpression mutants reveal that *atfA* is required for *A. fumigatus* germination and hypo-branching responses to LCOs. Our work here is the first to uncover, using gene-network predictions, a transcription factor responsible for a fungus regulatory response to LCOs. Future work will investigate if LCO perception and response occurs through the high-osmolarity glycerol (HOG) pathway as further predicted by MERLIN.

179 Copper homeostasis and *Cryptococcus neoformans* cell surface architecture *Corinna Probst*[1], Sarela Garcia-Santamarina[2], Jake Brooks[3], Inge van der Kloet[1], Andrew Alspaugh[1] 1) Duke University School of Medicine: Department of Medicine, Department of Molecular Genetics and Microbiology, Durham USA; 2) Instituto de Tecnologia Quimica e Biologica, Universidade Nova de Lisboa, Oeiras; 3) University of North Carolina at Chapel Hill, Department of Physics and Astronomy, Chapel Hill USA.

The trace element Copper (Cu) is an essential micronutrient, serving as a catalytic cofactor that drives iron uptake and distribution,

cellular respiration, ROS detoxification and other activities important for cell proliferation and survival. However, elevated levels of free non-bound copper are cytotoxic, causing ROS damage and mis-metalation of proteins. Therefore, functional copper homeostasis is essential for pathogenic microbes to quickly adapt to changing copper levels within their host during infection. In the opportunistic fungal pathogen *Cryptococcus neoformans* (*Cn*), the Cuf1 transcription factor is the central switch regulating Cu-responsive genes. The newly identified Cuf1-regulated copper-binding and release protein *Cn* Cbi1/Bim1 is a GPI-anchored copper-associated protein. Highly induced in copper limitation, Cbi1 plays a role in copper uptake through the copper transporter Ctr1. However, the mechanism of Cbi1 action is unknown. Interestingly, deletion of the *CBI1* gene affects cell wall integrity and architecture leading to altered macrophage activation and changes in the expression of specific virulence-associated phenotypes. The copper-deficient *cbi1*Δ mutant strain possesses an aberrant cell wall gene transcriptional signature as well as defects in chitin and chitosan deposition, cell wall carbohydrates previously shown to bind copper ions. In line with this finding, *Cn* strains defective in chitosan biosynthesis show an altered resistance profile when presented with copper stress. Based upon these findings, we suggest a new role for the fungal cell wall in regulating cellular copper levels, shielding the cell from states of excess copper, while serving as a copper storage site during conditions of extracellular copper limitation. Given its ability to bind and release copper and its localization on the cell surface of *Cn*, we further suggest that the Cbi1 protein is likely functioning in shuttling copper from the cell wall to the copper transporter for regulated copper uptake.

180 Connecting fungal genomes with the behavioral phenomes of ants, manipulated by *Ophiocordyceps* *Charissa de Bekker*[1], Ian Will[1], William Beckerson[1], Devin Burris[1] 1) University of Central Florida, Orlando, Florida, USA.

Transmission-promoting summiting behavior is a common parasitic manipulation, observed in a wide range of insect species infected by zombie-making parasites, including fungi. Yet, the molecular mechanisms that the fungi have evolved to hijack host behavior and the affected host pathways that give rise to altered behavioral phenotypes remain largely unknown. To provide a mechanistic perspective, we have developed *Ophiocordyceps camponoti-floridani* and its carpenter ant host as a model. Through our infection assays we found that *Ophiocordyceps*-infected individuals lose their ability to forage effectively, demonstrate a reduced communication, and undergo full-body tremors. Subsequently, towards the end if the infection, the ants climb towards an elevated position in which they latch on with their mandibles to facilitate fungal fruiting body formation and spore dispersal. To begin to unravel the fungal compounds that are involved in establishing these extended phenotypes, we combine comparative transcriptomics and metabolomics, with quantitative behavioral studies, micro-CT analyses and functional genetics assays. As such, we have begun to identify various candidate fungal compounds and ant host pathways that appear to be involved in the manipulated summiting of *Ophiocordyceps*-infected carpenter ants. These candidates include secreted enterotoxins, a protein-tyrosine phosphatase known to induce manipulations in caterpillars, an aflatrem derivative, and various novel small secreted proteins. We are currently testing the functions and involvement in manipulation of these compounds through knock outs, heterologous gene expression and protein production. As such, our integrative efforts are beginning to connect behavioral phenotypes of infected ants with the underlying fungal genes that give rise to those phenotypes.

181 *Phytophthora* zoospores display klinokinetic behaviour in response to a chemoattractant *Michiel Kasteel*[1, 2], Joris Sprakel[3], Tharun Rajamuthu[1, 2], Tijs Ketelaar[2], Francine Govers[1] 1) Wageningen University, Laboratory of Phytopathology; 2) Wageningen University, Laboratory of Cell Biology; 3) Wageningen University, Laboratory of Physical Chemistry & Soft Matter.

Phytophthora infestans, the causal agent of potato late blight, makes use of dispersal agents called zoospores to rapidly spread and infect. Being motile, these zoospores have the potential to actively track down their hosts using chemical cues such as sugars, amino acids and isoflavonoids. Identification of potential attractants has relied upon capillary systems in which the candidate chemoattractant is presented and aggregation at the source is scored. However, we do not know how these zoospores actually use their mobility to end up at these sources. In this study, we used high speed cameras to track zoospores over time and have quantified key trajectory parameters to describe their response to glutamic acid (Glu), an amino acid known to attract *P. infestans* zoospores. Zoospores innately swim in a run-and-tumble pattern with straight swimming stretches alternated by rapid turns. When exposed to Glu, tumbling frequency increases in a dose-dependent manner, eventually reaching a constant tumbling state. Glu did not affect flagellar drive nor run velocity, showing that the observed decreases in average velocity and active diffusion are solely attributable to an increase in tumbling frequency. We used the same experimental set-up to monitor zoospores of a mutant compromised in heterotrimeric G-protein signalling. We show their aberrant swimming behaviour to not be due to a defect in Glu-chemotaxis, but rather because they tumble at a high and consistent frequency. Alterations in tumbling frequency are part of a klinokinetic aggregation machinery well studied in bacteria, which we now show to also be a potential strategy of the zoospore homing response in oomycetes. The experimental set-up enables us to analyze chemotactic responses in detail and is instrumental for a greatly improved quantitative characterization of the *Phytophthora* homing response, thereby paving the way for studies into hampering or redirecting zoospores to reduce disease pressure.

182 RNA interference affects fungus-fungus interactions in the biocontrol agent *Clonostachys rosea* *Edoardo Piombo*[1], Ramesh Raju Vetukuri[2], Anders Broberg[3], Pruthvi Balachandra Kalyandurg[2], Sandeep Kushwaha[2,4], Dan Funck Jensen[1], Magnus Karlsson[1], Mukesh Dubey[1] 1) Department of Forest Mycology and Plant Pathology, Uppsala Biocenter, Swedish University of Agricultural Sciences, Uppsala, Sweden; 2) Department of Plant Breeding, Horticum, Swedish University of Agricultural Sciences, Lomma, Sweden; 3) Department of Molecular Sciences, Uppsala BioCenter, Swedish University of Agricultural Sciences, Uppsala, Sweden; 4) National Institute of Animal Biotechnology, Hyderabad, Telangana, India.

Clonostachys rosea is an antagonistic fungus with a proven role in the biocontrol of numerous insects, nematodes and fungi with a plant pathogenic activity. The antagonistic action of *C. rosea* is carried out through the production of toxic metabolites and reactive oxygen species, the induction of defence reactions on plant hosts, and the degradation of the target's cell wall with hydrolytic enzymes, such as chitinases. While it is proven that *C. rosea* reacts to different mycohosts by regulating the expression of precise host-dependent groups of genes, little is known regarding the role of RNA interference (RNAi) in regulating the biocontrol activity of this fungus. Since RNA interference is usually mediated by miRNA-like RNAs (milRNAs) produced through the cleaving of double strand RNAs by enzymes called "Dicers", we produced *C. rosea* deletion mutants for the two Dicer-like (dcl) enzymes identified in this fungus, and we verified that the Δdcl2 mutant has a diminished capacity of controlling the plant pathogens *B. cinerea* and *F. graminearum*. Afterwards,

we sequenced small and messenger RNAs from *C. rosea* Wild Type and mutants interacting with the fungal plant pathogens *Botrytis cinerea* and *Fusarium graminearum.* The results show how RNA interference is involved in *C. rosea* antagonistic action at multiple levels, both directly and indirectly. The Δ*dcl2* mutant had a lower expression of hydrolytic enzymes, secondary metabolite gene clusters and transporters involved in the removal of harmful compounds. Moreover, eleven milRNAs were identified in *C. rosea* WT but not in the Δ*dcl2* mutant, and they were predicted to target nine endogenous regulatory enzymes. Additionally, four proven virulence factors of *B. cinerea* and three of *F. graminearum* were predicted to be targeted by the dcl2-dependent milRNAs. All of them showed higher expression in the situations in which the targeting milRNAs were not produced, suggesting a putative role of fungal-fungal crossregulation in the biocontrol action of *C. rosea*.
These results improve our understanding of the complex interactions occurring between antagonistic fungi and plant pathogens, and they pose the base for future studies focusing on the role of cross-species RNAi-regulated mycoparasitic interactions.

183 *Lactobacillus*-secreted Yak1 inhibitor, 1-acetyl-beta-carboline, blocks *Candida albicans* morphogenesis and biofilm formation *Jessie MacAlpine*[1], Martin Daniel-Ivad[2], Zhongle Liu[1], Junko Yano[3], Nicole Revie[1], Robert Todd[4], Peter Stogios[5], Hiram Sanchez[6], Teresa O'Meara[7], Thomas Tompkins[8], Alexei Savchenko[5], Anna Selmecki[4], Amanda Veri[1], David Andes[6], Paul Fidel Jr.[3], Nicole Robbins[1], Justin Nodwell[2], Luke Whitesell[1], Leah Cowen[1] 1) Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada; 2) Department of Biochemistry, University of Toronto, Toronto, ON, Canada; 3) Center of Excellence in Oral and Craniofacial Biology, Louisiana State University Health Sciences Center School of Dentistry, New Orleans, LA, USA; 4) Department of Microbiology and Immunology, University of Minnesota Medical School, Minneapolis, MN, USA; 5) BioZone, Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, ON, Canada; 6) Department of Medical Microbiology and Immunology, University of Wisconsin, Madison, WI, USA; 7) Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI, 48109, USA; 8) Rosell Institute for Microbiome and Probiotics, 6100 Avenue Royalmount, Montreal, QC, Canada.

Interactions between bacteria and fungi are ubiquitous in nature, yet little is known about the phenotypic consequences of these interactions. In humans, the opportunistic fungal pathogen *Candida albicans* is a common member of the mucosal microbiota that can cause both superficial infections and life-threatening systemic disease. Vaginal candidiasis occurs in approximately 75% of healthy people with a vagina at least once in their lifetime, with fungal overgrowth often developing after a decline in bacterial abundance due to antibiotic use. *Lactobacillus* species are prominent constituents of the vaginal microbial community and the most common industrial probiotic. With the goal of identifying the mechanism(s) by which *Lactobacillus* affects *C. albicans* virulence, we observed that several species of *Lactobacillus* secrete a factor that can repress *C. albicans* hyphal morphogenesis, a cellular transition important for pathogenicity. Bioassay-guided fractionation linked this activity to 1-acetyl-beta-carboline (1-ABC), and genetic approaches identified the target of 1-ABC as the kinase Yak1. Additionally, we found beta-carbolines inhibited *C. albicans* biofilm formation both *in vitro* and *in vivo*.
To further explore the role of Yak1 in regulating filamentation, epistatic analysis was employed to assess whether Yak1 governs this developmental transition through the Ras1/Protein Kinase A (PKA) pathway. While overexpression of *TPK2,* a catalytic subunit of PKA, resulted in filamentous growth in the absence of an inducing cue, homozygous deletion of *YAK1* in this background blocked filamentation, suggesting Yak1 signals downstream of PKA. In follow up, we also selected mutants with a restored capacity to filament in the presence of 1-ABC, identifying amino acid substitutions in the putative phosphatase Oca6 and the transcription factor, Rob1. Additional genetic analyses suggested Oca6 functions upstream of Yak1, whereas Rob1 acts downstream of Yak1 in regard to regulation of morphogenesis. Interestingly, the importance of Yak1 in mediating filamentation appeared to be environmentally contingent as the kinase was dispensable for filamentation upon exposure to physiological concentrations of CO_2. Ongoing work is continuing to probe the role of Yak1 in regulating *C. albicans* morphogenesis in response to diverse environmental cues. Overall, these insights reveal *Lactobacillus*-secreted 1-ABC as a Yak1 inhibitor capable of blocking the yeast-to-filament transition in *C. albicans* and illuminate the complex circuitry by which Yak1 regulates a key virulence trait in this major human fungal pathogen.

184 Unlocking the biotech potential of the anaerobic fungi (Neocallimastigomycetes) *Michelle O'Malley*[1] 1) University of California, Santa Barbara.

Anaerobic fungi (Neocallimastigomycetes) are the primary colonizers of biomass within the digestive tract of large herbivores, where they have evolved unique abilities to break down lignin-rich cellulosic biomass through invasive, filamentous growth and the secretion of powerful lignocellulolytic enzymes. Despite these attractive abilities, considerably less genomic and metabolic data exists for gut fungi compared to well-studied anaerobic bacteria and aerobic fungi that hydrolyze cellulose. We have addressed these knowledge gaps by isolating and characterizing a collection of fungi from large herbivores using a combination of 'omics' tools. Hundreds of novel carbohydrate active enzymes (CAZymes) and components of fungal cellulosomes (enzyme complexes) were identified from several strains of anaerobic fungi, which were discovered through a combination of homology modeling and catabolite repression. Many of these CAZymes share high homology with those found in anaerobic bacteria, and likely arose through horizontal gene transfer. Additionally, high-resolution genomic sequences have revealed a rich set of biosynthetic genes across the fungi that likely regulate diverse processes from fungal development and maturation to microbial defense in the rumen microbiome. A wealth of diverse membrane transporters (SWEET, MFS, etc.) were also identified across anaerobic fungal genomes, which were verified to assist in sugar transport activity through heterologous expression in the yeast S. cerevisiae. Overall, our study has unmasked a rich repertoire of novel biomass-degrading enzymes, transporters, biosynthetic gene clusters, and a wealth of horizontally transferred genes within the rumen microbiome that can be used for engineering model microorganisms.

185 The complexity of Sweet – Competing carbon perception pathways in filamentous fungi *J. Philipp Benz*[1], Andre Fleissner[2], N. Louise Glass[3], Chaoguang Tian[4], Gustavo H. Goldman[1,5] 1) Technical University of Munich, Germany; 2) Technische Universität Braunschweig, Germany; 3) University of California at Berkeley, USA; 4) Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, China; 5) Universidade de São Paulo, Ribeirão Preto, Brazil.

Plant biomass is the most abundant carbon store on earth and a major food source for fungi. Enough evidence has accumulated to suggest that fungi can differentiate the polysaccharides present in plant biomass and thereby get the necessary information about its composition to decide which enzymes should be expressed to metabolize it. The information from the diverse signaling pathways

involved in the detection of the plant cell wall polymers has to be integrated by the fungi to prioritize and optimize efficiency. While much has been learned about the individual signaling pathways underlying this perception, we are only beginning to understand how they are connected and how this could explain the observable consequences, such as fungal carbon source biases, signaling cross-talk or carbon catabolite repression (CCR).
In my talk, I will present our latest findings regarding the intersections of signaling pathways for saccharides. On the one hand side, we have identified the F-box protein EXO-1 of *Neurospora crassa* as an important factor in the orchestration of the regulatory mechanisms underlying the correct response to the presence or absence of starch and glucose. In a following screen, we found evidence that F-box proteins are also involved in the integration of cellulose perception and CCR.
In another line of research, we are analyzing the cross-talk of cellulose and hemicellulose signaling. We have previously shown that cellulase expression is strongly inhibited in several filamentous fungi when mannan degradation products accumulate in the cell. We now analyzed the complex direct and indirect interactions of the cellulose transcription factors CLR-1 and CLR-2 with the regulator CLR-3 in more detail at the protein level. CLR-3 turns out to be a key component mediating the detection of inducer molecules, such as cellobiose and mannobiose, and the activation of CLR-1 and CLR-2.
Overall, the presented findings shape our view of fungal sugar perception pathways and contribute to a better understanding of the molecular processes with implications on the rational engineering of fungi for biotechnological applications.

186 The diets of biotrophs and opportunists in unhealthy hosts *James Kronstad*[1], Matthias Kretschmer[1], Djihane Damoo[1], Guanggan Hu[1], Eddy Sánchez-León1[1], Christopher Lee[1], Daniel Croll[2], Won Hee Jung[3] 1) University of British Columbia; 2) Université de Neuchâtel; 3) Chung-Ang University.

Fungal pathogens display a variety of life styles in terms of nutritional adaptation to the host environment. For example,some plant-associated fungi are biotrophs that are obligately dependent on nutrient acquisition from living hosts. We have employed the maize pathogen *Ustilago maydis* as a model to investigate biotrophy; this species has the experimental advantage that it can be grown in culture but is obligately dependent on plant infection to complete the sexual phase of its life cycle. Based on metabolic changes reported for diseased maize tissue, we hypothesized that *U. maydis* responds to combinations of different nutrients to support biotrophic growth. We tested this hypothesis and found that combinations of carbon sources trigger phenotypes associated with biotrophy. These phenotypes included the transcription of genes encoding effectors that are generally only expressed during biotrophic development, and the production of formation of melanin associated with sporulation in plant tumors. Additionally, we found that oxygen sensing and mitochondrial functions are important for the response to mixtures of carbon sources. These findings provide insights into the metabolic basis of biotrophy, and may support the development of methods to propagate obligate biotrophs in culture.

In parallel with the study of nutrient adaptation and biotrophy, we have also examined the ability of the opportunistic fungal pathogen *Cryptococcus neoformans* to adapt to the iron depleted environment during cryptococcosis in vertebrate hosts. Iron withholding is a critical component of nutritional immunity in vertebrates. In this context, a key feature of the response of *C. neoformans* to low iron is the elaboration of the polysaccharide capsule that is a major virulence factor. Key acquisition mechanisms for iron include the activity of a high affinity iron permease/ferroxidase system and uptake of heme via endocytosis. The regulation of these functions and others are controlled by the iron-responsive transcription factors Cir1 and HapX in association with the monothiol glutaredoxin Grx4. The use of a heme-responsive genetically encoded fluorescent sensor and mutant screens has provided a detailed view of endomembrane trafficking components that support heme use as an iron source. An understanding of these processes may provide opportunities for antifungal intervention.

187 Materialize fungi *Han Wosten*[1] 1) utrecht university.

Pure and composite fungal materials can be part of the transition from a linear to circular economy. A palette of such materials has been produced with foam-, elastomer-, and polymer-like properties, and properties of natural materials such as wood, cork and leather. So far, focus has mainly been on mechanical properties (e.g. compression strength and elasticity) and acoustic and thermal insulation. Fungal materials could exhibit other properties as well such as conductivity and bioactive properties such as antibacterial or immune-stimulatory properties.

188 How a fungus protects itself when producing a secondary toxic metabolite *Gustavo H. Goldman*[1] 1) Faculdade de Ciencias Farmaceuticas de Ribeirao Preto, Universidade de Sao Paulo, Brazil.

Aspergillus fumigatus causes a range of human and animal diseases collectively known as aspergillosis. *A. fumigatus* possesses and expresses a range of genetic determinants of virulence, which facilitate colonisation and disease progression, including the secretion of mycotoxins. Gliotoxin (GT) is the best studied *A. fumigatus* mycotoxin with a wide range of known toxic effects that impair human immune cell function. GT is also highly toxic to *A. fumigatus* and this fungus has evolved self-protection mechanisms that include (i) the GT efflux pump GliA, (ii) the GT neutralising enzyme GliT, and (iii) the negative regulation of GT biosynthesis by the *bis*-thiomethyltransferase GtmA. We identified a transcription factor (TF), RglT, important for *A. fumigatus* oxidative stress resistance, GT biosynthesis and self-protection, and virulence. RglT regulates the expression of several gli genes of the GT biosynthetic gene cluster, including the oxidoreductase-encoding gene gliT, by directly binding to their respective promoter regions. RglT is the main regulator of GliT and this GT protection mechanism also occurs in the non-GT producing fungus *A. nidulans*. However, *A. nidulans* does not encode GtmA and GliA. This work aimed at analysing the transcriptional response to exogenous GT in *A. fumigatus* and *A. nidulans*, and to identify additional components required for GT protection in *Aspergillus spp*. RNA-sequencing shows a highly different transcriptional response to exogenous GT with the RglT-dependent regulon also significantly differing between *A. fumigatus* and *A. nidulans*. However, we were able to observe homologues whose expression pattern was similar in both species (43 RglT-independent and 11 RglT-dependent). Based on this approach, we identified a novel RglT-dependent methyltranferase, MtrA, involved in GT protection. Taking into consideration the occurrence of RglT-independent modulated genes, we screened an *A. fumigatus* deletion library of 484 TFs for sensitivity to GT and identified 15 TFs important for GT self-protection. Of these, the TF KojR, which is essential for kojic acid biosynthesis in *Aspergillus oryzae*, was also essential for virulence and GT biosynthesis in *A. fumigatus*, and for GT protection in *A. fumigatus*, *A. nidulans*,

and *A. oryzae*. KojR regulates *rglT*, *gliT*, *gliJ* expression and sulfur metabolism in *Aspergillus* spp. Together, this study identified conserved components required for GT protection in *Aspergillus* species.
Funding: FAPESP, Brazil

189 Fungal pathogen effector-mediated dysbiosis to stimulate disease development in plant hosts *Bart Thomma*[1] 1) University of Cologne.

Beneficial plant-associated microbes are found in and on all organs of the plant and help to mitigate (a)biotic stresses. Plants are able to shape their microbiota and specifically attract beneficial microbes to suppress pathogens. Hence, the plant's microbiome can be considered an inherent, exogenous layer that complements its endogenous innate immune system.
Microbes typically secrete a plethora of molecules into their environment to promote niche colonization. Especially soil-dwelling microbes are well-known producers of antimicrobials that are exploited to outcompete microbial co-inhabitants in the soil. Also plant pathogenic microbes secrete a diversity of molecules into their environment for niche establishment.
Upon plant colonization, microbial pathogens secrete so-called effector proteins that promote disease development. While such effectors are typically considered to exclusively act through direct host manipulation, for instance through the suppression of host immune responses, we recently reported that the soil-borne fungal xylem-colonizing vascular wilt pathogen *Verticillium dahliae* exploits effector proteins with antibacterial properties to promote host colonization through the manipulation of beneficial host microbiota. Deeper analysis now reveals that *V. dahliae* has devoted part of its effector catalog to microbiota manipulation, through selective activities and life-stage dependent exploitation of particular effector proteins.

190 *Cryptococcus neoformans* Chitin Synthase 3 (Chs3) Plays a Critical Role in Dampening Host Inflammatory Responses *Camaron Hole*[1] 1) University of Tennessee Health Science Center, Memphis, TN.

Cryptococcus neoformans infections are significant causes of morbidity and mortality among AIDS patients and the third most common invasive fungal infection in organ transplant recipients. One of the main interfaces between the fungus and the host is the fungal cell wall. The cryptococcal cell wall is unusual among human pathogenic fungi in that the chitin is predominantly deacetylated to chitosan. Chitosan deficient strains of *C. neoformans* were found to be avirulent and rapidly cleared from the murine lung. Moreover, infection with a chitosan deficient *C. neoformans* lacking three chitin deacetylases (*cda1Δcda2Δcda3Δ*) was found to confer protective immunity to a subsequent challenge with a virulent wild type counterpart. In addition to the chitin deacetylases, it was previously shown that chitin synthase 3 (Chs3) is also essential for chitin deacetylase mediated formation of chitosan. Mice inoculated with *chs3Δ* at a dose previously shown to induce protection with *cda1Δcda2Δcda3Δ* die within 36 hours after installation of the organism. Mortality was not dependent on viable fungi as mice inoculated with heat-killed preparation of *chs3Δ* died at the same rate as mice inoculated with live *chs3Δ*, suggesting the rapid onset of death was host mediated likely caused by an over exuberant immune response. Histology, cytokine profiling, and flow cytometry indicates a massive neutrophil influx in the mice inoculated with *chs3Δ*. Mice depleted of neutrophils survived *chs3Δ* inoculation indicating that death was neutrophil mediated. Additionally, mortality is cell wall elicited as mice that received cell wall material isolated from *chs3Δ* died at the same rate as mice that received whole *chs3Δ*. Altogether, these studies lead us to conclude that Chs3, along with chitosan, plays critical roles in dampening cryptococcal induced host inflammatory responses.

191 *Botrytis cinerea* secretes small RNA containing extracellular vesicles that enter plant cells through clathrin-dependent endocytosis *Baoye He*[1], Qiang Cai[2], Hailing Jin[1] 1) University of California, Riverside; 2) Wuhan University.

Extracellular vesicles (EVs) are membranous structures that are involved in the release of hundreds of different molecules to the cellular outer space by diverse cells from all life domains, including fungi. Fungal EV cargoes contain many proteins, nucleic acids neutral lipids, glycans, and pigments. Many of these cargos can be recognized and accepted by specific "recipient" cells and modulate normal physiological processes as well as pathological progression. Here we demonstrate that the necrotrophic fungus Botrytis cinerea can also secret EVs during its infection period. Many fungal sRNA effectors are detected in Botrytis EVs, and these EVs can partially restore the virulence of Botrytis *dcl1/dcl2* mutant, which lost its ability to produce most of the Botrytis small RNAs (sRNAs). We further find that these vesicle-delivered small RNA effectors are taken up by plant host cells through clathrin-mediated endocytosis. After getting into plant cells, the Botrytis EVs cargo sRNAs can suppress host target gene expression. These results reveal that EVs can be used by Botrytis cinerea to deliver virulence factors into host plant cells.

192 Roles of candidalysin of *Candida albicans* in the gut permeability and brain pathology *Courtney Smith*[1], Eun Young Huh[2], Jenny Hsieh[3,4], Soo Chan Lee[1,4] 1) South Texas Center for Emerging Infectious Diseases (STCEID), Department of Molecular Microbiology and Immunology, University of Texas, San Antonio, TX; 2) Naval Research Unit – San Antonio (NAMRU-SA), Maxillofacial Injury and Disease Department; 3) Brain Health Consortium, University of Texas, San Antonio, TX; 4) Department of Neuroscience, Developmental and Regenerative Biology, University of Texas, San Antonio, TX.

Candida albicans is one of the most researched and clinically isolated commensal fungal pathogens that has a major public health impact. In the past few decades, there have been increasing amount of data that shows neurodegenerative diseases, such as Alzheimer's disease (AD), may have a microbial origin. AD is characterized as a progressive neurological disorder that that destroys memory along with other important mental functions; it is also associated with the accumulation of amyloid plaques. It has been documented that *C. albican*s has colonized approximately 89% of AD patient's brain biopsies. However, the mechanism as to how *C. albicans* migrates to the brain and what fungal factor(s) contribute to AD pathology remains unknown. Our data for both *in vivo* mouse and *in vitro* cell line models, it is suggested that a toxin secreted by *C. albicans*, called candidalysin, increases gut permeability of the epithelial barrier permitting the fungus to migrate from the gut to the brain. These results strongly suggest that candidalysin plays a key role in the migration of *C. albicans*. Currently, we are testing how candidalysin affects brain pathology and endothelial cell permeability. Therefore, this will improve our knowledge of fungal pathogenesis and its contribution of commensal fungi have on AD pathology.

193 Unravelling the gene networks coordinating core and host-specific infection programs in the polyphagous plant pathogenic fungus *Sclerotinia sclerotiorum* *Sylvain Raffaele*[1], Shantala Mounichetty[1], Lise Pomies[2], Stefan Kusch[3], Heba Ibra-

him[4], Maurinne Payet[1], Frederick Garcia[2], Adelin Barbacci[1], Laurence Godiard[1] 1) INRAE - LIPME Castanet Tolosan, France; 2) MIAT, INRAE Castanet Tolosan, France ; 3) RWTH Aachen University, Aachen, Germany; 4) KU Leuven, Leuven, Belgium.

The range of host that a pathogen can infect is a major determinant of disease epidemics. This trait is highly dynamic with patterns of reduction (specialization), shifts and expansion frequently observed in pathogen lineages. The white mold fungus *Sclerotinia sclerotiorum,* a major threat to oil and vegetable crops, can infect hundreds of plant species, including the model Brassicaceae *Arabidopsis thaliana*. Consistent with *S. sclerotiorum* evolution through host range expansion, closely related fungal species are unable to colonize *A. thaliana* and show high sensitivity to camalexin, a defense compound produced by Brassicaceae plants. By comparing *S. sclerotiorum* transcriptome during the infection of six plants from distinct botanical families, we revealed 1,657 fungal genes up-regulated *in planta*. Among those, 4.6% only form the core infection program up-regulated in all host species. Comparative genomic and transcriptomic analyses indicated that host range expansion in *S. sclerotiorum* involved lineage-specific genes as well as regulatory divergence in conserved genes. The promoter of *S. sclerotiorum* genes that gained induction on camalexin and *A. thaliana* were enriched in a cis-motif presumably targeted by a core fungal transcription factor (TF). Functional analysis of this TF supports a role in camalexin tolerance and host colonization. Using gene network modeling, we explored how this and other fungal TFs connect core and host-specific gene reprogramming to cause disease. Insights into the structure and evolutionary dynamics of virulence gene networks in broad host-range fungal pathogens will be discussed.

194 Analysis of commensal *Candida albicans* strains reveals genomic variability but conserved pathogenic potential *Teresa O'Meara*[1], Noelle Visser[1], Kevin Amses[2], Alexandra Web[3], Andrea Hodgins-Davis[1], Katura Metzner[1], Matthew O'Meara[3], Ryan Mills[3,4], Timothy James[2] 1) Department of Microbiology and Immunology, University of Michigan, Ann Arbor MI; 2) Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI; 3) Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI ; 4) Department of Human Genetics, University of Michigan, Ann Arbor, Michigan, Ann Arbor, MI 48109 .

Fungal pathogens like *Candida albicans* can cause devastating human disease. The risk of candidemia is complicated by the ubiquity of human colonization, the high rate of resistance to common antifungal therapies, and the toxicity of many antifungal compounds due to conservation of essential mammalian and fungal proteins. As the number of immunocompromised and hospitalized patients vulnerable to fungal infections increases, it is essential to discover new approaches for intervening in the transition of *C. albicans* from innocuous colonizer to lethal pathogen. Therefore, we undertook an analysis of the genetic variation in *C. albicans* commensal strains, with the goal of identifying genetic variants associated with increased virulence in systemic models of infection. By collecting a dense array of phenotypic data on diverse isolates from fecal and oral samples from healthy people, we characterized the structure of phenotypic variation among *C. albicans* isolates. Using a combination of karyotyping gels and novel synthetic long read technology, we characterized complex patterns of genetic variation in the species including SNVs and structural rearrangements. From this work, we demonstrate that healthy people are reservoirs for genotypically and phenotypically diverse *C. albicans* strains, and we were able to identify SNVs associated with specific *in vitro* phenotypes. However, the tested strains all retained the capacity to cause disease in systemic models of infection. This study provides a global view of commensal strain variation in *C. albicans,* and suggests that selection for commensalism in humans does not necessarily result in a fitness cost for invasive disease.

195 Metabolomic profiling of behaviorally manipulated insects infected by "zombie ant fungus" (*Ophiocordyceps*) *Ian Will*[1,2], Geoff Attardo[3], Charissa de Bekker[1,2] 1) Department of Biology, University of Central Florida, Orlando, USA; 2) Genomics and Bioinformatics Cluster, University of Central Florida, Orlando, USA; 3) Department of Entomology and Nematology, University of California - Davis, Davis, USA.

Ophiocordyceps camponoti-floridani, a species of "zombie ant fungus," infects and modifies the behavior of ants to further its own transmission at a lethal cost to the host. Manipulated ants perform a "death grip" biting and clinging behavior to attach themselves to plants. This behavior is understood as a fungal manipulation that benefits the parasite's growth and transmission. The underlying mechanisms of how these fungi can dysregulate animal behavior in such a coordinated manner has yet to be fully understood. To characterize possible compounds that relate to modified behavioral pathways, we performed liquid chromatography – mass spectrometry (LC-MS) on the head capsule of ants displaying the manipulated "death grip" behavior in the laboratory. By contrasting this against the present compounds in the heads of healthy ants, we aim to characterize candidate lipids (e.g. cell-cell communication and interaction), biogenic amines (e.g. neurotransmitters), and polyphenols/"natural products" (e.g. many toxic metabolites). We additionally link these metabolomics data with our previous transcriptomics study in a multi-omics approach to refine hypotheses about candidate mechanisms and fungal effectors at play.

196 The *Venturia inaequalis* effectorome is expressed in waves, and is dominated by expanded families with predicted structural similarity to avirulence effector proteins *Mercedes Rocafort*[1], Joanna K. Bowen[2], Berit Hassing[1], Murray P. Cox[3], Silvia de la Rosa[1], Brogan McGreal[2], Kim M. Plummer[4], Rosie E. Bradshaw[3], Carl H. Mesarich[1] 1) Bioprotection Aotearoa, School of Agriculture and Environment, Massey University, Palmerston North, New Zealand; 2) The New Zealand Institute for Plant and Food Research Limited, Mount Albert Research Centre, Auckland, New Zealand; 3) Bioprotection Aotearoa, School of Natural Sciences, Massey University, Palmerston North, New Zealand; 4) Department of Animal, Plant and Soil Sciences, La Trobe University, AgriBio, Centre for AgriBiosciences, La Trobe University, Bundoora, Victoria, Australia.

Venturia inaequalis is a subcuticular pathogen that causes one of the most devasting apple diseases, known as scab or black spot. The pathogen has a large repertoire of secreted proteins, with many of these anticipated to function as virulence factors (effectors) in promoting host infection or avirulence factors (Avr effectors) in triggering host resistance. Strikingly, most effector candidate (EC) proteins from *V. inaequalis* belong to expanded families ranging in size from five to 75 members. We performed the first comprehensive gene expression (RNA-seq) analysis of *V. inaequalis* during biotrophic colonization of apple leaves. Based on this analysis, we determined that genes encoding EC proteins are mostly expressed in waves corresponding to two specific infection stages: early and mid-late infection. The early expression wave is dominated by genes that encode EC families with an annotated functional domain. One such example is a family of 39 proteins with a stress up-regulated Nod19 domain. We hypothesize that this domain may be required for

modulating oxidative stress during host-colonization. Contrarily, most genes expressed during the mid-late wave encode expanded EC families with no or little amino acid similarity to other proteins. Therefore, to glean more information about their functions, we used AlphaFold2 to predict the structural fold of the most highly expressed member from each EC family. Strikingly, many EC families were predicted to share structural similarity to Avr effector proteins from other plant-pathogenic fungi. For example, members of the largest expanded EC family were predicted to have a β-sandwich fold with structural similarity to MAX (*Magnaporthe* AVRs and ToxB) effectors. Three further families were predicted to have structural similarity to members of the ToxA effector superfamily, while two other families were predicted to have structural similarity to LARS (*Leptosphaeria* AviRulence-Suppressing) effectors. Additionally, many EC families were predicted to adopt a similar structure to proteins with a killer protein 6 (KP6)-like and knottin-like fold. These results further reinforce the idea that fungal effectors share a limited number of structural folds, and potentially provide an enriched list of ECs from which Avr effectors can be identified. In conclusion, we show how the use of transcriptomics together with structural biology can be a powerful tool to identify and prioritize ECs for further studies.

197 The global movement of fungal and oomycete crop pathogens: mechanistic models, predictions and perils *Sarah Gurr*[1], Tom Chaloner[1], Dan Bebber[1] 1) University of Exeter.

Of the various challenges to food security, the threat of fungal (and oomycete) infection of our calorie and commodity crops outstrips that posed by bacterial and viral diseases combined (Fisher *et al*., 2012 *Nature* 484; Bebber *et al.,* 2013 *Nature Climate Change* 3; Fones *et al*., 2020 *Nature Food* 1).
We face a future blighted by known adversaries, by new variants of old foes and by new diseases. Modern agricultural intensification practices have heightened the challenge - the planting of vast swathes of genetically uniform crops, guarded by one or two inbred resistance (R) genes, and use of single target site antifungals has hastened emergence of new virulent and fungicide-resistant strains. Climate change compounds the saga as we see altered disease demographics - pathogens are moving poleward in a warming world (Bebber *et al.,* 2013 *Nature Climate Change* 3).
This presentation will highlight some current notable and persistent fungal diseases. It will consider the evolutionary drivers which underpin emergence of new diseases and manmade “accelerators” of spread. I will set these points in the context of a series of different disease models, initially with statistical correlative models, and thence with more recent mechanistic models - parametrised by data collected from pathogen, host, climate and with a temporal axis (Fones *et al*., 2020 *Nature Food* 1). Such models have enabled us to look across biological scales, that is from the global level to crop to host-pathogens *per se,* in our development of predictive movement models. In essence, the talk will cover the global distributions of crop pathogens (Bebber *et al.,* 2013 *Nature Climate Change* 3 11), their predicted movement (Bebber *et al.,* 2014 *New Phytologist* 202), crop disease saturation (Bebber *et al.,* 2014 *Global Ecology and BioGeography* 23) and thence our more recent work which aligns ecology with plant disease biology to (i) evaluate the risk of specialist and generalist pathogens (Chaloner *et al.,* 2020 *Nature Communications* 11) and, in particular, (ii) the demonstration that crop yields will increase at higher latitudes but likely decline in the tropics (recent versus 2027), but that such yield gains will be tempered by a greater burden of disease and by unfamiliar pathogens. Moreover, regions such as US, Europe and China will experience major changes in pathogens assemblages (Chaloner *et al*., 2021 *Nature Climate Change* 11).

198 Evolutionary Morphogenesis, Chytrids and the rise of The Fungi *Edgar Medina*[1], Lillian Fritz-Laylin[1] 1) Department of Biology, University of Massachusetts Amherst.

Morphogenesis comprises the cellular processes that generate organismal shape. Despite being sister lineages, the morphogenetic programs of multicellular Fungi and Animals are remarkably divergent in their *materia prima* (e.g. cell wall vs. epithelia) and its mechanisms. How morphogenetic programs evolved and diversified in these lineages remains a key question in evolutionary cell biology. Chytrids are members of the deepest lineages of Fungi, and their life cycle alternates between an animal-like ciliated zoospore cell that swims and crawls and a sporangial cell with fungal traits like a cell wall and hyphae. The growth of the sporangial cell results in a multinucleated compartment that is more than a hundred times larger than the initial uninucleated zoospore. During late development, the sporangia switches developmental programs and undergoes cellularization to produce numerous zoospores that are discharged through a pore in the cell wall. During zoospore formation, chytrid sporangia are transiently multicellular and undergo morphogenetic transformations akin to those of animal embryonic development. These transformations include a cytokinetic event that produces a multicellular sphere of tightly packed uninucleated cells. This morula-like sphere undergoes ciliogenesis and synchronous abscission to form motile zoospores. This combination of fungal traits, such as cell wall and hyphae, and an animal-like developmental program makes chytrids uniquely suited to trace the evolution of animal and fungal morphogenesis. We recently developed the chytrid *Spizellomyces punctatus* as a genetically tractable model system for evolutionary cell biology. We combine live-cell imaging of F-actin, microtubules, myosin-II and membrane dynamics with pharmacological perturbations to characterize the developmental program and morphogenetic transformations underlying chytrid cellularization. Further, we show that Chytrids retain key potential regulators of cellularization that are shared with animals but absent from Dikaryotic fungi. These results provide new insights into the ancestral morphogenetic toolkit present in the common ancestor of animals and fungi as well as innovations unique to the Fungal morphogenetic program.

199 A pandemic clonal lineage of the wheat blast fungus Sergio M Latorre[1], Thorsten Langer[2], Andrew J Foster[2], Vincent M Were[2], Adeline Harant[2], Angus Malmgren[2], Kurt Lamour[3], Joe Win[2], Sophien Kamoun[2], Nicholas J Talbot[2], *Hernán A Burbano*[1] 1) University College London, London, UK.; 2) The Sainsbury Laboratory, University of East Anglia, Norwich, UK.; 3) Department of Entomology and Plant Pathology, University of Tennessee, TN, USA..

Wheat, the most important staple crop worldwide, is threatened by wheat blast. This disease, caused by a wheat-infecting lineage of the blast fungus, *Magnaporthe oryzae*, originated in South America, before it spread to Bangladesh (2016) and Zambia (2017). Here, we reconstruct the genetic history of the wheat blast pandemic using genotyping (Tembo *et al*. 2021a) and whole-genome sequencing (Tembo *et al*. 2021b) data from African blast fungal isolates shared under the OpenWheatBlast initiative (www.openwheatblast.net). We show that the recent emergence of wheat blast in Asia and Africa was caused by a single genetic lineage of the wheat blast fungus closely related to South American isolates. Analysis of linkage disequilibrium patterns has revealed the clonal nature of this pandem-

ic lineage. In spite of its narrow genetic diversity relative to South American populations, the phylogenetic structure of the pandemic lineage suggests that the outbreaks in Zambia and Bangladesh originated by independent introductions. Genome analyses of virulence effector content have helped to predict candidate disease resistance genes that may have utility in controlling wheat blast. One of these, *Rmg8*, was effective against multiple isolates of the pandemic clone. Moreover, we found that Zambian wheat blast isolates are susceptible to strobilurin fungicides. However, they are at risk from resistance development and can mate with the prevailing local finger millet (*Eleusine coracana*) blast isolates, highlighting the evolutionary potential of the African outbreak to cause further damage to wheat production across the continent. These findings will inform management strategies for this devastating wheat disease and warrant further genomic surveillance to prevent and manage further outbreaks.

Tembo *et al*. 2021a. https://doi.org/10.5281/zenodo.4605959
Tembo *et al*. 2021b. https://doi.org/10.5281/zenodo.4637175

200 Convergent recognition of the *Magnaporthe oryzae* host specificity determinant *PWL2* in divergent grass species *Diana Gómez De La Cruz*[1], Helen Brabham[1], Vincent M Were[1], Inmaculada Hernández-Pinzón[1], Phon Green[1], Motoki Shimizu[2], Ryohei Terauchi[2,3], Nicholas J Talbot[1], Matthew Moscou[1] 1) The Sainsbury Laboratory, University of East Anglia, Norwich, UK; 2) Iwate Biotechnology Research Centre, Kitakami, Japan; 3) Kyoto University, Kyoto, Japan.

The blast fungus *Magnaporthe oryzae* is a devastating pathogen that infects and causes significant yield losses of economically important grass crops such as rice, wheat, barley, oat, and millet. Most *M. oryzae* isolates are host-specific, meaning their pathogenicity is restricted to a few species. However, important host jumps have occurred, such as the relatively recent emergence of wheat blast, which threatens wheat cultivation in South Asia and sub-Saharan Africa. Host range dynamics and species specificity have been shown to be determined by the presence of effectors that are recognised by corresponding plant resistance genes, making individual isolates non-adapted to most grass species. In barley, resistance against *M. oryzae* co-segregates with barley powdery mildew resistance at the *Mla* locus, highlighting a case of multiple pathogen recognition. Using fine mapping and forward genetics, we established that the barley intracellular immune receptor *Mla3* recognises the host-specificity determinant *PWL2* from *M. oryzae*. *PWL2* belongs to the *PWL* host specificity effector gene family, which exerts a major effect on the ability of the blast fungus to infect weeping lovegrass (*Eragrostis curvula*). Isolates of *M. oryzae* carrying *PWL2* are unable to cause disease on weeping lovegrass, whereas isolates carrying the loss-of-function allele *pwl2-2* are virulent on this host. While barley and weeping lovegrass maintain the same specificity of recognition, no homolog of *Mla* was identified in the latter. Our results show that convergent evolution of *PWL2* recognition has occurred in two divergent grass species, and highlight a complex evolutionary interaction between communities of plant and pathogenic fungi that lead to recognition of distinct pathogens by the same immune receptor.

201 Emerging tree pathogen *Phellinus noxius* has a long evolutionary history in eastern Asia, Australia, and the Pacific Islands *Olga Kozhar*[1], Mee-Sook Kim[2], Jorge Ibarra Caballero[1], Ned B. Klopfenstein[3], Phil G. Cannon[4], Jane E. Stewart[1] 1) Colorado State University, Fort Collins, CO; 2) USDA Forest Service, Pacific Northwest Research Station, Corvallis, OR; 3) USDA Forest Service, Rocky Mountain Research Station Moscow, ID; 4) USDA Forest Service, Forest Health Protection, Vallejo, CA.

Emerging pathogens and diseases they cause have increased exponentially over the last century, and it is critical to determine if these pathogens are native that were present in areas of emergence for a long time, or invasive that were recently introduced to those areas. Understanding the ecological and evolutionary processes promoting pathogen emergence can help to control pathogen and disease spread. Over the last few decades, brown root rot disease, caused by the root- and wood-rotting fungus *Phellinus noxius* of unknown origin, has been causing extensive damage to diverse trees in tropical/subtropical regions. Understanding the population structure, demographic history, and potential pathways of spread for *P. noxius* is an initial step to determine if *P. noxius* is an invasive pathogen that is causing the emergence of brown root rot disease. Little is known about the pathogen's population structure, diversity, and invasion routes. Using restriction site-associated DNA sequencing data, we characterized genetic relationships, pathways of spread, and evolutionary histories of *P. noxius* collected from 15 locations in eastern Asia, Australia, and the Pacific Islands. We analyzed patterns of genetic variation using Bayesian inference, maximum likelihood phylogeny, principal component analysis, and populations splits and mixtures measuring correlations in allele frequencies and genetic drift. In addition, we applied coalescent based theory using approximate Bayesian computation (ABC) with supervised machine learning. Population structure analyses revealed five distinct genetic groups of *P. noxius* with little admixture and with signatures of a complex recent and ancient migration history among study locations. ABC analyses indicated most likely pathogen spread from ghost population to Malaysia and the Pacific Islands (Guam and American Samoa), and with subsequent spread to Taiwan and Australia. Furthermore, ABC analyses indicate that major spread by *P. noxius* occurred 1,000s of generations ago, contradicting previous assumptions that it was recently introduced in many areas. Our results suggest that *P. noxius* has a long evolutionary history in eastern Asia, Australia, and the Pacific Islands, and recent pathogen and disease emergence is likely driven by anthropogenic and natural disturbances, including deforestation, land-use change, severe weather events, and introduction of exotic plants.

202 Genomic diversification of the specialized parasite of the fungus-growing ant symbiosis *Kirsten Gotting*[1], Daniel May[1], Jeffrey Sosa-Calvo[2], Lily Khadempour[3], Charlotte Francoeur[1], Margaret Thairu[1], Shelby Sandstrom[1], Caitlin Carlson[1], Marc Chevrette[1], Monica Pupo[4], Tim Bugni[1], Ted Schultz[2], J. Spencer Johnston[5], Cameron Currie[1] 1) University of Wisconsin-Madison, Madison, Wisconsin; 2) Smithsonian Institution, Washington, DC; 3) Rutgers University, Newark, New Jersey; 4) University of São Paulo, Ribeirão Preto, Brazil; 5) Texas A&M University, College Station, Texas.

Fungi shape the diversity of life. Characterizing the evolution of fungi is critical to understanding symbiotic associations across kingdoms. In this study, we investigate the genomic and metabolomic diversity of the genus *Escovopsis*, a specialized parasite of fungus-growing ant gardens. Based on 25 high-quality draft genomes, we show that *Escovopsis* forms a monophyletic group arising from a mycoparasitic fungal ancestor 61.82 million years ago (Mya). Across the evolutionary history of fungus-growing ants, the dates of origin of most clades of *Escovopsis* correspond to the dates of origin of the fungus-growing ants whose gardens they parasitize. We reveal that genome reduction is a consistent feature across the genus *Escovopsis*, largely occurring in coding regions, specifically in

the form of gene loss and reductions in copy numbers of genes. All functional gene categories had reduced copy numbers, but antimicrobial resistance and pathogenic virulence genes maintained functional diversity. Biosynthetic gene clusters contribute to differences among *Escovopsis* spp., and a similar diversity is also present in metabolomes of sister taxa in the Hypocreaceae. Taken together, our results indicate that *Escovopsis* spp. evolved unique genomic repertoires to specialize on the fungus-growing ant-microbe symbiosis. This genomic evolution represents an example of a eukaryotic genus evolving a reduced genomic toolkit while maintaining ancient host associations.

203 The maize mycobiome and implication on mycotoxin contamination in relation to climatic patterns *Bwalya Kataiti*[1,2], Bas J. Zwaan[1], Anne van Diepeningen[1], Pierre Schoenmakers [1], Paul Kachapulula[3], Henry Njapau[2], Sijmen E. Schoustra[3] 1) Wageningen University and Research, Wageningen, Netherlands; 2) National Institute for Scientific and Industrial Research, Lusaka, Zambia; 3) University of Zambia, Lusaka, Zambia.

Maize is often contaminated with an array of fungi at field. The implication is mycotoxin contamination. We investigated the fungal microbiome contaminating maize in the field in relation to climatic patterns and aflatoxin (AF) and fumonisin (FB) contamination thereof. The study was done over two seasons and two contrasting agroecological zones (AEZs) in Zambia. AEZ1 is comparatively drier and hotter than AEZ3 which is wetter and milder.
Maize samples were collected from 40 fields per season, spread across eight selected districts over the AEZs. AF and FB were analysed by High Pressure Liquid Chromatography. For mycobiome study, isolated DNA from maize kernels surface wash was analysed by amplicon sequencing targeting the fungal nuclear ribosomal internal transcribed spacer 1 region. Influence of AEZ and season on AF and FB was analysed by Kruskal Wallis non-parametric approach. Sequencing output data was processed using the Divisive Amplicon Denoising Algorithm 2 pipeline. Taxonomy was assigned to output sequences using the UNITE taxonomic fungal database. Principle coordinate and component analyses, non-metric multidimensional scaling and Phyloseq were used to study species abundances and relations with environmental variables.
Results revealed 87 fungal genera present on the maize mycobiome whose composition was climatic pattern related. Over the two seasons, *Fusarium* and *Sarocladium* had the highest relative abundance of 44% and 37%. PCA loading factors revealed *Fusarium* presence to be antagonistic with *Sarocladium*. *Aspergillus* had no clear antagonism or mutualism with *Fusarium*.
Fusarium was abundant throughout seasons and AEZs without any correlated environmental variables. Further, FB was detected throughout AEZs and seasons. Overall, FB contamination in season 1 was higher than season 2. *Aspergillus* was a low abundance genus across seasons and AEZs (0 – 10%). Its ingress into maize was higher in the hotter and drier zone during season 1 which had a dry spell. Overall, its abundance correlated with AF contamination. This resulted in high AF levels in maize in the hot drier zone in the first season with the dry spell. No AF was detected in second season and AEZs. Further, the AEZ with dry spell had higher levels of *Aspergillus, Penicillium, Meyerozyma, Ustilago* and *Kodamaea*.
Overall the maize mycobiome was diverse. The implication on the crop is AF contamination due to *Aspergillus* ingress into maize, depending on climatic conditions. Further observed implication is perpetual FB contamination due to *Fusarium* irrespective of climatic conditions.

204 Metagenomics approaches to understanding the synergistic roles of environment and chytrid infection in host extinction *Matthew Fisher*[1], Thomas Sewell[1] 1) Imperial College London.

Outbreaks of emerging infectious diseases are trained by local biotic and abiotic factors, with host declines occurring when conditions favour the pathogen. Extinction of the Tanzanian Kihansi spray toad (*Nectophrynoides asperginis*) in 2004 was contemporaneous with the construction of a dam, implicating habitat modification in the loss of this species. However, high burdens of a globally emerging infection, *Batrachochytrium dendrobatidis* (*Bd*) were synchronously observed implicating infectious disease in this toads extinction. Here, by shotgun sequencing skin DNA from archived toad mortalities and assembling chytrid mitogenomes, we prove this outbreak was caused by the *Bd*CAPE lineage and not the panzootic lineage *Bd*GPL that is widely associated with global amphibian declines. Molecular dating showed an expansion of *Bd*CAPE across Southern Africa overlapping with the timing of the extinction event. However, post-outbreak surveillance of conspecific species inhabiting this mountainous region showed widespread infection by *Bd*CAPE yet no signs of amphibian ill-health or species decline. Our findings show that despite efforts to mitigate the environmental impact caused by dams construction, invasion of the pathogen ultimately led to the loss of the Kihansi spray toad; a synergism between emerging infectious disease and environmental change that likely heralds wider negative impacts of fungal pathogens on biodiversity in the Anthropocene.

205 Pararesistance: a non-genetic mechanism of antifungal drug resistance *Jinglin Lucy Xie*[1], Kiran Chandrasekher[2], Judith Berman[3], Daniel Jarosz[1] 1) Stanford University School of Medicine, Stanford, CA; 2) Cornell University, Ithaca, NY; 3) Tel Aviv University, Tel Aviv, Israel.

Drug resistance is a major cause of treatment failure in infectious diseases and an emerging public health crisis. Although research has largely focused on identifying mutation-based mechanisms of drug resistance, cellular variation driven by epigenetic heterogeneity may be a hidden force promoting rapid adaptation to drug-induced stress. Recent studies have demonstrated that multiple chromatin-based and protein-based epigenetic states can be induced in response to stress. These heritable 'molecular memories' often confer a fitness advantage during re-exposure to stress. However, the mechanisms that promote the establishment and maintenance of such non-genetic states remain poorly characterized, especially in pathogens. Here, we describe a non-genetic mechanism of stress adaptation that accelerates the acquisition of drug resistance in a leading human fungal pathogen, *Candida albicans*. We discovered that a transient exposure to fluconazole, the most widely prescribed antifungal, elicits a sustained protective response in a subpopulation of cells. This mode of drug adaptation, which we term 'pararesistance', is induced by low doses of fluconazole, and facilitates the rapid emergence of resistance to high doses of fluconazole. Exposure to low levels of the protein denaturant guanidine hydrochloride or a suppressor of liquid-liquid phase separation 1,6-hexanediol blocks the induction, suggesting that pararesistance may be established via a prion-like mechanism. RNA-sequencing analysis showed pararesistant isolates share similar transcriptional profiles that are distinct from those of susceptible isolates. Importantly, a number of multidrug transporters such as *CDR1* are constitutively upregulated in pararesistant cells.

Consistent with this finding, pararesistant isolates exhibited increased efflux, resulting in decreased accumulation of Cdr1 substrate rhodamine 6G. Additionally, phenotypic characterization of 62 pararesistant isolates across 20 different growth conditions revealed that pararesistance confers resistance to a number of Cdr1 substrates in addition to fluconazole, including brefeldin A and terbinafine. These results suggest that the adaptive value of pararesistance is at least in part mediated by the upregulation of drug efflux. Together, this work presents a new paradigm for understanding non-genetic mechanisms that drive the rapid evolution of drug resistance, establishing a conceptual framework for developing novel therapeutic strategies that target evolutionary processes.

206 Targeting *Aspergillus fumigatus* hypoxia response pathways to potentiate contemporary antifungal therapies *Cecilia Gutierrez Perez*[1], Sourabh Dhingra[1,2], Steven M Kwansy[3], Timothy J Opperman[3], Robert A Cramer[1] 1) Dartmouth College, Hanover NH, USA; 2) Clemson University, Clemson, SC, USA; 3) Microbiotix Inc, Worcester, MA, USA.

Aspergillus fumigatus is a ubiquitous airborne filamentous fungus that is estimated to contribute to 600,000 deaths each year. There are currently only three contemporary antifungal therapies to treat invasive *Aspergillus* infections. Rapidly increasing resistance to first line therapy voriconazole highlights a significant need to develop novel antifungals with innovative mechanisms of action. Research from our lab has shown that the hypoxia response, mediated by the transcriptional regulator SrbA, is necessary for virulence and azole resistance in *Aspergillus fumigatus*. Therefore, identifying a compound that inhibits the SrbA mediated hypoxia response pathway would introduce a potentially novel antifungal that could potentiate azoles activity *in vivo*. We designed and performed a high-throughput screen by adapting a gpdA-luciferase reporter system to screen over 200,000 small molecule compounds for antifungal activity in the presence of fluconazole or hypoxic conditions. Using a secondary screen measuring enhanced fluconazole sensitivity and hypoxia specificity, we confirmed 50 compounds that fit all parameters to date. We then prioritized compounds that show limited human toxicity and an MIC≤10 μM for further investigation. Increased SrbA expression through expression of the N terminus bHLH transcription reduces susceptibility to several of these molecules. These data suggest that the compounds may act on the SrbA dependent hypoxia response. Since SrbA pathway inhibition increases azole sensitivity, we next tested the compounds against voriconazole-resistant clinical isolates and determined that combination therapy increases voriconazole efficacy. Using this high-throughput screen and follow up secondary screens we have identified compounds that are hypoxia specific and potentiate azole therapy with minimal human toxicity. Additionally, preliminary data suggests that several compounds are acting through the SrbA dependent hypoxia response pathway, a well characterized virulence factor. Through this work we are finding that we can identify novel antifungal compounds that act through innovative and well characterized biological mechanisms. One potential application of these findings is that these compounds can be used in combination therapy to potentiate azoles and combat antifungal resistance.

207 Exploiting synergistic and antagonistic drug interactions to improve treatment of systemic fungal infections Morgan Wambaugh[1], Rodrigo Costa[1], Magali Ayala[1], Steven Denham[1], Alifa Jakamartana[1], *Jessica Brown*[1] 1) University of Utah.

Background: Systemic fungal infections cause over a million deaths annually worldwide and have mortality rates of up to 90%. Many of patients have underlying immune system dysfunction due to HIV infection, cytotoxic cancer chemotherapy treatment, or other sources of immunosuppression, complicating treatment. However, the limited number of treatments and poor availability of some critical drugs handicap already long treatment regimes. For example, treatment of fungal meningoencephalitis caused by *Cryptococcus neoformans*, a primary killer of AIDS patients, involves more than six months on the antifungal azole drug fluconazole. **Objectives:** While cheap and broadly available, azoles such as fluconazole are primarily fungistatic - inhibiting cell growth rather than killing fungal cells - necessitating long treatment times. To improve systemic fungal infection treatments, we developed novel high-throughput methods to systematically identify FDA-approved drugs that act synergistically with fluconazole, exploiting these interactions to improve treatment efficacy. **Methods:** Combinations are considered synergistic if the minimum inhibitory concentration (MIC) of each drug is at least four-fold lower than the MIC of each individual drug. To identify synergistic interactions via high-throughput screening, we used our novel overlap^2 method (O2M), which identifies genetic "signatures" of synergistic interactions. We use these signatures to identify genes whose knockout phenotypes in the presence of a wide variety of drugs identifies those that interact synergistically with fluconazole. **Results:** We identified and validated 40 drugs that act synergistically with fluconazole. One of these, the anticholinergic drug dicyclomine, more than doubled the time-to-endpoint of mice with disseminated cryptococcosis when treated with fluconazole + dicyclomine compared to vehicle control or each drug alone. We will new present data on the molecular mechanisms underlying the synergistic interactions between fluconazole and our novel synergistic partners. We also investigate whether fluconazole and its synergistic partners are able to confer cross-resistance and/or cross-sensitivity. For example, we find that exposure to the common cholesterol lowering drug atorvastatin can induce fluconazole resistance. Our long-term goal is to rapidly designed synergistic drug combinations to combat systemic fungal infections and prevent treatment complications by identifying negative interactions.

208 Live cell imaging to understand fungicide mode of action *Gero Steinberg*[1], Martin Schuster[1], Sreedhar Kilaru[1] 1) Univ Exeter.

Cell biological analysis of genetically modified fungi is traditionally used to better understand the role of particular gene products in fungi. Considering the need for new fungicides against human and plant pathogens, we started to apply such combination of quantitative live cell imaging and molecular genetics to the study of fungicide mode of actions in various fungal pathogens. Here we will present unpublished results that demonstrate the power of this approach and reveal unexpected and new ways of fungicide activity in the fungal cell. This knowledge promises to help developing new strategies in pathogen control.

209 Predicting predictability of fungicide resistance evolution *Nichola Hawkins*[1], Bart Fraaije[1] 1) NIAB.

Pathogenic fungi have demonstrated incredibly high levels of adaptive potential, evolving to infect their hosts and to overcome control measures used by humans in agriculture and medicine. In order to manage the threat of disease control failure, it is important to take a proactive rather than a reactive approach, responding to the risk of resistance before it becomes a problem. For a given control measure, resistance management strategies should be informed by the level of resistance risk, and the levels of resistance and patterns of cross-resistance associated with the mutations most likely to emerge. However, this would mean predicting evolution in advance, and evolutionary biologists have long debated how predictable evolution may be. I have compared the levels of evolutionary predictability

in target-site mutations seen across plant pathogen species for different fungicide groups. The contrasting levels of predictability may relate to differences in the fitness costs and trade-offs, functional constraints, and cross-resistance patterns for the different genes. Further characterising the functional basis of these differences will help to improve resistance predictions, and assessments of confidence levels of such predictions, for future disease control measures.

210 Methionine synthase as a target for antifungal drug development *Jennifer Scott*[1], Benjamin Thornton[1], Jonathan Fowler[1], Rachael Fortune-Grant[1], Riba Thomas[1], Lydia Tabernero[1], Elaine Bignell[2], Jorge Amich[1,3] 1) Manchester Fungal Infection Group, University of Manchester, Manchester, UK; 2) MRC Centre for Medical Mycology, University of Exeter, Exeter, UK; 3) Mycology Reference Laboratory, National Centre for Microbiology, Instituto de Salud Carlos III, Majadahonda, Spain..

Mycoses pose an urgent threat to human health and are responsible for approximately 1.6 million deaths annually. Current therapeutic options for life threatening fungal infections, such as those caused by *Aspergillus fumigatus*, *Candida albicans* and *Cryptococcus neoformans*, are severely limited. Even under antifungal treatment these infections have mortality rates of ~50% and rising antifungal resistance exacerbates the need for the development of novel drugs.

Fungal pathogenic potential is strongly influenced by their metabolic versatility. Therefore, pathways required for cellular metabolism and nutrient homeostasis in host tissues are fundamental for infection and may represent exciting targets for antifungal drug discovery. As the conditions encountered throughout the course of infection, and consequently the fungal metabolic requirements, may vary significantly, it is crucial to validate targets in established infections.

We recently optimised a genetic model to allow characterisation and validation of prospective antifungal drug targets in growing *A. fumigatus* hyphae and established *in vivo* infections. We used the model to investigate methionine synthase (MetH), which has been described as essential for viability or virulence in *A. fumigatus*, *C. albicans* and *C. neoformans*. We showed that downregulation of *metH* expression triggered a complex metabolic imbalance, beyond methionine auxotrophy, which inhibited growth and thus virulence in two *in vivo* models of established infection. In addition, a structure-based virtual screening predicted differential druggability between the human and fungal enzymes, which could guide the design of novel specific inhibitors with reduced off target binding.

Following the validation of MetH as a promising antifungal target this work initiates the early stages of drug development. We have optimised the expression and purification of soluble MetH and designed a simple, cost-effective enzymatic assay suitable for high throughput screening. From our virtual screening, we have identified fragments which have a high probability to specifically bind to regions crucial for MetH's enzymatic activity.

Taken together our results support methionine synthase's potential as a druggable, specific and broad-spectrum antifungal drug target. We provide initial results that will permit hit identification using complementary approaches: high throughput screening of compound libraries and directed design of binding fragments.

211 Unanticipated drug-fungal interactions and their potential to impact the outcome of infection Parker Reitler[1], Jessica Regan[2], Tracy Peters[3], Christian DeJarnette[4], *Glen Palmer*[5] 1) University of Tennessee Health Sciences Center, Memphis, TN; 2) University of Tennessee Health Sciences Center, Memphis, TN; 3) University of Tennessee Health Sciences Center, Memphis, TN; 4) St. Jude Children›s Research Hospital, Memphis, TN; 5) University of Tennessee Health Sciences Center, Memphis, TN.

While there are well defined risk factors for many types of invasive fungal infections (IFIs) including neutropenia, diabetes and HIV infection, their occurrence within high-risk individuals and clinical outcomes are often highly unpredictable. *Candida* sp. are also a leading cause of mucosal disease including vulvovaginal candidiasis (VVC), an idiopathic condition with 5-10% of women suffering distressing and painful recurrent infections for unknown reasons. Given the limited therapeutic options available, it is critical to identify, understand and mitigate factors that contribute to an individual's risk of infection, and of a poor outcome. As eukaryotes, fungi share much of the same cell biology with mammals, with many physiological responses, biochemical and signaling pathways well conserved. As such, many of the drugs designed to induce a physiological response in human cells, may induce an analogous response in fungi. Furthermore, as endogenous residents of the G.I. tract and other mucosal surfaces, *Candida* species are directly exposed to the drugs consumed by their human host. Yet, the impact of most drugs upon fungal physiology and pathogenicity is poorly characterized, or in most cases completely unknown. As such, we have begun to systematically evaluate how exposure to non-antifungal drugs can modulate the physiology, immunogenicity and/or antifungal sensitivity of *Candida albicans*, and to determine if such interactions are sufficient to impact the outcome of infection. A series of phenotypic screens were conducted with a comprehensive collection of ~2700 drugs approved for human use, to identify those that undermine the antifungal efficacy of fluconazole or caspofungin, or which affect cell wall composition or integrity. These studies have revealed that a significant fraction of non-antifungal drugs affect fungal phenotype and drug sensitivity *in vitro*, often with little or no impact on growth. Furthermore, many do so at therapeutically relevant concentrations. The atypical antipsychotic, aripiprazole, was identified as decreasing fluconazole susceptibility by ~32-fold through a Tac1p-dependent mechanism, as well as modulating cell wall composition. Treatment with therapeutically relevant concentrations of aripiprazole also enhanced the virulence of *C. albicans* in a mouse model of disseminated infection. Collectively, these data reveal the potential for non-antifungal medications administered to colonized or infected individuals, to influence the clinical outcomes of IFIs.

213 DIVERSIFY - A flexible multispecies approach for construction of efficient heterologous fungal cell factories *Uffe Mortensen*[1], Katherina Vanegas[1], Tomas Strucko[1], Fabiano Contesini[1], Michael Nielsen [2], Zofia Jarczynska[1] 1) Technical University of Denmark; 2) Novozymes.

Recent sequencing of a large range of fungal species has revealed large repertoires of putative biotechnologically relevant genes and secondary metabolite gene clusters. However, characterization analysis of the genes and products are often complicated by the fact that many fungi do not propagate well at laboratory conditions; and if they do, many genes may be silent. In addition, it will most likely be necessary to develop genetic tools from scratch to allow for gene characterization directly in the host organism. Similarly, if a product

turns out useful, a new fungus is not very likely to perform well in industrial bioreactors. It may therefore be beneficial to examine gene functions and produce novel products in a well-characterized production host where the genic tools are already in place. However, often the commercial potential of these species is impeded by difficulties to predict host physiological and metabolic compatibility with a given product, and lack of adequate genetic tools. It may therefore be advantageous to produce the compounds heterologously in well-characterized hosts with well-developed genetic toolboxes. A complication of this strategy is that the biochemistry of the new processes and the new products may not fit the physiology of the new host. For example, the product may be toxic or the new proteins may not fold efficiently in the new host. We therefore envision that efficient heterologous production can be established with a higher success rate, if the new processes are implemented in several different hosts in parallel. To this end, we have established a flexible platform, DIVERSIFY, for multi-species heterologous gene expression. Moreover, relevant gene expression cassettes compatible with the platform can quickly be assembled from libraries of parts, directly into the fungal strain, in construction steps that can easily be automated.

214 4-color live-cell imaging and other novel microscopy tools reveal dynamic sub-cellular distributions of core clock components in *Neurospora crassa* *Ziyan Wang*[1], Bradley Bartholomai[1], Jennifer Loros[2], Jay Dunlap[1] 1) Department of Molecular & System Biology, Geisel School of Medicine at Dartmouth, Hanover, NH; 2) Department of Biochemistry & Cell Biology, Geisel School of Medicine at Dartmouth, Hanover, NH.

Circadian rhythms are endogenous daily oscillations driven by a molecular clock that help organisms better coordinate their metabolism and behaviors with the environment. Organisms from fungi to animals share a similar phosphorylation-driven transcription/translation negative feedback loop (TTFL) as the core clock mechanism, which describes an oscillator composed of positive and negative elements. Research on the filamentous fungus, *Neurospora crassa*, has provided answers to many fundamental clock-related questions. In *N. crassa*, the White Collar Complex (WCC) is a heterodimeric transcription factor and serves as the positive element. Frequency (FRQ) forms a complex with the FRQ-interacting RNA Helicase (FRH) and Casein Kinase-1 (CK-1a), known as the FFC complex, which is the negative element.

Molecular components of the circadian clock have been described over thirty years of genetic and molecular biological studies. However, little is known about their dynamics and regulation at the sub-cellular level. In recent years, live-cell imaging has become a new hotspot in circadian research because of its high spatiotemporal resolution. A few characteristics of *N. crassa,* such as difficulties in heterologous protein expression, rapid growth rate, and tendency to form large masses of fused hyphae, have made it a difficult organism to use for live-cell microscopy on a circadian time scale. We are developing strategies to overcome such difficulties in applying live-cell imaging to *Neurospora* circadian research.

We have now successfully developed and applied constitutive promoters at various expression levels for expression of heterologous proteins and cellular compartment markers, as well as bright fluorescent tags across the visible light spectrum in *N. crassa*. In addition, we have optimized and tested photoconvertible and photoswitchable fluorescent proteins for monitoring a subset of proteins in *N. crassa*. With mTagBFP2 tagged BML (cytoskeleton), mNeonGreen tagged Cdc11 (septa), mApple tagged WC-2 (nuclei), and iRFP670 tagged SON-1 (nuclear envelop), we can use 4-color living cell imaging in fungal system to look at different cellular compartment. Through multicolor live-cell imaging of core clock components (together with a nuclear marker) with these novel microscopy tools, we can potentially elucidate the dynamics of their subcellular localization and protein-protein interactions in high spatiotemporal resolution.

215 A platform for functional analysis for *Candida albicans* strain variation *Yinhe Mao*[1], Max Cravener[1], Norma Solis[2], Scott Filler[2], Aaron Mitchell[1] 1) Department of Microbiology, University of Georgia, Athens, GA; 2) Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, CA.

Candida albicans is a major human fungal pathogen. The clinical isolate SC5314 and its derivatives have been well studied, and have revealed molecular mechanisms of cellular processes and pathogen-host interaction. However, most studies have not accessed the extensive genetic variation among *C. albicans* isolates and its functional consequences. Different clinical isolates of *C. albicans* display striking differences in phenotypes, genotypes, and regulatory network structure. Mutations can affect both ORFs and non-coding sequences. What are the determinants of this extensive diversity? We created a new platform to investigate strain variation based on genotype-phenotype relationships. An SC5314 genomic DNA library was produced in lambda phage, which maintains 20 kb inserts stably. We have introduced the library into other clinical isolates by two different approaches. The first approach uses CRISPR-Cas9 techniques to integrate the library at the *MDR1* locus. We created about 2600 homozygous transformant strains in a weakly filamentous strain, P75010, and they were screened for the ability to form hyphae and damage host cells. The second approach is to generate autonomously replicating clones capable of autonomous maintenance instead of integrating into the genome, based on the strategy of Bijlani et al., mSphere. 2019 Mar 6;4(2):e00103-19. Lambda phage libraries were added with selection marker and origins of replication at one end and terminal telomere repeats at both ends. The autonomously replicating mutants showed a high copy number of genes, providing a powerful new tool to investigate pathway components and identify strain variation. Together, the low copy complementation library and the high copy overexpression library provide exciting tools to explore *C. albicans* strain diversity.

216 FungiDB: Free online informatic tools for fungal and oomycete biologists Evelina Basenko[2], *Omar Harb*[1], David Roos[1] 1) University of Pennsylvania; 2) University of Liverpool.

FungiDB (https://FungiDB.org) is a free online resource enabling browsing, querying, and sophisticated mining of genomic-scale datasets. FungiDB is component of The Eukaryotic Pathogen & Vector Bioinformatics Resource Center (VEuPathDB.org) that integrates genomic, transcriptomic, proteomic, epigenetic, population diversity, phenotypic, and other types of data for eukaryotic microbes (pathogenic & nonpathogenic, free-living & parasitic), as well as the hosts and vectors of human disease. As one of the largest VEuPathDB databases, FungiDB encompasses hundreds of genomes including yeast, filamentous fungi and oomycetes. A user-friendly web interface with embedded bioinformatics tools supports in silico experiments leveraging public data, along with private datasets uploaded by the user into My Workspace via the VEuPathDB Galaxy interface. With FungiDB, you can browse genomes and examine gene record pages, create searches to mine Omics-scale datasets, annotation, and the results of automated analyses (protein domains, orthology predictions from OrthoMCL.org, metabolic pathways, etc.) Expert knowledge of genes, phenotypes, publications, and other gene-centric data may be captured as User Comments, via bulk data submission, or through the integrated Apollo annotation platform. New datasets

can be nominated for integration by emailing the FungiDB help desk or via social media. FungiDB also offers a range of tutorials and webinars for novice and expert users, and will host a Help Desk (in-person and virtual) during all poster sessions at FGC, to answer any questions that meeting participants may have.

217 Fungal Bioreporters to Monitor Outcomes of Host-Cell Interactions *Neta Shlezinger*[1] 1) The Hebrew University.

Fungal pathogens infect over 1 billion human patients and cause over 1.6 million fatalities annually. Among them, Aspergillus fumigatus has become the leading mold infecting immunocompromised patients. Due to the combination of limited effective pharmaceutical options, the rise in at-risk patient populations and treatment-refractory cases, proper treatment of fungal infections has become a growing health concern. Specifically, the emergence of multi-drug resistant pathogenic species now poses a critical unmet medical need. Moreover, fungal pathogens present a particular challenge because they are eukaryotes and share significant homology with their human hosts, necessitating the development of innovative, non-cross-reactive, state-of-the-art therapies.
While various evidence point to early infection as a key event in the eventual progression to disease, recent studies show that during this stage, highly adaptable and dynamic host and fungal cells engage in complex, diverse interactions that contribute to well-documented heterogeneous outcomes of infection. However, current methodologies to probe host-pathogen interactions rely on measurements of bulk populations, thereby overlooking this diversity that can trigger different outcomes.
Fluorescence-based techniques enable researchers to monitor physiologic processes, specifically fungal cell viability and death, during cellular encounters with the mammalian immune system with single event resolution. By incorporating two independent fluorescent probes in fungal organisms either prior to, or ensuing experimental infection in mice, or cultured leukocytes, it is possible to distinguish and quantify live and killed fungal cells to interrogate genetic, pharmacologic, and cellular determinants that shape host-fungal cell outcomes.
We leverage these new tools to conduct “dual omics” approaches to simultaneously capture both host and pathogen molecular changes and specific cell subsets that underlie distinct infection outcomes, to reconstruct the repertoire of host and pathogen strategies that prevail at critical stages of early infection.

218 Development of a rapid and reversible system for targeted protein depletion in the filamentous fungus, *Fusarium graminearum* *John Ridenour*[1], Devin Wright[1], Michael Divine[1], Michael Freitag[1] 1) Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR.

Development requires nuanced regulation of transcription in response to environmental cues, but it is not fully understood how fungi orchestrate these responses. Conditional depletion of a protein is a powerful tool to determine how specific regulators contribute to development. The plant-derived, auxin-inducible degron (AID) system has been used to selectively degrade proteins in a variety of organisms. In the presence of auxin, the F-box protein, TIR1, recognizes and mediates degradation of an AID-tagged protein via canonical ubiquitination and shuttling to the proteasome. In this study, we engineered an AID system in the filamentous fungus, *Fusarium graminearum*, and demonstrate that a target protein can be reversibly depleted using this system. Suz12 is a conserved component of Polycomb Repressive Complex 2 (PRC2) and essential for proper development in *F. graminearum*. Here, we first generated a degradable version of Suz12 (dSuz12) by fusing GFP and a codon-optimized, minimal AID tag to the N-terminus of Suz12. We next generated a codon-optimized version of TIR1 and combined the two genes by crossing. Integration of both, dSuz12 and TIR1, have minimal impact on growth. Following induction of dSuz12 depletion by the synthetic auxin, naphtaleneacetic acid (NAA), we observed loss of subnuclear localization of dSuz12 as well as defects in secondary metabolism, both comparable to those observed in a *suz12* deletion strain. Defects in secondary metabolism were alleviated after NAA wash out, demonstrating dSuz12 depletion is reversible. We will report on additional molecular data obtained with dSuz12 and other degradable targets, including an essential protein kinase, that validate the system and help elucidate mechanisms regulating transcription and development in fungi. Together, our results provide a novel approach to deplete specific proteins in *F. graminearum* and reveal important insights into the activity of PRC2.

219 Genes of unknown function conserved across fungi: a call for action Asaf Salamov[1], Igor Shabalov[1], *Igor Grigoriev*[1,2] 1) US DOE Joint Genome Institute, LBNL, Berkeley, CA; 2) Plant and Microbial Biology Department, University of California Berkeley, Berkeley, CA.

The ever-increasing number of sequenced genomes presents us with an exciting opportunity to discover highly conserved gene families of unknown function and characterize them experimentally. Over 18 million proteins encoded in ~1300 fungal genomes from JGI MycoCosm were clustered into families using cascaded MMseqs2 with default parameters (Steinegger et al, 2017). A subset of 142 clusters of proteins with the following properties has been selected: (i) proteins conserved across large phylogenetic distances, i.e. present in either >50% of all fungal genomes or >90% of genomes in the clade; (ii) proteins of unknown function, with neither known Pfam domains except for the domains without specific function, like DUF or UPF, nor Blastp hits against Swissprot; (iii) at least 20% of genes of each selected cluster are transcriptomics based models.
We have detected these gene families across the kingdom Fungi and invite the international research community to functionally characterize their individual members and propagate their annotations across the Fungal Tree of Life (https://mycocosm.jgi.doe.gov/conserved-clusters/run/run-2020;FRJbyJ). Investigators can register, login, click on any cluster, and add notes to any protein from the list along with the methods used for functional characterization. Once characterized and annotated, each of such proteins enables us to expand the annotation to the entire cluster composed of proteins from many fungal species. Besides the targeted single protein biochemical characterization this list could focus more high-throughput methods on predicting function of these proteins: structure-based functional predictions or gene knockouts or various multiomics capabilities for characterization for sequenced genomes through JGI Community Science Program. Examples of these approaches will be presented.

220 Novel GCaMP6 imaging of cytosolic free calcium dynamics reveals stress-specific signalling responses in the fungal pathogen, *Candida albicans* *Callum J Parkin*[1], Claudiu Giuraniuc [2], Mariana Almeida [2], Mark D Fricker [3], Srishti Chawla[2], Silvia Wehmeier[2], Tina Bedekovic[1,2], Neil Gow[1,2], Alexandra C Brand[1,2] 1) MRC Centre for Medical Mycology at the University of Exeter, Exeter, UK; 2) School of Medicine, Medical Sciences & Nutrition, University of Aberdeen, Aberdeen, UK; 3) School of Plant Sciences, University

of Oxford, Oxford UK.

Calcium (Ca^{2+}) ions are an important second messenger for stress adaptation responses and growth in eukaryotic cells. In fungi, Ca^{2+}-signalling is poorly understood due to sequestration of Ca^{2+} reporter dyes into the vacuole but the availability of genetically encoded Ca^{2+} indicators (GECIs) offers new insights into Ca^{2+}-induced cell responses in real time. We have engineered the GCaMP6 construct for use in the fungal pathogen, *Candida albicans*, and used it to reveal dynamic cytoplasmic Ca^{2+} spiking in cell populations and individual yeast and hyphae for the first time. Growing yeast cells emit Ca^{2+} spikes of <10 s in duration at a rate dependent on extracellular [Ca^{2+}]. Ca^{2+} spikes are inhibited by chelation of extracellular Ca^{2+} with BAPTA, acidic pH and treatment with the Ca^{2+}-channel blocker, verapamil. GCaMP6 also revealed slower changes in the resting level of intracellular Ca^{2+} in a treatment-dependent manner.

Exposure of cells to stress compounds elicited differential GCaMP6 response signatures. The anionic surfactant, SDS, caused an instant, population-wide burst of spiking and a rise in resting levels. Cells did not adapt to repeated exposure to SDS, which led to ~10% cell death per treatment. Hyperosmotic shock (1 M NaCl) inhibited spiking and caused cell shrinkage on repeated exposure but cell viability was unaffected. Spiking ceased while resting levels rose in cells exposed to physiologically-relevant oxidative stress (5 mM H_2O_2) but this response diminished on repeated treatment, suggesting activation of an adaptive response. Adaptation was not seen in cells lacking the key antioxidant transcription factor, Cap1, or the phosphatase, calcineurin. Interestingly, the oxidative stress response was not seen in the *hog1Δ* mutant. These results are consistent with the role of the Cap1 and calcineurin signalling pathways in adaptation to oxidative stress and suggest that the *hog1Δ* mutant is pre-adapted to oxidative stress induced by 5 mM H_2O_2.

Live-cell imaging of GCaMP6 reporter activity is a useful tool for dissecting the role of Ca^{2+} in the response pathways of *C. albicans,* to diverse environmental challenges by observing stress-induced Ca^{2+} signatures in individual cells.

221 Combined Pan-, Population-, and Phylo-Genomic Analysis of *Aspergillus fumigatus* Reveals Population Structure and Lineage-Specific Diversity *Lotus Lofgren*[1,2], Robert Cramer[3], Jason Stajich[2] 1) Duke University, Durham, NC; 2) University of California Riverside, Riverside, CA ; 3) Dartmouth, Hanover, NH .

Aspergillus fumigatus is an important agent of human fungal disease, where virulence heterogeneity is thought to be at least partially structured by genetic variation between strains. While population genomic analyses based on reference genome alignments offer valuable insights into how gene variants are distributed across populations, these approaches fail to capture intraspecific variation in genes absent from the reference genome. Pan-genomic analyses based on de novo assemblies offer a promising alternative to reference-based genomics, with the potential to address the full genetic repertoire of a species. Here, we use a combination of population genomics, phylogenomics, and pan-genomics to assess population structure and recombination frequency, phylogenetically structured gene presence-absence variation, evidence for metabolic specificity, and the distribution of putative virulence and antifungal resistance genes in *A. fumigatus*. We provide evidence for three distinct populations of *A. fumigatus*, with low levels of outcrossing and a skewed MAT1-1:MAT1-2 ratio of approximately 7:5. The three clades are structured by both gene variation (SNPs and indels) and distinct gene presence-absence variation with unique suites of accessory genes present exclusively in each clade. Accessory genes displayed functional enrichment for nitrogen, phosphorus, and carbohydrate metabolism, and the three clades displayed unique profiles of presence-absence and copy number variation for metabolic genes and genes involved in nutrient mining, hinting that populations may be stratified by environmental niche specialization. Similarly, the distribution of antifungal resistance genes and resistance alleles were often structured by phylogeny. Despite low levels of outcrossing, *A. fumigatus* demonstrated a large pan-genome with a core:accessory ratio of approximately 67.2:32.8, and included many genes unrepresented in the Af293 reference genome. These results highlight the inadequacy of single-reference based approaches for capturing intraspecific variation, and the power of combined genomic approaches to elucidate population structure, genetic diversity, and the putative ecological drivers of clinically relevant fungi.

222 Fusarium effectome analysis reveals high diversity of effectors and direct relationship with the fungus lifestyle and their strategies for host colonization/infection *Domingo Martínez-Soto*[1], Houlin Yu[1], Gengtan Li[1], Shira Milo-Cochavi[1], Sean Sullivan[1], Kelly S. Allen[1], Li-Jun Ma[1] 1) University of Massachusetts.

Plants, representing more than 80% of human food, are essential for the health of human lives. Unfortunately, plant diseases caused by pathogenic microorganisms create severe yield losses and threaten food security globally. A group of cosmopolitan filamentous ascomycetes fungi, members within the species complex of *Fusarium oxysporum* (FOSC) can cause host-specific vascular wilt diseases in more than 100 crop species including tomato, cotton, watermelon, banana, etc.; leading to billions of dollars annual yield losses. Host-specific virulence is controlled by horizontally acquired accessory chromosomes (ACs) among FOSC genomes. Similar to other plant pathogens, FOSC species possess or have acquired additional genetic weapons, such as effector genes directly involved in the colonization (adaptation and defense) and infection (attack) of their plant hosts, through the acquisition of ACs. Using a pangenome approach, this study analyzes the effectome of 24 Fusarium species and compared them to seven other model fungi genomes. Based on computational analysis and manually curation of all effectomes, we classify Fusarium effectors into four large groups:
i) “Colonizers and attackers”, involved in plant compounds degradation and get nutrients (pectinases, cutinases, cellulases, Six, etc.);
ii) “Targets”, directly involved in the modification and modulation of host process (chorismate mutase, *inter alia*);
iii) “Helpers” or “facilitators” involved in helping to fungal cells during the host colonization (oxidoreductase, hydrophobins, repellent proteins, etc.); and
iv) “Protectors”, involved in the maintenance and protection of the fungal cells against antifungal proteins produced by the host (chitin-binding proteins, LysM, cerato-platanin, etc).
Interestingly, we observed: *a*) a high diversity of annotations for the effector proteins; *b*) FOSC share with other phytopathogens effectors involved in colonization, but FOSC have specific effectors for the attack, e.g. Six and Foa; *c*) only 8% of the functional domains or functional annotation are conserved in the effectors of FOSC, the rest of effectors are present in some species, or they are specie-specific; and *d*) the diversity of effector proteins is directly related with the Fusarium lifestyles and their strategies for host colonization/infection (e.g. *Fusarium* pathogens have more Six, Foa, Pectinases, Glycosyl hydrolases, etc. than *Fusarium* endophytes). Details on the functional importance of these effectors based on transcriptome data, mutagenesis, and heterologous expression using tobacco plants

will be presented. We believe the pan-effectome analysis will not only help to understand the molecular mechanisms that underpin host-plant interactions and will also assist the development of novel disease management strategies to increase global food security.

223 An orthologous gene coevolution network provides insight into eukaryotic cellular and genomic structure and function *Jacob Steenwyk*[1], Megan Philips[1], Yang Feng[2], Swapneeta Date[1], Todd Graham[1], Judith Berman[2], Chris Hittinger[3], Antonis Rokas[1] 1) Vanderbilt University, Nashville, TN; 2) Tel Aviv University, Ramat Aviv, Israel; 3) University of Wisconsin-Madison, Madison, Wisconsin.

Orthologous gene coevolution—which refers to gene pairs whose evolutionary rates covary across speciation events—is often observed among functionally related genes. We present a comprehensive gene coevolution network inferred from the examination of nearly three million orthologous gene pairs from 332 budding yeast species spanning ~400 million years of eukaryotic evolution. Modules within the network provide insight into cellular and genomic structure and function, such as genes functioning in distinct cellular compartments and DNA replication. Examination of the phenotypic impact of network perturbation across 14 environmental conditions using deletion mutant data from the baker's yeast *Saccharomyces cerevisiae* suggests that fitness in diverse environments is impacted by orthologous gene neighborhood and connectivity. By mapping the network onto the chromosomes of *S. cerevisiae* and the opportunistic human pathogen *Candida albicans*, which diverged ~235 million years ago, we discovered that coevolving orthologous genes are not clustered in either species; rather, they are most often located on different chromosomes or far apart on the same chromosome. The budding yeast coevolution network captures the hierarchy of eukaryotic cellular structure and function, provides a roadmap for genotype-to-phenotype discovery, and portrays the genome as an extensively linked ensemble of genes.

224 Births, deaths and survival of a retrotransposon family in the face of repeat induced point mutations (RIP) *Ivar Westerberg*[1], Aaron Vogan[1], Lorena Ament-Velásquez[2], Hanna Johannesson[1] 1) Uppsala University; 2) Stockholm University.

The filamentous fungus *Podospora anserina* is a part of a species-complex with six other species. *P. anserina* is known to harbor a relatively small abundance of repeats (seven percent of the genome), and also possess the host defence mechanism known as repeat induced point-mutations (RIP). RIP induces C-to-T mutations in repetitive regions of the genome, and hence can introduce nonsense mutations into TEs. Typical classification relies on a number of assumptions, such as constant rates of evolution, which are violated by RIP due to its stochastic nature. Thus, classifying TEs in many fungal species is a complex and imprecise process. In this study, we utilized a combination of a sequence similarity network (SSN) approach together with the more commonly used alignment-based classification to explore the evolution of an LTR retrotransposon family, Crapaud, across the Podospora species-complex. LTR-retrotransposons rely on an RNA-mediated transposition mechanism and contain protein domains for reverse transcription and integration as well as structurally important long terminal repeat sequences that provide sequence promotors and transcription termination sites. Our initial results revealed multiple variants of the terminal repeats, where half of the terminal repeat was conserved and the other diverged, throughout the Podospora genomes. Additionally, we identify several full length variants of Crapaud that are present in multiple copies throughout the species-complex. This result is indicative of the variants either actively transposing or having done so at some point in the evolution of the species-complex. The identification of these variants reveals a recent radiation of Crapaud within the species-complex, with some variants specific to one or a few species. The utilization of SSNs to classify these repeats showcase the utility of the addition of a sequence similarity clustering method to the typical methods of studying TE evolution in genomes with RIP. These results also provide key insights into the evolution of LTR retrotransposons and how they manage to evade the host defence RIP. Crapaud is abundant in the species-complex and present in structurally important regions such as the centromeres. The evolution and radiation of this LTR retrotransposon thus give further insights into the overall genome evolution of the species-complex.

225 The distributed genome of *Fusarium oxysporum*: mix and match of core and accessory chromosomes *Like Fokkens*[1], Petra Houterman[2], Martijn Rep[2] 1) Wageningen University; 2) University of Amsterdam.

Fusarium oxysporum (*Fo*) is an asexual, opportunistic pathogen that can cause wilting and rot symptoms on a variety of economically important crops and ornamentals. Over the last years, characterization of the pangenome of *Fo* has revealed an enormous genetic diversity in this species complex. In addition to a set of conserved core chromosomes, *Fo* has a wealth of transposon-rich accessory chromosomes that are present in only a small subset of strains. Some of these accessory chromosomes are associated with the ability to infect a particular host. Horizontal transfer of these chromosomes makes this ability accessible to other strains, particularly to non-pathogens. We expect that the probability that new diseases arise through horizontal transfer depends on the capacity to maintain a compartmentalized genome: to colocalize genes that are important for infection, and to avoid invasion by transposons brought in by foreign chromosomes.

Using a combination of long- and short read sequencing, we study cross-talk between core and accessory chromosomes, particularly of chromosomes associated with host-specificity, in tomato and cucurbit-infecting *Fo* . We find that all tomato-infecting strains share the same host-associated chromosomes, albeit in different configurations. In contrast, cucumber-, watermelon- and melon-infecting strains mix-and-match horizontally transferred chromosomes. To complement comparative genomics studies in natural isolates we also performed an experimental evolution study in which we compare strains that received a chromosome through horizontal transfer in the lab with wild-type tomato-infecting strains and quantify genomic rearrangements and transposon activity in the period directly after a strain receives a foreign chromosome.

226 A Pangenomic assessment of a Cercospora beticola global population *Nathan Wyatt*[1], Rebecca Spanner[1,3], Viviana Rivera-Varas[2], Gary Secor[2], Melvin Bolton[1] 1) Sugarbeet and Potato Research Unit, United States Department of Agriculture, Fargo, ND 58102, United States.; 2) Department of Plant Pathology, North Dakota State University, Fargo, ND 58102, United States.; 3) Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile; ANID—Millennium Science Initiative—Millennium Institute for Integrative Biology (iBIO), Santiago, Chile.

Cercospora beticola, causal agent of Cercospora leaf spot (CLS), is the most economically damaging pathogen of sugar beet (*Beta vulgaris* subsp. *vulgaris)*. Population genetic analysis have shown *C. beticola* populations to have high levels of genetic diversity and

gene flow between local and geographically distant populations. Relationships among pathogen populations can reveal important information relevant to disease epidemiology including sources of origin, population dynamics, and patterns of dispersal. This information is crucial for predicting the capacity of particularly virulent strains to disperse to geographically distant locations. To gain an understanding of *C. beticola* population dynamics on a global scale, a large population of *C. beticola* isolates from geographically distinct regions was subject to whole genome sequencing. Using this global data set, we conducted a pangenomic analysis to provide novel insights into the pathogen's evolution, genome dynamics, and virulence genes relevant to crop disease outbreaks. Core and accessory genomic components were assessed as well as population-specific presence/absence variation to provide a foundation for characterizing the adaptive landscape of the genome of *C. beticola*. Further, a genome wide association study (GWAS) approach was employed with this population to identify genomic loci associated with adaptation to CLS management practices. Preliminary results of a GWAS identified four strong marker trait associations in the *C. beticola* genome corresponding to virulence on an economically important sugarbeet cultivar. These results set the foundation for larger comparative population genomic analysis assessing origin, patterns of dispersion, and shifts in the global population of *C. beticola*.

227 Complete Genome Sequences and Genome-Wide Characterization of *Trichoderma* Biocontrol Agents Provide New Insights into their Evolution and Variation in Genome Organization, Sexual Development and Fungal-Plant Interactions Wan-Chen Li[1], Ting-Chan Lin[1], Chia-Ling Chen[1], Hou-Cheng Liu[1], Hisn-Nan Lin[1], Ju-Lan Chao[1], Cheng-Hsilin Hsieh[1], Hui-Fang Ni[2], Ruey-Shyang Chen[3], *Ting-Fang Wang*[1] 1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; 2) Department of Plant Protection, Chiayi Agricultural Experiment Station, Council of Agriculture, Chiayi, Taiwan; 3) Department of Biochemical Science and Technology, National Chiayi University, Chiayi, Taiwan.

Trichoderma spp. represent one of the most important fungal genera to mankind and in natural environments. The genus harbors prolific producers of wood-decaying enzymes, biocontrol agents against plant pathogens, plant-growth-promoting biofertilizers, as well as model organisms for studying fungal-plant-plant pathogen interactions. Pursuing highly accurate, contiguous, and chromosome-level reference genomes has become a primary goal of fungal research communities. Here, we report the chromosome-level genomic sequences and whole-genome annotation datasets of four strains used as biocontrol agents or biofertilizers (*T. virens* Gv29-8, *T. virens* FT-333, *T. asperellum* FT-101 and *T. atroviride* P1). Our results provide comprehensive categorization, correct positioning and evolutionary detail of both nuclear and mitochondrial genomes, including telomeres, AT-rich blocks, centromeres, transposons, mating-type loci, nuclear-encoded mitochondrial sequences, as well as many new secondary metabolic and carbohydrate-active enzyme gene clusters. We have also identified evolutionarily-conserved core genes contributing to plant-fungal interactions, as well as variations potentially linked to key behavioral traits such as sex, genome defense, secondary metabolism and mycoparasitism. The genomic resources we provide herein significantly extend our knowledge not only of this economically important fungal genus, but also fungal evolution and basic biology in general.

228 A global pangenome analysis of tan spot (*Pyrenophora tritici-repentis*) reveals an open genome and virulence factors nested in mobile elements Ryan Gourlie[1], Megan McDonald[2], Mohamed Hafez[1], Rodrigo Ortega-Polo[1], Kristin Low[1], Wade Abbott[1], Stephen Strelkov[3], *Reem Aboukhaddour*[1] 1) Agriculture and Agri-food Canada, Cereal Pathology, Lethbridge, Alberta, Canada; 2) University of Birmingham, School of Biosciences, Edgbaston, Birmingham, United Kingdom; 3) University of Alberta, Faculty of Agricultural, Life, and Environmental Sciences, Edmonton, Alberta, Canada.

Pyrenophora tritici-repentis (Ptr) is one of the most destructive wheat pathogens in the world; its genome is a mosaic of the presence and absence of effectors, and hence Ptr can serve as a model for examining the evolutionary process behind acquisition of virulence in necrotrophs to explain new disease emergence. In this work, we took advantage of a diverse collection of 41 Ptr pathogenic isolates from different global origins and applied the short and long read sequencing technologies to dissect the Ptr genome. Ptr exhibited an open-pangenome with 43% of genes in the core set, 57% of its predicted genes defined as accessory. A clear distinction between pathogenic and non-pathogenic genomes was observed in size, gene content, and phylogenetic relationships. Ptr genome exhibited major chromosomal rearrangements, including chromosomal fusion, translocation, and segment duplications. An intraspecies translocation of ToxA, the necrosis-inducing effector coding gene, was confirmed, as ToxA and a 143 Kb crypton, was localized on two different chromosomes in the Ptr species. Additionally, ToxB, the gene encoding the chlorosis inducing effector, was clustered as three copies on a 294 Kb transposable element in the coding isolate. ToxB and its carrying transposon were missing from the ToxB non-coding reference isolate, but the homolog toxb and the transposon were present in another non-coding isolate. The Ptr genome also exhibited a 'one-compartment' organization and but may still possess a 'two-speed genome' facilitated by copy-number variation as reported in other fungal pathosystems. This study provides the most comprehensive insights into Ptr genome and highlights the structural organization of Ptr genome as an open pangenome with 'one-compartment'.

229 Allorecognition genes drive reproductive isolation in *Podospora anserina* S. Lorena Ament-Velásquez[1], *Aaron A. Vogan*[1], Alexandra Granger-Farbos[2], Eric Bastiaans[1, 3], Ivain Martinossi-Allibert[1], Sven J. Saupe[2], Suzette de Groot[3], Martin Lascoux[4], Alfons J. M. Debets[3], Corinne Clavé[2], Hanna Johannesson[1] 1) Systematic Biology, Department of Organismal Biology, Uppsala University; Uppsala, Sweden.; 2) Institut de Biochimie et de Génétique Cellulaire, UMR 5095 CNRS, Université de Bordeaux; Bordeaux, France.; 3) Laboratory of Genetics, Wageningen University & Research; Wageningen, The Netherlands.; 4) Plant Ecology and Evolution, Department of Ecology and Genetics, Uppsala University; Uppsala, Sweden..

Allorecognition, the capacity to discriminate self from conspecific non-self, is a ubiquitous organismal feature typically governed by genes evolving under balancing selection. Using population level whole genome sequencing and laboratory mating assays, we show that in the fungus *Podospora anserina*, allorecognition loci controlling vegetative incompatibility (*het* genes), define two reproductively isolated groups through pleiotropic effects on sexual compatibility. These two groups emerge from the antagonistic interactions of the unlinked loci *het-r* (encoding a NOD-like receptor) and *het-v* (encoding a methyltransferase). Using a combination of genetic and ecological data, supported by simulations, we provide a concrete and molecularly defined example whereby the origin of reproductively isolated groups in sympatry is driven by pleiotropic genes under balancing selection.

230 An NLR-like system delimits individuals in the basidiomycete *Coprinopsis cinerea* *Ben Auxier*[1], Julia Marschall[1], Alfons Debets[1], Duur Aanen[1] 1) Wageningen University.

Fusion between hyphae has potential benefits, but to limit risks should be restricted to be within an individual. To acheive this, sustained fusion is restricted based on the identity of polymorphic allorecognition genes. The genes responsible for this non-self recognition have been unknown in Basidiomycetes. Since Basidiomycetes experience an extended dikaryotic phase, non-self recognition likely functions differently from known mechanisms of ascomycetes. We present results of genetically mapping the first known basidiomycete non-self recognition locus in the model mushroom *Coprinopsis cinerea*. Using set of ~600 F1 offspring, combined with independant backcross lines, we identify a region on chromsome 5 whose alleles are strongly associated with nonself recognition. Fine-mapping of this region combined with genomic comparisons of additional *C. cinerea* isolates provide evidence that nonself recognition is driven by ancient polymorphic alleles of an NLR-like system. The polyallelic locus we identify appears to involve a Leucine Rich Repeat, a novel finding for fungal non-self recognition. We speculate this locus may form part of a reader-writer system, allowing the mating and cohabitation of two genomes, yet retaining the identity in all parts of the life cycle. These results provide a first understanding of how Basidiomycetes regulate individuality.

231 Self- and non-self-recognition for cell fusion and heterokaryon incompatibility in the industrial filamentous fungus *Aspergillus oryzae* *Jun-ichi Maruyama*[1] 1) The University of Tokyo.

Aspergillus oryzae is the industrial filamentous fungus used in Japanese traditional food fermentation. Though sexual reproduction has not been found in *A. oryzae*, we previously identified two mating types of strains, indicating the potential for sexual reproduction. Cell fusion is the first process in sexual reproduction as well as parasexuality, both of which are important for crossbreeding.
We have developed methods employing auxotrophic complementation and BiFC techniques to investigate the cell fusion in *A. oryzae*. By affinity purification, we identified FsiA as a Pezizomycotina-specific protein interacting with orthologues of MAP kinase Fus3 and transcription factor Ste12, which are commonly involved in fungal cell fusion. FsiA participates in regulating the expression of cell fusion-related genes, revealing a complex regulation specific to filamentous fungi where cell fusion also occurs vegetatively.
In *A. oryzae*, numerous strains are selectively used for different fermentation purposes such as sake, soy sauce, and *miso*. Recently, we found a correlation between strain phylogeny and compatibility/incompatibility features. Time-course observation demonstrated that cell death occurs upon cell fusion in the incompatible strain pairing, and notably, incompatible cell fusion immediately leads to mitochondria fragmentation. In the deletion of mitochondrial fission-related genes, viable heterokaryons were observed in the incompatible strain pairing. This is the first finding that mitochondrial fission is functionally involved in the heterokaryon incompatibility in filamentous fungi, which will highlight a potential strategy to overcome the incompatibility barrier for industrial crossbreeding.
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232 Functional Amyloids Are Widespread in Fungal Biofilm Adhesins *Peter Lipke*[1], Melissa Garcia-Sherman[1], Yves Dufrene[3], Stephen Klotz[2] 1) CUNY Brooklyn College; 2) U. Arizona, Tucson, AZ; 3) U. catholique de Louvain, Louvain-la-Neuve, BE.

Amyloid structures assemble through a periodic repeating type of bonding called “cross-β,” in which identical sequences in many protein molecules form β-sheets that interdigitate through side chain interactions. Cross-β bonding requires identical sequences on many molecules; therefore, the bonds form only when threshold concentrations of identical sequences are present. Cross-β bonds cluster adhesins on fungal cell surfaces into patches with extremely high ligand-binding avidity. The clusters on individual cells also interact *in trans* to form cell-cell adhesions. Therefore, compounds that inhibit amyloid formation also inhibit fungal adhesion, aggregation, and biofilm formation. We present a model for how amyloid-like cross-β bonds form *in trans* between adhering cells.
These cell-to-cell bonds are highly stable and are species- or strain-specific. A wide variety of fungal adhesins have evolved to form such cross-β bonds, which form in biofilms and stabilize them against flow. This property allows biofilms to include members with identical adhesins, and to marginalize heterologous strains and species (conforming to Dawkins’ “greenbeard” theory of altruism). Thus, amyloid-like bonds are widespread feature of abscesses in pathological mycoses and in environmental biofilms. The cross-β bonds are bound by the PRR Serum Amyloid P component, which skews macrophages toward non-inflammatory responses. This finding helps explain the lack of inflammation in deep mycoses. On the other hand, there are now reports of association of CNS fungal infections with Alzheimer’s disease. We speculate that fungal amyloids may help seed pathological amyloids as well.

233 A chitin polysaccharide monooxygenase functions in trans with a plasma transmembrane protein to trigger allorecognition upon cell contact *Adriana Rico Ramirez*[1], Tyler Detomasi[2], Pedro Gonçalves[1,5], Michael Marletta[2,3,4], N. Louise Glass[1] 1) Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA; 2) Department of Chemistry, University of California, Berkeley, CA, USA; 3) California Institute for Quantitative Biosciences, University of California, Berkeley, CA, USA; 4) Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA; 5) Present address: Department of Cell Biology and Anatomy, College of Medicine, National Cheng Kung University, Tainan City, Taiwan.

Allorecognition is the ability of cells to differentiate self from non-self. In *Neurospora crassa*, three allorecognition systems function as checkpoints during germling and hyphal fusion by restricting cell fusion between genetically incompatible strains. These cell fusion allorecognition checkpoints regulate the capacity of cells to undergo chemotropic interactions, undergo cell wall dissolution upon contact, and the ability to form a viable heterokaryon after cell fusion. The second checkpoint acts upon cell contact to assess genetic compatibility and regulate cell wall dissolvement and consequent cell fusion. The cell wall remodeling (*cwr*) region consists of two linked genes that are under severe linkage disequilibrium, *cwr-1* (NCU01380) and *cwr-2* (NCU01382). *cwr-1* encodes a protein with a polysaccharide monooxygenase domain (PMO), homologous to chitin-active copper dependent AA11 PMO from *Aspergillus oryzae*. *cwr-2* encodes a predicted transmembrane protein with domains of unknown function. Phylogenetic analysis of a wild population of *N. crassa* showed that *cwr-1/cwr-2* alleles fall into six different haplogroups. Cell wall dissolution and fusion occur between strains that have *cwr-1* with *cwr-2* alleles from the same haplogroup, while non-allelic interactions between *cwr-1* of one haplogroup and *cwr-2* of a different haplogroup causes cell fusion arrest. We expressed the PMO domains of the different haplogroups of *cwr-1*, as well the

complete predicted CWR-1 protein in a heterologous system and confirmed that all six haplogroups CWR-1 PMOs oxidatively degraded both the α and β alloforms of chitin. In addition, we found that the CWR-1 PMO domain is sufficient to cause cell fusion arrest and we predict that this domain confers specificity by interacting in trans with CWR-2. However, mutations in the CWR-1 histidine brace, which is essential for PMO catalytic activity, did not abolish the cell fusion checkpoint between incompatible strains. *In vitro* analysis showed that these amino acids are critical for catalytic activity, indicating that CWR-1 PMO catalytic activity itself is not the mediating factor in allorecognition. PMO domain modeling from the six haplogroups showed regions with high levels of structural variability between the different haplogroups. By constructing CWR-1 chimeras, we identified the V86-D202 region as important for allorecognition, suggesting that this CWR-1 region confers the checkpoint specificity when interacting in trans with CWR-2.

234 Interspecies interactions of *Neurospora crassa* and *Botrytis cinerea* are mediated by a conserved cell-cell communication mechanism Hamzeh Hammadeh[1], Antonio Serrano[1], Natascha Stomberg[1], Ulrike Brandt[1], *Andre Fleissner*[1] 1) Technische Universitaet Braunschweig.

Cell fusion is essential for the development of most eukaryotic organisms, its molecular basis is, however, only poorly understood. An established model organism to study cell-cell-fusion is *Neurospora crassa*. Germinating spores of this fungus grow towards each other and fuse to form a supracellular network. This type of cell-cell fusion is common in many other filamentous ascomycete fungi. Fusion germlings of *N. crassa* employ an unusual signaling mechanism, in which the two fusion partners coordinately alternate between signal sending and signal receiving, thereby establishing a kind of cellular dialog. This process involves the alternating recruitment of the MAP kinase MAK-2 and the SO protein to the plasma membrane. To test if this mechanism is conserved in other fungi, we characterized the roles of the MAK-2 and SO homologs in the grey mold *Botrytis cinerea*. Comparable to *N. crassa*, both proteins are required for germling interactions. In addition, we observed an identical alternating membrane recruitment of the two proteins in interacting cell tips, suggesting that the "cell dialog" signaling mechanism is conserved. When *N. crassa* and *B. cinerea* spores are mixed, interactions between the two species are frequently observed, which result in mutual interspecies attraction and cell-cell contact. However, interspecies fusion was never observed. These findings suggest that the so far unknown signal and receptor that mediate cell-cell communication are also conserved, and that so far uncharacterized downstream mechanisms have evolved, that prevent interspecies fusion after cell-cell contact. In addition, we found that the presence of *N. crassa* can reprogram developmental decisions in *B. cinerea*. In the grey mold, cell fusion and pathogenic growth appear to be mutually exclusive. When confronted with *N. crassa*, however, *B. cinerea* also undergoes fusion under growth conditions, which usually trigger infectious growth. We hypothesize that the pathogenic development may be suppressed in the presence of the so far unknown fusion signals.

235 Competing for cheating escalates the deleterious effects of reproductive parasitism Alex Grum-Grzhimaylo[1, 2], Alger Jorritsma[1], Eric Bastiaans[1], Alfons Debets[1], *Duur Aanen*[1] 1) Wageningen University; 2) Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands.

Using experimental evolution in the fungus *Neurospora crassa* we earlier demonstrated that free fusion of mycelia favours cheater lineages (Bastiaans et al., 2016). Subsequently we showed that cheating is due to loss-of-function mutations in fusion genes, most often the *so* gene (Grum-Grzhimaylo et al., 2021). Δ*so* mutants are unable to initiate fusion but – at low frequency – retain access to wild-type mycelia that fuse with them. Δ*so* mutants cheat during asexual spore formation by taking a larger share of the spores produced by the heterokaryon, but negatively impacting its total spore production. Here, we show that Δ*so*-cheater mutants in parallel evolution lines convergently acquired an additional loss-of-function mutation in the conidiation gene *acon*-2 and replaced the Δ*so* mutant, implying that the Δ*so* Δ*acon*-2 double mutant has a selective benefit over the Δ*so* mutant. We show that there is no heterokaryon formation between Δ*so* Δ*acon*-2 and Δ*so* mutants, implying that the competitive benefit of Δ*so* Δ*acon*-2 does not depend on fusion. Instead, we show that Δ*so* Δ*acon*-2 has a higher mycelial growth rate than the Δ*so* mutant. The replacement of the Δ*so* mutant by the double mutant results in a polymorphism between Δ*so* Δ*acon*-2 and wild type. Δ*so* Δ*acon*-2 obtains a competitive benefit against the wild type similarly as the Δ*so* cheater: at low frequency the wild type fuses with the Δ*so* Δ*acon*-2 mutant, which subsequently has a benefit during conidiation. As the mixture between the Δ*so* Δ*acon*-2 mutant and wild type has even more crippled sporulation than the mixture of wild type and Δ*so* mutant, our results show that competing for cheating can escalate the deleterious effects of reproductive parasitism on total spore production.

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236 Fungal Chemical Warfare: How Secondary Metabolites Influence Relationships in Maize Associated Fungi *Tim Satterlee*[1], Trevor Mitchell[1], Jaci Hawkins[1], Anthony Glenn[1], Scott Gold[1] 1) USDA/ARS.

Abstract: Contamination of maize by mycotoxins is a global problem affecting food safety and security worldwide. Exposure to mycotoxins can lead to a variety of health problems for both humans and animals. Additionally, there is a large economic cost associated with mycotoxin contamination including reduced product market value and lower animal performance. Two mycotoxins commonly contaminating maize are aflatoxin and fumonisin that are produced by the plant pathogens *Aspergillus flavus* and *Fusarium verticillioides,* respectively. Multiple studies have found these pathogens together in field colonized maize but have not examined their direct interaction. Another maize-associated fungus that comes into contact with these mycotoxigenic fungi is the endophyte *Sarocladium zeae.* Pyrrocidine, produced by *S. zeae,* was recently shown to inhibit fumonisin production in *F. verticillioides.* In this study, we evaluated pairwise interactions between *A. flavus, F. verticillioides* and *S. zeae.* Our results indicated that when grown in proximity, *F. verticillioides* can inhibit the growth of *A. flavus,* and that fumonisin is the primary cause of this growth inhibition. While *F. verticilliodes* inhibition was not seen with aflatoxin, production of both fumonisin and aflatoxin was suppressed in the presence of the antagonist's primary mycotoxin. The responses also varied with *A. flavus* demonstrating a localized response to fumonisin where a more general response was seen

with aflatoxin treated *F. verticillioides.* While pyrrocidine had no effect on aflatoxin production, *S. zeae* produced other unidentified compound(s) that inhibit the growth of both *A. flavus* and *F. verticillioides.* This compound(s), unlike pyrrocidine, inhibits aflatoxin production in *A. flavus.* This work gives insights into the ecological role of fungal secondary metabolites in the interspecies battle for resource acquisition.

237 Laccase expression in the dung fungus *Coprinopsis cinerea* with 17 natural laccase genes Chelsea Cumagun[1], Zemin Fang[2], Shanta Subba[1], Michael Unger[1], *Ursula Kües*[1] 1) Molecular Wood Biotechnology and Technical Mycology, University of Goettingen, Goettingen, Germany; 2) School of Life Sciences, Anhui University, Anhui, China.

Laccases are phenoloxidases that can oxidize phenolic and aromatic compounds and occur in bacteria, fungi, insects, and plants. Among fungi, wood-rotting and litter-decaying Basidiomycetes are considered to be main producers of laccase in nature. Under different environmental conditions, such fungi secrete various forms of this enzyme, being either laccase isoforms encoded by the same gene, isoenzymes encoded by different laccase genes, or allozymes encoded by different alleles of a gene. The ink-cap mushroom *Coprinopsis cinerea* for example has 17 different laccase genes divided into 2 subfamilies. Different monokaryotic strains of the fungus can have inactivated copies of some of these genes. Most often, laccase gene *lcc15* is affected from gene inactivations, but *lcc4* or *lcc7* may also be inactivated. Functionally, the different laccases in the species are only at the beginning to be understood. Laccase genes in *C. cinerea* are differentially expressed during growth on distinct media and at different temperature regimes, during fungal differentiation and as defense in confrontation with other microbes. Monokaryotic strains usually express Lcc1 and Lcc5 as main laccases, under stress at lower temperature of 25-28 °C much higher than at 37 °C as the best growth temperature. Lcc9 is active at neutral pH and can also be expressed in cultures in traces while full expression is encountered as response in presence of competitors. By special features such as unusual pH preferences, laccases of *C. cinerea* are of interest for biotechnological applications. This requires good production rates of properly glycosylated enzymes. Enzymes can be overexpressed in *C. cinerea* upon cloning their genes behind highly active promoters, transformation of the constructs into suitable monokaryotic strains and cultivation of transformants under favorable environmental conditions, with yields up to 30 U/ml culture supernatant depending on the gene used for cloning. Crossing of transformants can further enhance laccase yields with dikaryons expressing a single enzyme or mixtures of laccases when transformants of different laccase genes were mated.

238 Production of Organic Acids in Engineered Aspergillus *Jon Magnuson*[1], Scott Baker[1], Aivett Bilbao[1], Kristin Burnum-Johnson[1], Joseph Cottam[1], Ziyu Dai[1], Shuang Deng[1], Yuqian Gao[1], Joonhoon Kim[1], Young-Mo Kim[1], Nathalie Munoz-Munoz[1], Kyle Pomraning[1], Ana Robles[1], Errol (Robby) Robinson[1], Bobbie-Jo Webb-Robertson[1], Jeremy Zucker[1] 1) Pacific Northwest National Laboratory.

Our team employs *Aspergillus niger* and *A. pseudoterreus* species for the production of various native and non-native organic acids due to their high productivity potential and tolerance to very low pH. Within the multi-institution Agile BioFoundry consortium, we have implemented the Design Build Test Learn (DBTL) approach to develop and improve strains for the production of various biofuels and bioproducts (chemicals). *Aspergillus niger* is our platform for the biosynthesis of the monomer 3-hydroxypropionic acid (3HP) utilizing a three gene heterologous pathway that routes production through beta-alanine as an intermediate. Through a few DBTL cycles, we have generated a large number of strains that produce up to 40 g/L of 3HP. We have analyzed a selection of these strains via multi-omics (Test) and utilized bioinformatics, modeling and advanced computational techniques (Learn) to identify additional gene targets to improve 3HP production. The overall strategy, results and lessons learned through this DBTL approach will be discussed.

239 MY-CO SPACE: An artistic-scientific vision on how to build with fungi *Vera Meyer*[1], Bertram Schmidt[1], Carsten Pohl[1], Christian Schmidts[2], Sven Pfeiffer[3] 1) Technische Universität Berlin, Germany; 2) University of the Arts Berlin, Germany; 3) Bochum University of Applied Sciences, Germany.

MY-CO SPACE is a collaborative work of the interdisciplinary ArtSci collective MY-CO-X, that enables an artistic-scientific discussion about a future social significance of fungi for the creation of places and spaces. MY-CO SPACE is a wooden fungal sculpture that makes it possible to experience living in a space capsule as well as in a fungal fruiting body. The wooden construction with fungal panels was built from biological materials and is biodegradable. The living space of approx. 20 sqm, can be divided by the guests themselves into sleeping, reading and working areas. It is not a completely enclosed space but a retreat and study space that lives from and deals with the contact with the outside world. The habitable sculpture is reminiscent of a space station and transports the work of Galina Balashova (born 1931), the architect of the Soviet space programme, into the 21st century. Galina Balashova was responsible for the interior design of the manned spacecraft Soyuz and the Mir space station. Her central design question in the 1960s-1980s was: “How can physical-technical structures and essential living functions be integrated in the smallest space in such a way that people can live and work under conditions of weightlessness and extreme physical stress?”
MY-CO SPACE as a sculptural habitat translates this question to today’s challenges: „How can biological-technical structures and essential living functions be integrated in the smallest possible space in such a way that people can still live and work light-hearted under conditions of limited resources?“ This architectural artwork thus strives for a different point of view and a process of interaction in which humans are involved in a conscious as well as unconscious conversation with their environment, a point of view that tacitly implies a flattening of hierarchies between the different agents and authors - human as well as non-human. MY-CO SPACE is therefore a built reflection on a cooperation with biological systems that store, transform and recycle organic matter and energy, and an exploration of fungi as a future lightweight building material resistant to fire, shock and water, and whose modification through biotechnology is possible. It is the urgency of the planetary situation and the issues we now face that require a holistic approach and close collaboration between art and science.

240 Use of a cell-free expression (CFE) to fast characterize fungal enzymes in the wood decomposer *Rhodonia placenta* *Jesus Castano Uruena*[1], Joshua Goering[1], Irina Novikova[2], James E. Evans[2], Jiwei Zhang[1] 1) Department of Bioproducts and Biosystems Engineering, University of Minnesota, Twin Cities, MN; 2) Pacific Northwest National Laboratory, Richland, WA.

Brown rot fungi are efficient wood degraders that rely on ROS (Reactive Oxygen Species; e.g., ·OH) generated by the Fenton reaction to break down the lignin barrier. This system adapted the mechanisms used by white rotters for lignocellulose hydrolysis, but it may

have also developed unique enzymes to tolerate ROS. Using crude enzymes, our previous work indicated that glycosyl hydrolases and pectinases are tolerant of ROS in the brown-rot fungus *Rhodonia placenta*, relative to white-rot and soft-rot fungi. However, this hasn't been validated using purified enzymes, and these brown rot enzymes with promising application potential haven't been fully characterized. A high-throughput method to produce high quality/quantity proteins for enzyme characterization is essential for this research. In our work, we targeted 14 key glycosyl hydrolases and oxidoreductases that are important for early wood depolymerization in *R. placenta*, and used a wheat germ-based CFE system to synthesize these enzymes. Using predicted coding sequences of *R. placenta* from the public JGI-MycoCosm genomic annotation database, we successfully synthesized all of the 14 enzymes. Four of them (~30% success rate) were characterized with the expected enzyme activities, namely an α-L-arabinofuranosidase, a benzoquinone reductase (BQR), a ferric reductase, and a heme-thiolate peroxidase (HTP). Further characterization of the optimal pH (4-6) and temperature (35°C-45°C) indicated that they were typical fungal enzymes. Among them, the α-L-arabinofuranosidase was remarkably stable at a broad range of pH values (3-8) and showed high stability to temperature (60°C), whereas the other enzymes were relatively sensitive. This α-L-arabinofuranosidase also showed clear resistance to potential inhibitors such as EDTA, NaN_3, or metal ions. More importantly, it was remarkably tolerant of ROS generated by the Fenton reaction compared to the other enzymes that were highly damaged by the oxidative treatment. These results suggest that brown-rot enzymes like α-L-arabinofuranosidases may withstand the pressure of a harsh environment characterized by the presence of ROS in fungi, and this implies a potential synergistic effect between oxidative and hydrolytic degradation during brown-rot. The CFE method and distinctive features revealed by this work certainly render brown-rot enzymes promising tools for new biotechnological applications.

241 Title to come *Debbie Yaver*[1] 1) Nature›s Fynd.

242 Understanding DNA Uptake by Anaerobic Fungi *Tejas Navaratna*[1], Jessy Gonzalez[1], Michelle O'Malley[1] 1) UC Santa Barbara.

Anaerobic fungi, found in the rumen of herbivores, are powerful degraders of lignocellulosic biomass and are equipped with a broad suite of enzymes to accomplish this goal. These organisms are found in diverse multi-kingdom communities and have been shown to produce a range of biotechnologically valuable products, including short- and medium-chain fatty acids and bioactive small molecules. However, a major bottleneck remains in the translation of anaerobic fungi to the industrial setting as there is a lack of robust genetic engineering strategies to generate protein diversity, introduce heterologous genes, and create specific knockouts for functional enhancement.

A necessary first step for genetic manipulation is the introduction of foreign DNA and/or nucleoprotein complexes. The life cycle of anaerobic fungi involves production of motile, unicellular zoospores that due to their thinner cell wall, are potentially more amenable to genetic manipulation than mature life stages. We show that zoospores can be analyzed and sorted by fluorescence-activated cell sorting (FACS) to gain insights into the size-dependence of DNA entry. We constructed a library of fluorescent dsDNA probes and interrogated uptake by natural competence and electroporation across a range of parameters. Combined with insights into survival post-transformation, these data show robust zoospore entry across the length range. Furthermore, we tested cell entry and nuclear localization of a GFP-Cas9 ribonucleoprotein complex by FACS and fluorescence microscopy, and all together, these findings inform a systematic approach for transformation of anaerobic fungi.

243 Growing the future of biomaterials: learning from fungal genetics to tune Fine Mycelium™ *Rachel Linzer*[1], Phil Ross[1], Leslie Decker[1], Sean Sierra-Patev[2], Olivia Bewsey[1], Lindsey Filowitz[1], Janne Kerovuo[1] 1) MycoWorks, Emeryville, CA; 2) Clark University, Worcester, MA.

Fungal mycelium-based biomaterials is an emerging field with promising opportunities for innovative material properties and sustainability. Mycoworks, a biomaterials company based in Emeryville, CA, is currently producing Fine Mycelium™, a biomaterial alternative for fashion and other applications that currently use animal leathers or synthetic, plastic-based analogues. Cultivating fungi for food, medicine and as biotechnological "cellular factories" to yield desired molecules has a long history at commercial scale, and a host of well-established methods exist for these applications. However, there are fewer settled methods available for producing fungal biomaterials, creating a burgeoning space, open for innovation.

At MycoWorks, we have launched a nascent research program using genetics to drive the development of novel ways to tune aesthetic and mechanical properties of our biomaterials and improve our growth process. Early genomic efforts have focused on insights from the full-genome sequence of our current working fungal strain, a white-rot wood-decay basidiomycete in the *Ganoderma lucidum sensu lato* species complex. Initial analyses have identified multiple putative hydrophobins, proteins that play diverse roles in the fungal lifecycle with potential to influence the hydrophobicity of surfaces. With the identification of these and other candidate gene regions, we can explore genetic engineering efforts aimed at more targeted process interventions. Additionally, we are developing a high-throughput screening platform compatible with future "-omics" approaches to test a collection of fungal strain candidates for growth factors conducive to our process and gene targets to "tune" Fine Mycelium™ properties. Informed by fungal ecology, we are continuing to collect candidates and tap into the diversity of fungal genetics to grow the future of biomaterials.

244 Mycotecture off planet: fungi as a building material on the Moon and MarsMycotecture off planet: fungi as a building material on the Moon and Mars *Lynn Rothschild*[1,9], Christopher Maurer[2], Monika Lipińska[3], Debbie Senesky[4], Ivan Paulino-Lima[5], Jessica Snyder[5], Martyn Dade-Robertson[3], Anil Wipat[3], Maikel Rheinstädter[6], Eneko Axpe[4], Christopher Workman[7], David Cadogan[8], James Head[9] 1) NASA Ames Research Center, Moffett Field, CA ; 2) redhouse studio, Cleveland, OH; 3) Newcastle University, Newcastle upon Tyne, UK; 4) Stanford University, Stanford, CA; 5) Blue Marble Space Institute of Science at NASA Ames Research Center, Moffett Field, CA; 6) McMaster University, Hamilton, ON, Canada; 7) DTU, Kongens Lyngy, DENMARK; 8) Moonprint Solutions, Dover DE; 9) Brown University, Providence, RI.

A turtle carries its own habitat. While reliable, it costs energy and is not easily adapted to the environment. NASA makes the same

trade-off when it transports habitats and other structures needed to lunar and planetary surfaces. In contrast, a bird builds its home at destination using sustainable manufacturing and *in situ* materials. In a NASA Innovative Advanced Concepts (NIAC) Phase 1 study, we introduced the use of structures grown by fungal mycelial biocomposites at destination.

Mycelial materials are known thermal insulators, fire resistant, and unlike plastics and glues, do not outgas. They are more flexible and ductile than regolith alone. The density and material properties are tuned during production. The material could be used dry, wet, frozen with water or as part of a self-produced biocomposite which would allow such enhancements as radiation protection and a vapor seal. Even better, it is self-replicating so the habitat could be extended at a future date, and thus also be self-repairing. Some form of this material could be used for a habitat at destination, furniture, storage, additional buildings, and the shell of multiple rovers. As a stand-alone material or in conjunction with agglutinated or sintered regolith, a mycotectural building envelope could significantly reduce the energy required for building because in the presence of feedstock and water, it would grow itself. After the arrival of humans, additional structures could be grown with feedstock of mission-produced organic waste streams. When protected, the mycomaterials could have a long life, but at the end of its life cycle the material could be become fertilizer for mission farming or production of new mycomaterials. Radiation has been considered a “show stopper” for human missions, but some black fungi not only survive, but may thrive in space radiation. We could supplement our mycomaterials by engineering the mycelia to bind materials such as metals as we did in Phase 1.

While our habitat shell is designed to be inert, we can envision its extension into a living state participating actively in waste recycling, oxygen production, and detoxification similar to a living roof. Other benefits of mycotecture for NASA include production of furniture and fabrics on site, to water purification. Terrestrial spin-offs include quickly deployable, warm safe shelters to house refugees. We are currently exploring the use of mycotecture to increase sustainability in the restaurant, Azurmundi.

245W Casein kinase 1 and disordered clock proteins form functionally equivalent phospho-based circadian modules in fungi and mammals Daniela Marzoll[1], Fidel Serrano[1], Anton Shostak[1], Carolin Schunke[1], *Axel Diernfellner*[1], Michael Brunner[1] 1) Heidelberg University Biochemistry Center, Heidelberg, Germany.

Circadian clocks adjust physiology and metabolism to the 24-h day-night cycle. Eukaryotic circadian clocks are based on transcriptional-translational feedback loops. Core components, such as FRQ in *Neurospora crassa* and PERs in animals are not conserved. We show that CK1 is sufficient to promote hyperphosphorylation of FRQ and mPER2 on a circadian timescale. CK1 targets a large number of low affinity sites. The slow phosphorylation relies on site-specific recruitment of CK1 and access of intrinsically disordered segments of FRQ or mPER2 to the bound kinase, and on CK1 autoinhibition. We propose that the clock proteins FRQ or PERs and CK1 form functionally equivalent phospho-based timing modules in the core of the circadian clocks of fungi and animals.

246T Conformational Changes in the Circadian Negative Arm Correlate with Dynamic Interactomes Involved in Diverse Biological Processes *Jacqueline Pelham*[1], Alexander Mosier[1], Samuel Altshuler[1], Christopher Kirchoff[1], William Fall[1], Lisa Baik[2], Joanna Chiu[2], Jennifer Hurley [1,3] 1) Department of Biological Sciences, Rensselaer Polytechnic Institute, Troy, NY; 2) Department of Entomology and Nematology, University of California Davis, CA; 3) Center for Biotechnology and Interdisciplinary Sciences, Rensselaer Polytechnic Institute, Troy, NY.

The widely conserved circadian clock employs a transcriptional/translational negative feedback loop (TTFL) to anticipate environmental changes due to the Earth’s diurnal cycle. While an astounding amount of physiology is coordinated by this feedback loop, the intricacies of the conserved molecular oscillator are poorly understood. In *Neurospora crassa* the source of circadian output has been canonically accepted as transcriptional activation by the positive arm. However, over 40% of oscillating *Neurospora* proteins do not have rhythmic mRNA, establishing circadian post-transcriptional regulation through unknown sources, a phenomena which is conserved in higher eukaryotes. To this end we are investigating the *Neurospora*negative arm protein FREQUENCY (FRQ) as a potential source of this regulation. Given the pervasive conservation of the intrinsically disordered protein (IDP) nature of negative-arm clock proteins, we hypothesized that post-transcriptional regulation may stem from conformational shifts in negative-arm proteins that time vacillations in the constituents of negative-arm macromolecular complexes to time cellular physiology. Our investigation of the negative arm clock protein FRQ demonstrated temporal conformational fluidity correlated with daily changes in physiologically diverse macromolecular complex components. FRQ interactors that are classified as IDPs were more likely to interact with FRQ at their nadir, suggesting FRQ may tune post-transcriptional regulation via the control of interactor stability. An analogous investigation of the macromolecular complexes centered around *Drosophila melanogaster* PERIOD (dPER) and human PERIOD (hPER2) found a similar number and physiological diversity of interacting partners in higher eukaryotes. Short linear motifs (SLiMs) associated with the interactors localized to disordered and phosphorylated regions on the PERs and FRQ, with disordered interactors oscillating in the macromolecular complexes over circadian time. This oscillation correlated with oscillations in post-transcriptionally regulated proteins, suggesting the negative arm may tune cellular physiology and proteostasis post-transcriptionally via oscillations in the circadian negative-arm macromolecular protein complexes.

247F Quantitative phosphoproteomic analysis of appressorium development by the rice blast fungus *Magnaporthe oryzae* Neftaly Cruz-Mireles[1], Miriam Osés-Ruiz[1,2], Paul Derbyshire[1], Nicholas J. Talbot[1], *Frank L.H. Menke*[1] 1) The Sainsbury laboratory; 2) IMAB, Public University of Navarre.

Rice blast is one of the most devastating plant diseases and a major risk to global food security. To infect rice, the blast fungus *Magnaporthe oryzae* develops a specialised dome-shaped infection structure called an appressorium. The appressorium generates enormous turgor of up to 8.0 MPa which is applied as physical force to rupture the host plant cuticle. Previous work has shown that the Pmk1 mitogen activated protein kinase is essential for development of appressoria and invasive growth *in planta*. Despite being discovered more than two decades ago, very little is known about how Pmk1 precisely controls infection-related development. We used discovery phosphoproteomics to identify a set of nearly 2000 phospho-proteins in germinating conidia during appressorium development. We generated a large spectral library of over 6800 phosphorylation sites which provides a valuable resource for understanding the regulation of appressorium development. From our spectral library we selected 440 phospho-peptides for analysis by parallel

reaction monitoring (PRM) to accurately quantify changes in phosphorylation, comparing *Dpmk1* mutants with the wild type Guy11. During a time series between 0-6 h post germination, we identified 30 proteins as potential direct Pmk1 targets. This subset includes previously reported members of the Pmk1 pathway such as the transcription factors Hox7 and Mst12 that are required for appressorium development and function, respectively, as well as novel proteins required for plant infection. To verify whether these are direct Pmk1 targets, we used an analogue-sensitive mutant of Pmk1 (*pmk1*^{AS}) to selectively inhibit Pmk1-dependent phosphorylation from 1h after conidial germination. Phosphorylation of MAPK motifs in several targets were nearly completely abolished in the presence of the ATP-analogue NA-PP1 at 2h, 3h and 4h post germination, confirming these sites as Pmk1-dependent. Our work lays the basis for a step-change in understanding of the regulation of appressorium development and shows the power of quantitative phospho-proteomics to identify new regulators of appressorium development in an important plant fungal pathogen.

248W Anaerobic fungi are an untapped source of biotechnologically relevant membrane proteins *Susanna Seppala*[1], Igor Podolsky[1], Justin Yoo[1], Elizabeth Schauer[1], Taylor Gierke[1], Jennifer Brown[1], Kevin Solomon[1], Michelle O'Malley[1] 1) University of California, Santa Barbara.

Sustainable biotechnology seeks to employ microbial cell factories, such as *Saccharomyces cerevisiae*, to produce a variety of value-added molecules from a wide range of affordable, abundant, and renewable feedstock. To do so, the microbial cell factory organism has to possess membrane proteins that recognize and take up the substrate in question, that prevent the escape of any intermediates, and that efficiently clear metabolites that could inhibit production. Using transcriptomics and genomics, we find that non-model early diverging anaerobic fungi from the phylum Neocallimastigomycota, are an untapped source of potentially useful membrane proteins. Anaerobic fungi are found in the intestinal tract of herbivorous animals, and using transcriptomic and genomic sequencing, we found that they possess a variety of genes encoding membrane-embedded proteins that likely allow the slow-growing fungus to thrive in the competitive environment of the animal gut. These membrane proteins include unique receptors and transporters that can be leveraged for a range of biotechnological applications. Specifically, we found that anaerobic fungi possess genes encoding sugar transporters from the Sugars Will Eventually be Exported Transporter (SWEET) family. SWEETs are ubiquitous in plants but have rarely been observed in microorganisms, and are overlooked as a tool for biotechnology. Furthermore, we found that anaerobic fungi possess genes encoding fluoride transporters that can be leveraged as tools for selection and biocontainment of model microbial cell factories. Lastly, we recently discovered that anaerobic fungi express genes encoding 'prokaryotic' small multidrug resistance transporters that can potentially be used to mitigate toxic effects related to the production of hydrophobic bioproducts in yeast cell factories. In conclusion, anaerobic fungi are an untapped source of membrane-embedded proteins that enable the further development of efficient cell factories for the conversion of lignocellulosic biomass into value-added molecules.

249T Pyrolyzed substrates induce aromatic compound metabolism in the post-fire fungus, *Pyronema domesticum* *Monika S. Fischer*[1], Frances Grace Stark[1], Timothy D. Berry[2], Nayela Zeba[2], Thea Whitman[2], Matthew F. Traxler[1] 1) University of California, Berkeley, CA; 2) University of Wisconsin, Madison, WI.

Wildfires are a fundamental disturbance in many ecosystems, and their frequency and severity are increasing in many regions of the world. Fire affects soil surface carbon stocks by volatilizing much of the available carbon into CO_2 and transforming remaining surface carbon into complex, polyaromatic, pyrolyzed organic matter (PyOM). Below the soil surface, fires generate a substantial necromass at depths where the heat is sufficient to kill soil organisms but does not catalyze the formation of PyOM. *Pyronema* species strongly dominate soil fungal communities, and have been widely observed to fruit on the soil surface within weeks to months after fire. Yet the carbon pool (i.e., necromass or PyOM) that fuels their rise in abundance is unknown. We used a *Pyronema domesticum* isolate from the catastrophic 2013 Rim Fire (CA, United States) to ask whether *P. domesticum* is capable of metabolizing PyOM. *P. domesticum* grew readily on agar media where the sole carbon source was PyOM (specifically, pine wood PyOM produced at 750°C). Using RNAseq, we investigated the response of *P. domesticum* to PyOM and observed a comprehensive induction of genes involved in the metabolism and mineralization of aromatic compounds, typical of those found in PyOM. Lastly, we used 13C-labeled 750°C PyOM to demonstrate that *P. domesticum* is capable of mineralizing PyOM to CO2. Collectively, our results indicate a robust potential for *P. domesticum* to liberate carbon from PyOM in post-fire ecosystems and return it to the bioavailable carbon pool.

250F Inhibitor targeting the Prp8 intein splicing of *Cryptococcus neoformans* Anil Tharappel[1], Zhong Li[1], Xiangmeng Wu[1], Qing-Yu Zhang[1], *Hongmin Li*[1] 1) University of Arizona, Tucson.

Drug resistance is a major concern in the treatment of cryptococcosis, caused by *Cryptococus neoformans and C. gatti*. Alternative drug targets are necessary. Splicing of inteins from proprotein precursors is crucial for activities of essential proteins hosting intein elements in many organisms including human pathogens such as *C. neogormans and C. gattii*. Since there is no intein splicing mechanism reported in human beings, targeting the intein splicing would be an attractive strategy to combat deadly diseases such as cryptococcosis in immunocompromised individuals especially with increasing concern of drug resistance. In this work, a resazurin based growth inhibition assay was employed to screen 97,000 small molecules in wild type *C. neoformans* H99 (*Cne*-WT) and its inteinless mutant strain (*Cne*-Mut). Among the hit molecules, CMN was found to be potent with 50% inhibitory concentration (IC_{50}) of 0.6 μg/ml and minimum inhibitory concentration (MIC) of 1.5 μg/ml against *Cne*-WT whereas in *Cne*-Mut, the MIC was 16-fold higher. CMN showed inhibitory activity on intracellular infection of *Cne*-WT in macrophages. The specificity of CMN towards the target was confirmed by protein-based intein splicing assay employing split nanoluciferase-intein fusion protein with an IC_{50} of 4.6 μg/ml. Also, the binding of CMN to pure recombinant intein was demonstrated by thermal shift assay (TSA). CMN is found to inhibit intein splicing *in vitro* in split GFP-Prp8 intein assay and *in vivo* in *Cne*-WT. CMN was fungi-static in nature and showed synergistic effect with known antifungal drug amphotericin B (AmB). CMN was absorbed and releases slowly in mouse when given orally and attained a T_{max} of 9.6 h and $T_{1/2}$ of 7.2 h. The fungal load in the lung was reduced by 60% when *Cne*-WT infected BALB/c mice were treated orally with CMN. Overall, CMN represents a potent antifungal with novel mechanism of action to treat *Cne* infection.

251W Characterization of *Phanerochaete chrysosporium* mutants resistant to *Bagassa guianensis* wood extractives *Delphine Noël*[1], Duy Vuong Nguyen[1], Antonio Fernández-González[1,2], Nadine Amusant[3], Mathieu Schwartz[1,4], Eric Gelhaye[1], Mélanie Mo-

rel-Rouhier[1], Rodnay Sormani[1] 1) Université de Lorraine, INRAE, IAM, F-54000 Nancy, France; 2) Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín (EEZ), Agencia Estatal CSIC, c/ Profesor Albareda, 1, 18008 Granada, Spain; 3) UA, AgroParisTech, UMR Ecofog, CIRAD, CNRS, INRAE, BF701, Kourou, France; 4) Université de Bourgogne-Franche Comté, CNRS, INRA, Centre des Sciences du Goût et de l'Alimentation, 17 rue Sully, 21065 Dijon, France.

Wood decaying fungi are ever more attractive as they possess an array of enzymes able to degrade lignocellulosic material. However, during wood degradation processes, fungi have to cope with toxic molecules released from wood and defined as "extractives". Thousands of wood extractives molecules have been discovered but very little is known concerning their putative antifungal activity. In that context, tropical wood species appear as models of choice to study wood extractives toxicity due to their strong durability and high amounts of extractives. To highlight the molecular targets of such extractives in fungi, a collection of *Phanerochaete chrysosporium* mutants was generated using UV mutagenesis and screened for resistance to *Bagassa guianensis* wood extractives. Whole genome resequencing of two mutants resistant to *B. guianensis* wood extractives highlighted one common mutated gene. After checking this sequence among the 34 mutants, it appears that this gene is mutated in 28 of them. It does not have any functional annotation but contains a putative DENN domain. Based on a phylogenetic analysis, the protein coded by that gene was identified as an orthologous protein to the human DENND6 protein that activates the RAB14 GTPase through guanine nucleotide exchange factor (GEF) activity. Interestingly, the most frequent mutation in this gene among the mutant collection is the substitution -E234K-, that concerns an amino acid located at the interface of interaction between DENND6 and RAB14. The recombinant proteins of the *Pc*DENND6, *Pc*DENND6 E234K and the two RAB GTPases proteins of *P. chrysosporium* were produced heterologously, purified and characterized for GEF and GTPase activities. To complete these biochemical data, a physiological approach based on CO_2 measurement allowed us to show a higher growth of the mutant strain compared to the WT. This supports the hypothesis that the mutant strain acquired an advantage over the WT to grow on wood, likely because of resistance to extractives.

252T Isolation of mutants *resistant to itraconazole* in the white-rot fungus *Phanerochaete chrysosporium* RP78 leads to identification of alleles in *CYP51/ ERG11* *Rodnay Sormani*[1], Antonio Fernandez-Gonzalez[1,3], Thomas Roret[1,4], Delphine Noël[1], Duy Vuong Nguyen[1], Elena Hego[1], Sophie Valière[2], Mélanie Morel-Rouhier [1], Gelhaye Eric[1] 1) Université de Lorraine, INRA, IAM, F-54000 Nancy, France; 2) US1426, GeT-PlaGe, Genotoul, Castanet-Tolosan, France; 3) Present address: Estación Experimental del Zaidín: Granada, Andalucía, Spain; 4) Present address: CNRS, LBI2M, Sorbonne Universités, Roscoff, France.

Phanerochaete chrysosporium is a model of white-rot fungus able to degrade all wood components. As we have access to the genome sequence of the strain *P. chrysosporium* RP78, a forward genetic strategy using next-generation sequencing can be a powerful tool to decipher the molecular basis of white-rot fungi physiology. As a proof of concept, we chose to analyze the resistance to itraconazole in a pool of mutants using a whole genome resequencing strategy. Itraconazole is a well-known antifungal drug belonging to the triazole family, compounds inhibiting ergosterol biosynthesis by acting on lanosterol 14-alpha-demethylase CYP51 in other fungal species. Random mutagenesis has been done and mutants with *resistant to itraconazole* phenotype have been selected and their genomic DNA has been sequenced. Mutations leading to amino acid substitutions have been identified in the *CYP51* encoding gene. Those mutations limit the inhibitory effect of itraconazole and allow *rit* mutants to have ergosterol production in presence of itraconazole. From this work, another interesting point is the possibility to produce and characterize mutants in *P. chrysosporium* RP78, opening further studies to better understand the molecular basis of the white-rot fungus living model.

253F Methionine synthase as a target for antifungal drug development *Jennifer Scott*[1], Benjamin Thornton[1], Jonathan Fowler[1], Rachael Fortune-Grant[1], Riba Thomas[1], Lydia Tabernero[1], Elaine Bignell[2], Jorge Amich[1,3] 1) Manchester Fungal Infection Group, University of Manchester, Manchester, UK; 2) MRC Centre for Medical Mycology, University of Exeter, Exeter, UK; 3) Mycology Reference Laboratory, National Centre for Microbiology, Instituto de Salud Carlos III, Majadahonda, Spain..

Mycoses pose an urgent threat to human health and are responsible for approximately 1.6 million deaths annually. Current therapeutic options for life threatening fungal infections, such as those caused by *Aspergillus fumigatus*, *Candida albicans* and *Cryptococcus neoformans*, are severely limited. Even under antifungal treatment these infections have mortality rates of ~50% and rising antifungal resistance exacerbates the need for the development of novel drugs.

Fungal pathogenic potential is strongly influenced by their metabolic versatility. Therefore, pathways required for cellular metabolism and nutrient homeostasis in host tissues are fundamental for infection and may represent exciting targets for antifungal drug discovery. As the conditions encountered throughout the course of infection, and consequently the fungal metabolic requirements, may vary significantly, it is crucial to validate targets in established infections.

We recently optimised a genetic model to allow characterisation and validation of prospective antifungal drug targets in growing *A. fumigatus* hyphae and established *in vivo* infections. We used the model to investigate methionine synthase (MetH), which has been described as essential for viability or virulence in *A. fumigatus*, *C. albicans* and *C. neoformans*. We showed that downregulation of *metH* expression triggered a complex metabolic imbalance, beyond methionine auxotrophy, which inhibited growth and thus virulence in two *in vivo* models of established infection. In addition, a structure-based virtual screening predicted differential druggability between the human and fungal enzymes, which could guide the design of novel specific inhibitors with reduced off target binding.

Following the validation of MetH as a promising antifungal target this work initiates the early stages of drug development. We have optimised the expression and purification of soluble MetH and designed a simple, cost-effective enzymatic assay suitable for high throughput screening. From our virtual screening, we have identified fragments which have a high probability to specifically bind to regions crucial for MetH's enzymatic activity.

Taken together our results support methionine synthase's potential as a druggable, specific and broad-spectrum antifungal drug target. We provide initial results that will permit hit identification using complementary approaches: high throughput screening of compound libraries and directed design of binding fragments.

254W Mutation in the ribosomal protein gene eL42 results inCycloheximide resistance in the Ophiostomatales *Brenda Wingfield*[1], Mike Wingfield[1], Duong Anh Duong[1] 1) University of Pretoria.

Resistance to the antibiotic Cycloheximide has been reported from a number of fungal taxa. In particular some yeasts are known to be highly resistant to this antibiotic. Early research showed that this resistance resulted from a transition mutation in one of the 60S ribosomal protein genes. In addition to the yeasts, most genera and species in the Ophiostomatales are highly resistant to this antibiotic, which is widely used to selectively isolate these fungi. As whole genome sequences are now available for numerous members of the Ophiostomatales, the opportunity arose to determine whether the mechanism of resistance in this order is the same as that reported for yeast genera such as *Kluyveromyces*. We examined all the available genomes for the Ophiostomatales and discovered a transition mutation in the ribosomal protein eL42 gene. This causes the substitution of the amino acid Proline to Glutamate in position 56 of the predicted protein. This is the most likely the cause of this antibiotic resistance across the Order. This change across all genera in the Ophiostomatales suggests that the mutation arose early in the evolution of these fungi.

255T Structure of the translating *Neurospora* ribosome arrested by cycloheximide Lunda Shen[1], Zhaoming Su[2], Kailu Yang[2], Cheng Wu[1], Thomas Becker[3], Deborah Bell-Pedersen[1], Junjie Zhang[1], *Matthew Sachs*[1] 1) Texas A&M University; 2) Stanford University; 3) Ludwig-Maximilians University Munich.

Ribosomes translate RNA into proteins. The protein synthesis inhibitor cycloheximide (CHX) is widely used to inhibit eukaryotic ribosomes engaged in translation elongation. However, the lack of structural data for actively translating polyribosomes stalled by CHX leaves unanswered the question of which elongation step is inhibited. We elucidated CHX's mechanism of action based on the cryo-electron microscopic structure of actively translating *Neurospora crassa* ribosomes bound with CHX at 2.7 Å resolution. The ribosome structure from this filamentous fungus contains clearly resolved ribosomal protein eL28, like higher eukaryotes but unlike budding yeast, which lacks eL28. Despite some differences in overall structures, the ribosomes from *Neurospora*, yeast, and humans all contain a highly conserved CHX-binding site. We also sequenced classic *Neurospora* CHX-resistant alleles. These mutations, including one at a residue not previously observed to affect CHX-resistance in eukaryotes, were in large subunit proteins uL15 and eL42 that are part of the CHX binding pocket. In addition to A-site tRNA, P-site tRNA, mRNA, and CHX that are associated with the translating *N. crassa* ribosome, spermidine (SPD) is also present near the CHX-binding site close to the E site on the large subunit. The tRNAs in the peptidyl transferase center are in the A/A site and P/P site. The nascent peptide is attached to the A-site tRNA and not the P-site tRNA. The structural and functional data obtained show that CHX arrests the ribosome in the classical PRE translocation state and does not interfere with A-site reactivity.

256F Sre1, a transcription factor controlling ergosterol biosynthesis, stimulates response to nickel, an important micronutrient for fungi *Amber Matha*[1], Tuyetnhu Pham[2], Xiaofeng Xie[1], Robert Maier[1], Xiaorong Lin[1,2] 1) Department of Microbiology, University of Georgia, Athens, GA; 2) Department of Plant Biology, University of Georgia, Athens, GA.

Nickel is an abundant element on Earth. While humans do not produce enzymes that require nickel as a cofactor, many microbes use nickel for various enzymatic activities. This presents a unique avenue for development of selective antimicrobial therapies. There are nine nickel-requiring proteins known in microbes, but only one, urease, is known in Kingdom Fungi. Interestingly, even in the absence of urease, nickel supplementation drastically stimulates growth of the human fungal pathogens *Cryptococcus* and *Candida* species. Thus, we hypothesize that nickel is an unrecognized micronutrient for fungi. To investigate regulation of nickel homeostasis in *Cryptococcus neoformans*, we analyzed cryptococcal transcriptome in response to nickel. We found that the ergosterol biosynthesis pathway is upregulated by nickel. Consistently, Sre1, a transcription factor that upregulates ergosterol biosynthesis pathway in response to hypoxia, is required for growth on nickel even though *sre1*Δ cells do not accumulate more nickel than the wild type. Two *sre1*Δ suppressors identified on Ni showed aneuploidy on chromosomes 5 and 11 respectively. Interestingly, *ERG25* gene is present on chromosome 11 and overexpression of this ergosterol biosynthetic enzyme known to be downstream of Sre1 indeed fully rescues *sre1*Δ's growth defect in hypoxia, nickel, and virulence in animals. We found a few genes highly upregulated by nickel are present in chromosome 5. These genes are likely related to mitochondrial function. Surprisingly, disruption of Pas2, another transcription factor that regulates metabolism rewiring critical for hypoxia adaptation independent of Sre1, rescues the growth defect of *sre1*Δ in Ni. We hypothesize that the rescue of growth on Ni in the *sre1*Δ*pas2*Δ mutant may be due to changes in metabolism and mitochondrial function. The findings from our work lead us to propose that nickel stimulates ergosterol biosynthesis pathway as well as aerobic respiration, and either pathway could potentially compensate the loss of Sre1. On Ni media, the absence of Sre1 causes ergosterol level decrease and suppression of aerobic respiration genes, which leads to growth inhibition. This work has uncovered an unrecognized role of nickel in fungal growth independent of urease and the regulatory network controlled by Sre1 that regulates nickel homeostasis. This and future work may also lead to the discovery of novel nickel-requiring enzymes in fungi.

257W Oxygen mediated cell-cell heterogeneity and antifungal drug susceptibility in *Aspergillus fumigatus* biofilms *Kaesi Morelli*[1], Caitlin Kowalski[2], Robert Cramer[1] 1) Dartmouth College, Hanover, NH; 2) University of Oregon, Eugene, OR.

Aspergillus fumigatus is a filamentous fungus commonly found in compost and soil which can cause invasive disease in immunocompromised individuals. Infections with strains whose conidia are susceptible to antifungals *in vitro* frequently fail to respond to treatment *in vivo.* This is in part due to *A. fumigatus* adopting a biofilm mode of growth *in vivo*. As *A. fumigatus* biofilms mature, steep oxygen gradients form due to increased fungal oxygen consumption. These zones of low oxygen correlate with regions of hyphae with reduced translational activity and subsequent antifungal drug resistance. However, treatment of *A. fumigatus* biofilms increases oxygen availability in the biofilm and subsequently increases antifungal susceptibility of fungal biofilms. Moreover, reoxygenation of the biofilm exogenously also increases antifungal susceptibility. However, the mechanisms underlying reactivation of antifungal drug susceptibility upon reoxygenation remain ill defined. To address this question, we utilized RNA sequencing to identify transcripts responsive to reoxygenation in *A. fumigatus* biofilms. Transcripts highly differentially expressed in reoxygenated biofilms include genes involved in active transmembrane transport, positive regulation of transcription, and nitrogen metabolism. Genes of interest that we have selected for characterization include genes with domains predicted to be involved in both heme-binding or metabolite-binding as well as phosphorelay or transcription factor domains. CRISPR mediated gene replacement of candidate genes and characterization of reoxygenation

phenotypes has revealed promising candidates critical for oxygen mediated biofilm antifungal drug susceptibility. Collectively, these data report a critical role for oxygen in the antifungal drug response and highlight the importance of defining mechanisms of cell-to-cell heterogeneity in complex filamentous fungal biofilms.

258T Manganese Transporters and Virulence in *Candida albicans* *Asia Wildeman*[1], Valeria Culotta[1], Brendan Cormack[2] 1) Johns Hopkins School of Public Health; 2) Johns Hopkins School of Medicine.

All kingdoms of life require transition metals including Zinc, Iron, Copper, and Manganese. Pathogens who inhabit mammalian hosts must attain these essential nutrients within competitive environments. The mammalian immune system restricts nutrient availability upon infection with an invading pathogen through a defense mechanism named nutritional immunity. *Candida albicans* is an opportunistic and polymorphic fungal pathogen that must access trace metals as nutrients within the restrictive environment of the human body. *C. albicans* has a gene family of four NRAMP transporters, a gene class that was originally identified in human immune cells for restricting microbial growth by withholding metal nutrients. Three out of the four *C. albicans* NRAMP transporters are uncharacterized. We have found that two members of this family, Smf12 and Smf13, are manganese (Mn) transporters that have non-redundant roles in Mn acquisition, protein glycosylation, and superoxide dismutase (SOD) activity and that both *smf12Δ* and *smf13Δ* mutants have defects in polarized growth in liquid culture and virulence in the murine model of systemic infection. This work establishes a strong linkage between the nutritional requirement of Mn and virulence in a fungal pathogen.

259F Temperature-specialized function of glycogenins in *Cryptococcus neoformans* *Liza Loza*[1], Thomas Hurtaux[1], Daphne Ko[1], Tamara Doering[1] 1) Washington University in St. Louis.

The basidiomycete yeast *Cryptococcus neoformans* is ubiquitous in the natural and urban environment. Upon inhalation of spores by a host, fungi can access the lungs but are generally cleared or sequestered in granuloma-like structures by the response of an intact immune system. In immunocompromised people, by contrast, fungi can proliferate and disseminate via the central nervous system, causing an often-fatal meningoencephalitis. Systemic cryptococcosis kills ~180,000 people globally each year, accounting for 15% of AIDS-associated mortality.

The cryptococcal cell encounters various stressors both in the environment and during its forays through the human body as an opportunistic pathogen. Carbohydrate storage molecules are a conserved eukaryotic strategy to address one such stressor: low nutrient availability. Glycogen is a glucose storage polymer that is catabolized for energy when extracellular carbon sources are scarce. It is comprised of a glycogenin protein dimer surrounded by alpha-1,4-linked glucose chains with alpha-1,6-linked branchpoints. As well as being the structural core of glycogen, glycogenin initiates glycogen synthesis by transferring a glucose monomer from UDP-glucose to itself in a unique self-glucosylation reaction.

As part of my thesis work, I have identified two glycogenins in *C. neoformans*, Glg1 and Glg2. Deletion of the gene encoding Glg1 results in a dramatic virulence defect in a mouse model of cryptococcal infection. The virulence profile of the *GLG2* deletion mutant is similar to that of wild type, but it has a striking survival defect inside phagocytes *in vitro*. Both mutants exhibit a modest glycogen synthesis defect; a greater defect is seen in the double mutant, supporting the idea that these proteins share a function. The glycogen-deficient phenotypes of all three strains occur at 37°C but not at 30°C, suggesting a temperature-dependent function that is relevant to pathogenesis. Preliminary studies with catalytic and acceptor mutant strains indicate that Glg1 is the preferred acceptor for both glycogenins.

Experiments to explore the possibility of an interaction between Glg1 and Glg2 and investigate the temperature-dependent phenotypes of my strains are ongoing. In addition to implicating glycogenin activity in cryptococcal virulence, this work has revealed intriguing differences between glycogen synthesis in *C. neoformans* and the well-studied pathway in *S. cerevisiae*.

260W Capsule glycosylation in *Cryptococcus neoformans* *Thomas Hurtaux*[1], Liza Loza[1], Tamara Doering[1] 1) Washington University in St. Louis, St. Louis, MO.

Cryptococcus neoformans is an environmentally ubiquitous fungus that is also an opportunistic pathogen. As such, it causes severe meningoencephalitis in immunocompromised patients, contributing to roughly 200,000 deaths per year (including 15% of all AIDS-related deaths). This emphasizes the need for a better understanding of *C. neoformans* pathogenesis and its interaction with the host cells. In that regard, one unique characteristic of *C. neoformans* is the presence of a capsule, an outer layer of polysaccharides surrounding the cell. These polysaccharides, termed glucuronoxylomannan (GXM) and glucuronoxylomannogalactan (GXMGal), have been shown to contribute to the virulence of *C. neoformans*.
Although the composition of the cryptococcal capsule has been well studied, we do not yet fully understand its biosynthesis. Upstream precursors such as activated sugars, as well as their transport, have been extensively investigated. But little is known about the enzymes responsible for the formation of GXM and GXMGal. In this project, we aim to identify and study these unknown glycosyltransferases. To do so, we combined *in silico* approaches, virulence studies and phenotyping to first identify potential targets. We then recombinantly expressed candidate proteins in several heterologous systems (*E. coli*, *P. pastoris* and mammalian cells). Canonically, glycosyltransferases present a transmembrane domain that can prove challenging for expression and solubilization. To circumvent that issue, we have expressed various truncated proteins that lack this hydrophobic domain. Subsequently, in order to characterize enzymatic activity, we are currently using three different techniques: a luminescence-based assay (Glo-Assay), a chromatographic assay (HPAEC-PAD), and a radio-labeled assay.
By deciphering the enzymatic biosynthesis of GXM and GXMGal, we hope to shed new light on the fundamental biology and pathogenesis of *C. neoformans*. Ultimately, this may help the design of new drugs that target glycosyltransferases and advance the battle against cryptococcosis.

261T Analysis of Roles for CPC-2 in Degrading the Plant Cell Wall Carbohydrate Cellulose *Anthony Silva*[1], Katherine Borkov-

ich[1] 1) UC Riverside, Riverside, CA.

Cross pathway control (CPC) in *Neurospora crassa* is crucial for responding to amino acid starvation. This pathway contains several proteins, including the RACK-1 homolog CPC-2. Our laboratory has investigated roles for CPC-2 as a Gb subunit during heterotrimeric G protein signaling. We also recently reported that mutants lacking *cpc-2* do not possess detectable cellulase (glucose release from cellulose) activity after growth on cellulose. As well, qPCR analysis with the D*cpc-2* mutant revealed normal mRNA levels for major cellulases *cbh-1* (NCU07340), *cbh-2* (NCU09680), beta glucosidase gh3-4 (NCU04952), and endoglucanase gh5-1 (NCU00762). In order to identify the enzymes affected in the *cpc-2* mutant, and to potentially pinpoint the reason for the absence of glucose release cellulase activity, we analyzed secretomes for levels of beta-glucosidase, cellobiohydrolase and endoglucanase activity. The endoglucanase and cellobiohydrolase enzymes function by hydrolyzing b1-4 linkages on cellulose to allow for breakdown into simpler sugars. Beta glucosidases are enzymes that cleave b1-4 linkages in disaccharides such as cellobiose to produce glucose. Overall, endoglucanase activity was the most affected of the three enzymes with activity in the *cpc-2* mutant only 50% of wild type. Beta glucosidase activity was found to be 65% of wild type and cellobiohydrolase activity in the D*cpc-2* mutant was 80% of wild type. These results, together with the findings showing normal levels of mRNAs for cellulases in the D*cpc-2* mutant, further support that CPC-2 is involved in post-transcriptional regulation of cellulase enzymes, with the largest impact on endoglucanases. Results from experiments to follow up on these findings will be presented. Further analysis is also being conducted to test epistasis between *cpc-2* and the three Galpha subunit genes during regulation of cellulase activity. These findings will further elucidate the interactions occurring between CPC-2 and the Ga subunits during growth on cellulose.

262F Lipid flippase mediated Cryptococcus-host interaction during pulmonary cryptococcosis *Siddhi Pawar*[1,2], Yina Wang[1], Orchi Dutta[2], Varsha Gadiyar[2], Raymond Birge[1,2], Chaoyang Xue[1,2] 1) Public Health Research Institute, New Jersey Medical School, Rutgers University, Newark, NJ ; 2) Department of Microbiology, Biochemistry, and Molecular Genetics, New Jersey Medical School, Rutgers University, Newark, NJ .

Cryptococcus neoformans is a facultative intracellular pathogen that infects the lung and disseminates to the central nervous system in immunocompromised patients. Alveolar macrophages are the first line of defense against *C. neoformans* infection. However, the molecular basis of macrophage recognition and interaction with this yeast pathogen remains incompletely understood. Our previous studies on the mechanism of antifungal drug resistance identified Cdc50, a regulatory subunit of lipid translocase (flippase), not only mediates drug resistance, but also is essential for virulence in *C. neoformans*. We found that loss of Cdc50 increases exocytoplasmic phosphatidylserine (PS) accumulation, making it susceptible to phagocytosis and rendering an avirulent phenotype in a murine model of cryptococcosis. Mice infected with *cdc50Δ* mutant cells induced high Th1 and Th17 cytokine production, increased fungal clearance in the lungs and prevented dissemination to secondary organs. We hypothesize that the accumulation of PS on *cdc50Δ* cell surface induces macrophage recognition and phagocytosis, which helps clear the infection in the lung. We are testing this hypothesis using other fungal strains with altered PS exposure to study their interaction with macrophages in both cell line J774 and murine primary macrophages. In collaboration with Dr. Raymond Birge at Rutgers, we are also evaluating the activation of PS receptors in IFN-γ chimeric reporter cell lines. If our hypothesis is confirmed, we will block the PS receptor activation using PS inhibitors to study PS mediated cryptococcus-host recognition. Overall, our study may lead to a novel target for antifungal development.

263W Lipid flippase regulation of antifungal drug resistance and virulence in *Cryptococcus neoformans* Chengjun Cao[1], Wei Huang[1], Siddhi Pawar[1], Yina Wang[1], *Chaoyang Xue*[1] 1) Rutgers University.

Echinocandins show fungicidal activity against common invasive mycoses but are ineffective against cryptococcosis. The underlying mechanism for echinocandin resistance in *Cryptococcus neoformans* remains poorly understood. Our forward genetic screen identified Cdc50, the regulatory subunit of lipid flippase, as a key contributor of caspofungin resistance. Further suppressor screen identified a mechanosensitive channel protein Crm1 (Caspofungin Resistant Mutation 1) that interacts with Cdc50. Mutagenesis studies showed that *crm1*D restored caspofungin resistance in *cdc50*D. Caspofungin-treated *cdc50*D cells exhibited abnormally high intracellular calcium levels ([Ca^{2+}]c) and heightened activation of the calcineurin pathway. Deletion of *CRM1* in the *cdc50*D background normalized the abnormally high [Ca^{2+}]c. Together, these results demonstrate that Cdc50 and Crm1 regulation of the calcineurin pathway and cytoplasmic calcium homeostasis may underlie caspofungin resistance in *C. neoformans*. In addition, we also found that Cdc50 is essential for fungal virulence. The *cdc50*Δ mutant cells are rapidly engulfed by macrophages in vitro and are cleared in the infected lung in a murine model of systemic cryptococcosis. We are currently testing the hypothesis that the accumulation of phosphatidylserine on the outer leaflet of lipid bilayer membrane contributes to both the increased sensitively of *cdc50*Δ cells against caspofungin and the loss of fungal virulence.

264T Role of Arv1 protein in sterol metabolism and pathogenicity of the chestnut blight fungus *Cryphonectria parasitica* *Soumyadip Kundu*[1], Chathuri Mohottige[1], Todd Mlsna[1], Angus Dawe[1] 1) Mississippi State University.

Intracellular sterol redistribution is a very important step in the lipid homeostasis of organisms. Lipid homeostasis is also directly linked to the organizational arrangement in the plasma membrane (PM) of the cells. Previous studies in the budding yeast *Saccharomyces cerevisiae* have demonstrated that the ARV1 (ACAT-related enzyme-2 required for viability 1) protein is a major regulator of the sterol transport mechanism from the endoplasmic reticulum to the plasma membrane, thus contributing to the structural organization of the PM, rendering it resistant to anti-fungal compounds as well as maintaining the integrity of the ER. This study is aimed to assess the significance of ARV1 in the plant pathogenic fungus *Cryphonectria parasitica* (*CpARV1)* and investigate its role in the pathogenesis and virulence of the fungus. *C. parasitica* is the major causative agent of Chestnut blight, which has wreaked havoc on the American chestnut species. Genomic analysis has revealed that the *CpARV1* gene is very closely linked to another gene that putatively encodes a cynamide hydratase (*CpCAH1)*. An initial gene deletion event using a prior gene prediction for unrelated work resulted in the elimination of both genes and a highly deformed phenotype in *C. parasitica* that was fully recoverable by complementing with the deleted region. Expression analysis through both qPCR and endpoint PCR has determined that the specific lack of *CpARV1* was primarily responsible for the debilitated phenotype of the double mutant, with no transcript detectable from the putative hydratase gene. Subsequent complementation of the *CpARV1* gene was also observed to restore the wild-type phenotype. Mass spectrometry-based

methods including single ion monitoring were employed to analyze the sterol content of the wild type strain, *CpARV1* deletion strain and the *CpARV1* complemented strain. The Single Ion Monitoring results clearly indicated a substantial decrease in sterol content of the *CpARV1* mutant strain compared to wild type EP155 (approximately half the peak height and area in the raw chromatogram) thus confirming a role for *CpARV1* in sterol homeostasis. It has been previously shown that infection of *C. parasitica* with virulence-attenuating hypoviruses altered intracellular lipid content and protein secretion. We have also explored sterol content in *C. parasitica* infected with CHV1-EP713 and found decreased sterol content (peak heights and areas approximately similar to that of the mutant strains in the raw chromatogram) suggesting a potential connection between the hypovirus-infected phenotype and *CpARV1*. Current ongoing mass-spectrometric analyses are targeted at ascertaining wider metabolomic differences between the strains. Transmission electron microscopic study is also being performed to analyze the ER integrity of the different strains.

265F Differential effects of G-protein subunits on multiple cellulase enzymes in *Neurospora crassa* *Abel Vargas*[1], Yagna Oza[1], Katherine Borkovich[1] 1) University of California, Riverside; Riverside, CA.

Fungi use heterotrimeric G-protein signaling to sense and respond to environmental sources of carbon. Production of cellulases occurs in the presence of cellulose and the absence of a preferred carbon source, such as glucose. *Neurospora crassa* secretes multiple classes of cellulases to fully degrade cellulose into glucose, including beta-glucosidases, endoglucanases, and cellobiohydrolases. These different classes of cellulases act on different parts of the cellulose chain, and there are multiple enzymes belonging to each class. Previous research conducted on *N. crassa* has implicated heterotrimeric G-protein subunits in production of the enzymes required for cellulose to be metabolized all the way to glucose (glucose release activity). In particular, *gna-1* and *gna*-3 gene-deletion mutants have no detectable glucose release cellulase activity when transferred to cellulose-containing medium after initial growth on medium containing glucose. Given that *N. crassa* produces multiple cellulases, we furthermore wanted to test the effects of G protein mutations on the different classes of cellulases, as well as possible epistatic relationships between the subunits during regulation of these enzymes. Results from glucose release, beta-glucosidase, endoglucanase, and cellobiohydrolase assays will be presented.

266W Lignocelluloses and solid waste substrates transformed by wood-decay fungi for production of natural compounds *Taina Lundell*[1], Tuulia Mali[1], Eero Kiviniemi[1], Hans Mattila[1], Janina Österman-Udd[1] 1) University of Helsinki, Helsinki, Finland.

Wood-decay fungi of Basidiomycota demonstrate applicability for bioconversion and biological treatment of plant biomass and lignocelluloses. The fungi adapt to changes in environmental and laboratory conditions and may be cultivated even on mixtures of solid plant-based and waste substrates. Fungal metabolic potentiality is also seen in their ability to produce diverse bioactive compounds and secondary metabolites. We recently opened the transcriptome and proteome of the white rot fungus *Phlebia radiata* on spruce wood during decomposition (1, 2) and fungal metabolic and gene expression response under anaerobic, ethanol-producing fermentative conditions (3).
One aim is to adopt the fungus and other isolates in bioreactor conversions of waste lignocelluloses and plant-based industrial side-streams for production of biofuels like bioethanol, and added-value natural compounds, organic acids and aromatic compounds. Specific interest is in metabolic profiles of wood-decay fungi with different decomposition traits, and efficiency in degradation of plant biomasses.
For these purposes, we have tested various agricultural, municipal, and wood-based wastes like straw, core board, sawdust, and disposed construction wood (4). Culture atmosphere was the major regulator for primary metabolism together with influencing on differential expression of the wood-decaying, carbohydrate-active enzyme encoding genes (3). Ethanol production was prominent in phlebioid fungi on solid lignocellulose cultures under oxygen depleted conditions (4).
Results on our recent experiments on the effect of reactive oxygen species on overall gene expression and accumulation of natural products by the fungi will be presented. Experimental findings of fungal single species (5) and co-cultures on production of enzyme activities and metabolites indicate that both the lignocellulose substrate and fungal interactions are major effectors guiding the decomposition events. The functional resilience of these fungi on varying wood and waste substrates will aid us in designing more sustainable solutions for production of biofuels, enzymes, and natural compounds.
References: [1]Mäkinen M (2019) BMC Genomics 20:430, [2]Kuuskeri J (2016) Biotechnol Biofuels 9:192, [3]Mattila H (2020) Biotechnol Biofuels 13:26, [4]Mattila H (2017) Bioresource Technol 225:254-261, [5]Veloz Villavicencio E (2020) Microorganisms 8:73

267T A RiPPing time: Exploring a novel peptide from *Zymoseptoria tritici* *Rosie Ford*[1], Gary Foster[1], Andy Bailey[1] 1) University of Bristol, Bristol, United Kingdom.

The fungal capacity for production of anthropogenically useful compounds has been recognised by mankind for several decades, with peptides formed by non-ribosomal peptide synthetases (NRPS), including penicillin, cephalosporin, and the cyclosporines, transforming modern medicine and undoubtedly extending human lifespans. It is therefore unsurprising that continued interest lies in fungal peptides. Fungal ribosomally synthesised and post-translationally modified peptides (RiPPs), were first characterised in 2007 following analysis of amanitin, despite the application of bacterial and animal RiPPs commercially prior to this. Subsequently, more and more peptides have been re-evaluated in terms of their biosynthetic mechanism, resulting in the re-classification of peptides, previously designated as products of NRPS, as RiPPs formed by post-ribosomal peptide synthesis. Of the known fungal RiPPs, most hold a toxic function whether this is against plants, mammals, or nematodes. A biosynthetic gene cluster for a novel fungal RiPP has recently been discovered within the genome of the wheat pathogen *Zymoseptoria tritici*. The RiPP in the IPO323 strain of the fungus has 9 copies of the repeat YVIPVD within its precursor peptide, but the repeat number varies between different strains indicating the presence of some level of selection on the peptide, the direction of which is currently unknown. Ongoing studies aim to discover the structure of this RiPP, its full biosynthetic pathway and its role in this important wheat pathogenic fungus. Functional investigations carried out to date, using a novel strain in which the gene encoding the RiPP precursor peptide has been deleted, have yielded negative results. The fungus showed no alteration in virulence against the disease-susceptible wheat variety Riband. A feeding experiment was performed with locusts, but they showed no difference in preference for leaves infected with wild-type or RiPP mutant fungus. In plate-based bioassays no obvious antibiotic function was detected. Knockout strains of each gene within the RiPPs biosynthetic gene cluster are currently in development. Intermediate products in RiPP biosynthesis are to be tested for modifications to pathogen virulence, and importantly will be used to piece

together RiPP peptide assembly, ultimately facilitating a deeper understanding of fungal RiPP synthesis more widely.

268F Identifying unique metabolite patterns during wood decay by brown rot fungi using metabolomics *Jesus Castano Uruena*[1,2,4], Nathalie Muñoz-Muñoz[3], Young-Mo Kim[3], Jonathan S Schilling[2] 1) Department of Bioproducts and Biosystems Engineering, University of Minnesota, Twin cities, MN; 2) Department of Plant and Microbial Biology, University of Minnesota, Twin cities, MN; 3) Pacific Northwest National Laboratory, Richland, WA; 4) Marine and Coastal Research Institute, INVEMAR, Santa Marta, Colombia.

White and brown rot fungi are important organisms for the cycling of nutrients and carbon locked in forest trees. Although significant genetic differences have been found between white and brown rot fungi, there is little knowledge about how the metabolomes are affected by the type of decay, and whether there are characteristic metabolite signatures that could be associated with a specific decay mode. The genetic differences, particularly the CAZy and oxidoreductase gene shedding in brown rotters with respect to white rotters, intuitively suppose differences in sugar profiles. Additionally, a significant expansion in polyketide synthase genes in brown rot fungi imply a higher production of secondary metabolites such as furanones, pyranones, and quinones. In this research, we grew two brown rot fungi (*Gloeophylum trabeum* and *Rhodonia placenta*) and two white rot fungi (*Trametes versicolor* and *Pleurotus ostreatus*) from different clades and analyzed their metabolomic profiles at different decay stages (early vs late) to identify differences during decay related to decay stage or rot type. Interestingly, we found a very identifiable metabolome signature for brown rot fungi only at late decay stages. On the contrary, white rot fungi did not show common metabolite patterns at any decay stage. Nonetheless, we found a potential biomarker compound, galactitol, which was only detected in white rot fungi. Also, white rot fungi performed better at removing phenolic compounds that were initially present in undecayed wood. One of the most interesting differences between brown rot and white fungi was the increased production of pyranones and furanones in brown rot fungi which could give these organisms some competitive advantages unique to their lifestyle and it is probably related to the expansion of polyketide synthase genes in these fungi. Likewise, as expected, we found a higher number of differentially abundant sugars and carboxylic acids in brown rot compared to white rot fungi at later decay stages, which agrees with the higher decomposition rates observed in brown rot fungi, and their ability to lower the pH more than white rot fungi. Finally, at the global level, we found that all four fungi accumulated furans (e.g. furfural, 5-methylfurfural), sugars, and carboxylic acids as decay progressed. In general, our findings showed there is an important correlation between the metabolite profiles and the type of wood rot, organism, and decay stage.

269W Functional characterization of the GATA-type transcription factor PaNsdD in the filamentous fungus *Podospora anserina* and its interplay with the sterigmatocystin pathway Ling Shen[1, 2], Thomas Gaslonde[3], Catherine Roullier[4], Huijuan Wang[1], Jennifer Bodin[3], Francois-Hugues Porée[5], Gwenael Ruprich-Robert[1], *Florence Chapeland-Leclerc*[1] 1) Université de Paris, CNRS, Laboratoire Interdisciplinaire des Energies de Demain (LIED - UMR 8236), F-75013 Paris, France; 2) Shenzhen University, Shenzhen Key Laboratory of Microbial Genetic Engineering, College of Life Sciences and Oceanography, 518000, Shenzhen, China; 3) Université de Paris, CNRS, Cibles Thérapeutiques et Conception de Médicaments (CiTCoM - UMR 8038), F-75006 Paris, France; 4) Université de Nantes, Mer Molécules Santé, EA 2160, F-44035, Nantes, France; 5) Université de Rennes 1, ISCR, UMR 6226 CNRS, Laboratoire de Chimie Thérapeutique, Faculté de Pharmacie, F-35065 Rennes, France.

The model ascomycete *Podospora anserina*, featured by its strict sexual development, is a prolific but yet unexploited reservoir of natural products. The GATA-type transcription factor NsdD has been characterized by the role in balancing asexual and sexual reproduction, and governing secondary metabolism in filamentous fungi. In the present study, we functionally investigated the NsdD ortholog PaNsdD in *P. anserina*. Compared to the wild-type strain, vegetative growth, ageing processes, sexual reproduction, stress tolerance and interspecific confrontations in the mutant were drastically impaired, owing to the loss of function of PaNsdD. In addition, the production of 3-acetyl-4-methylpyrrole, a new metabolite identified in *P. anserina* in this study, was significantly inhibited in the *ΔPaNsdD* mutant. We also demonstrated the interplay of PaNsdD with the sterigmatocystin biosynthetic gene pathway, especially as the deletion of *PaNsdD* triggered the enhanced red-pink pigment biosynthesis that only occurs in the presence of the core polyketide synthase-encoding gene *PaStcA* of the sterigmatocystin pathway. Taken together, these results contribute to a better understanding of the global regulation mediated by PaNsdD in *P. anserina*, especially with regard to its unexpected involvement in fungal ageing process and its interplay with sterigmatocystin pathway.

270T *cexA* and its regulatory processes – a closer look into the citric acid production mechanism of *Aspergillus niger* *Aline Reinfurt*[1,2], Valeria Ellena[1,2], Matthias Steiger[1,2] 1) Austrian Centre of Industrial Biotechnology (ACIB GmbH), Vienna, Austria; 2) Institute of Chemical, Environmental and Bioscience Engineering, TU Wien, Vienna, Austria.

Aspergillus niger is an important filamentous fungus used for the industrial production of citric acid. One of the main contributors to high citric acid accumulation by the fungus is the citrate transporter CexA. It belongs to the major faciliatory superfamily subclass DHA1 which act as drug-H^+ antiporters[1]. Since *cexA* and its regulators are essential within the citric acid production process, it is important to study their regulatory mechanism, which is the focus of this work. LaeA for one is known to be a major regulator of the *cexA* gene. It regulates the expression via methylation levels of the histones H3K4 and H3K9[2]. There are indications that other transcriptional regulators such as AmyR and XlnR are also involved in the regulation of *cexA* on a transcriptional level. Another factor that affects citric acid production is the amount of manganese that is present during the production process. Observations showed that the fungus develops a certain pellet-like morphology under manganese limitation conditions and that this limitation is decisive for high citric acid accumulation by *A. niger*[3,4]. However, the exact mode of action of manganese in the cell is not clear. The transcriptional influence of manganese on *cexA* and *laeA* is investigated in order to find out more about the connection between manganese limitation and citric acid production.

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271F Elucidating the biosynthesis of ribosomally synthesized backbone N-methylated macrocyclic peptides *Lukas Sonderegger*[1], Emmanuel Matabaro[1], Markus Künzler[1] 1) Institute of Microbiology, Department of Biology, ETH Zürich, Switzerland.

Backbone N-methylation and macrocyclization are desired modifications of peptide therapeutics, as they are known to improve cell permeability, target selectivity and proteolytic stability of peptides. The most famous example of a peptide therapeutic with these modifications is cyclosporin A, a non-ribosomal peptide produced by the fungus *Tolypocladium inflatum,* which is used as an immunosuppressant. Other peptide natural products displaying backbone N-methylation are the borosins, a new family of ribosomally synthesized and post-translationally modified peptides (RiPPs). The precursor proteins of these peptides contain a characteristic SAM-dependent peptide α-N-methyltransferase domain, which iteratively methylates the core peptides located at their C-termini. After completion of methylation, the borosin core peptide is cleaved off and, at least in some cases, macrocyclized by specific endoproteinases. The founding members of this RiPP family are the omphalotins, 12-amino acid macrocycles with nine backbone N-methylations. These peptides are produced by the mushroom *Omphalotus olearius* and are known to exhibit strong toxic activity against nematodes. The mushrooms *Lentinula edodes* (Shiitake) and *Dendrothele bispora* produce highly homologous peptides referred to as lentinulins and dendrothelins. The biosynthetic gene clusters of these peptides encode not only the respective peptide precursor proteins but also a conserved prolyl oligopeptidase and several additional enzymes that are not yet characterized but thought to be involved in the biosynthesis of these peptides. We established the heterologous expression of the precursor protein and the prolyl oligopeptidase in the yeasts *Pichia pastoris* and *Saccharomyces cerevisiae*, which was found to be sufficient for the production of the peptides in these hosts. *In vitro* experiments with the purified precursor protein and prolyl oligopeptidase, however, suggest that the prolyl oligopeptidase is not able to process the full-length precursor protein and that additional enzymes are involved in this process. We are aiming at the identification of these enzymes to further shed light on the biosynthetic pathway of these peptides and pave the way towards the biotechnological production of novel backbone N-methylated macrocyclic peptides with advantageous pharmacological properties.

272W Effect of *LaeA* on the secondary metabolism in the filamentous fungus *Podospora anserina* *Huijuan WANG*[1], Thomas Gaslonde[2], Pierre Grognet[3], Gwenaël Ruprich-Robert[1], Florence Chapeland-Leclerc[1] 1) Université de Paris, CNRS, Laboratoire Interdisciplinaire des Energies de Demain (LIED - UMR 8236), F-75013 Paris, France; 2) Université de Paris, CNRS, Cibles Thérapeutiques et Conception de Médicaments (CiTCoM - UMR 8038), F-75006 Paris, France; 3) Institute for Integrative Biology of the Cell (I2BC, UMR 9198) CEA, CNRS, Université Paris Saclay 91400 Orsay cedex France.

Secondary metabolites (SMs) are low molecular weight natural products. The filamentous fungus *Podospora anserina* contains more than 40 putative biosynthetic gene clusters (BGCs) in the genome. Yet, the SMs produced by this fungus are poorly characterized. BGCs are controlled by complex regulatory networks. Their regulations depend on multilevel fashions, including transcriptional regulation, epigenetic control and environmental signal stimuli. *LaeA* (loss of aflR expression A) is a global transcription factor, belonging to the velvet complex, which coordinates development and SM production in response to variation in light levels in some filamentous fungi.
We focus here on the functional characterization of *LaeA* in *P. anserina.*
For that, we have undertaken the construction of *LaeA* deletion (*ΔPaLaeA*) and overexpression (*OE-PaLaeA*) mutants.
We will present the phenotypical characterization of these mutants, especially focusing on fungal growth in various conditions (as different carbon sources or different stresses) and sexual reproduction.
Moreover, by using HPLC-UV-MS approach, three metabolites were specifically found in *ΔPaLaeA*. Their identification is currently in progress and will be discussed in our presentation.

273T Primary, secondary and tertiary metabolites, proteins and carbohydrates *Jens Frisvad*[1] 1) Technical University of Denmark.

Many words have been suggested to denote small molecules (metabolites) and few words have been used to denote different kinds of macromolecules such as proteins and polysaccharides. Primary metabolites, primary proteins, and primary polysaccharides are present in nearly all kinds of organisms, or at least in large groups of organisms, such as bacteria, archaea, plants, fungi, algae, animals etc., but certain groups of organisms such as anaerobic fungi have adapted their primary metabolism to function under anoxic condition. Examples of primary metabolites in fungi are oxaloacetate present in the mitochondria of all aerobic fungi and tryptophan. Secondary metabolites (SMs), secondary proteins, secondary polysaccharides are the results of conspicuous outwards directed differentiation, and they are taxonomically restricted, often being only produced by few species (for example only 1- 30 species of filamentous fungi) in major taxonomic groups. They are accumulated products, often secreted (the secretome) or deposited on the cell wall or in the cell membrane (the depositome). Metabolites, proteins, polysaccharides that are produced by one species, but used by another species are tertiary (one example of a tertiary metabolite is the milkweed toxin used by the monarch butterfly to protect it against being eaten by certain birds). Other words used to denote primary metabolites are general metabolites and other words to denote secondary metabolites are specialized metabolites, special metabolites, small molecules, idiolites, natural products, natural compounds, while such expressions have not been coined for proteins and polysaccharides. Examples of secreted fungal SMs are citrinin and penicillin while deposited SMs include penitrem A and aflavinin. Examples of secondary proteins are PAF (*Penicillium rubens* antifungal protein) and the bubble protein from *Penicillium brevicompactum*, and exoenzymes and hydrophobins. Examples of fungal secondary polysaccharides are pullulan and mannocarolose. A common word for all secondary metabolites, secondary proteins and secondary carbohydrates is extrolites. Ruderal selected fungi, such as most yeasts, Mucoromycetes, *Neurospora*, produce few and simple SMs (for example carotenoids and simple amino acid derived SMs), stress selected fungi also produce few SMs, while competition selected fungi can often (potentially) produce a large number of simple and very complex SMs.

274F Evolution-driven combinatorial chemistry by genetics using fungal natural product gene clusters *Pablo Cruz-Mo-*

rales[1], Carolina Cano-Prieto[1], Agustina Undabarrena[1], Ana Calheiros de Carvalho[1], Dushica Arsovska[1], Xiaowei Li[1], Jay Keasling[1] 1) Technological University of Denmark.

The availability of affordable genome sequencing platforms, efficient genome mining, analytical methods and synthetic biology tools has granted unprecedented access to microbial natural products, mostly from bacteria. Despite these advances, Fungi which have larger and distinctively different chemical repertoires remain an underexploited source of natural products.

Our team aims to develop genome mining methods and yeast heterologous expression platforms for fungal natural products using a combination of phylogenomics and synthetic biology. Our approach is based in the observation that natural product biosynthetic gene clusters that produce chemically related molecules can be classified into families (BGCFs). Evolutionary analysis of these families reveals that they are formed by groups of highly conserved enzymes that evolved to generate common intermediates which were diversified by a set of accessory enzymes which were acquired through millions of years. Their gain and loss led to the production of metabolites that are filtered by natural selection and fixed within species populations depending on their environments. Our goal is to exploit this evolutionary mechanism to gain access to the chemical diversity encoded in entire BGCFs, capture it into vials and use it for production of drugs and other bioproducts.

Here we will present our efforts to systematically identify and classify fungal BGCFs, define their taxonomic distribution and predict chemical diversity using phylogenomics. We plan to use this information to generate biosynthetic blueprints for combinatorial DNA libraries for heterologous production in yeast. The goal is to produce many molecules that can then be screened for their bioactivities.

275W Pathogenic fungi at the crossroads of metal starvation and oxidative stress *Valeria Culotta*[1], Francisco Hernandez[1], Yiran Wang[1] 1) Johns Hopkins University Bloomberg School of Public Health.

Upon entering an animal host, a microbial pathogen is immediately deprived of its essential micronutrient iron. Mammals respond to infection through a clinical condition known as anemia of inflammation, whereby available pools of iron are rapidly sequestered, starving the invading microbe of this micronutrient required for growth. Successful fungal pathogens adapt by activating innovative means for capturing host iron in spite of widespread iron limitation. We observe that a low iron environment can also signal “SOS” to fungal pathogens, alerting them to prepare for additional host inflammatory insults including chemical attacks of reactive oxygen species (ROS). When iron-starved, diverse *Candida* species including members of the CTG clade and *Candida auris* secrete an extracellular form of superoxide dismutase (SOD) enzyme that has no obvious role in iron homeostasis but is essential for guarding against host attack by ROS. These iron-regulated SODs represent members of a family of so-called Cu-only SODs that are unique to fungi and closely related oomycetes. These SODs are all extracellular and effectively combat the ROS attack of host phagocytes as well as participate in fungal signaling involving ROS. In numerous fungal pathogens for plants, animals and insects, the Cu-only SODs are important virulence factors. In addition to Cu-only SODs, iron-starved *Candida albicans* secretes small (≤1 kDa) soluble metal-reactive molecules into the extracellular environment. These include copious levels of riboflavin (vitamin B2) which can act in redox reactions to reduce extracellular Cu(II) and Fe(III) to the reduced Cu(I) and Fe(II) forms required for fungal uptake of the metal. Iron-starved *C. albicans* also secrete a small highly charged metal binding molecule that is not a metal reductant but binds extracellular Cu and Fe with high affinity. These multi-layer responses to iron starvation including secretion of anti-oxidant SODs and small molecules to modify extracellular metals, can help the fungal pathogen cope with the stressful environment imposed by its host.

276T Interaction of the bZIP-type transcription factors NapA and RsmA in the regulation of oxidative stress defence and sterigmatocystin production of *Aspergillus nidulans* Bernadett Bákány[1,2,3], Yin Wen-Bing[4,5], Beatrix Dienes[3], Tibor Nagy[6], Éva Leiter[1], Tamás Emri[1], Nancy P. Keller[5,7], *István Pócsi*[1] 1) Department of Molecular Biotechnology and Microbiology, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary; 2) Doctoral School of Molecular Medicine, University of Debrecen, Debrecen, Hungary; 3) Institute of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary; 4) State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; 5) Department of Medical Microbiology and Immunology, University of Wisconsin, Madison, USA; 6) Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen, Hungary; 7) Department of Bacteriology, University of Wisconsin, Madison, USA.

Basic leucine zipper (bZIP) transcription factors are of critical importance in the environmental stress response of eukaryotes. In this work, we studied the effect of gene deletions and overexpressions of two bZIP transcription factors, NapA and RsmA, in the oxidative stress response and sterigmatocystin production of *Aspergillus nidulans*.
We found that NapA is crucial in the neutralization of oxidative stress by negatively regulating the intracellular reactive species production and positively modulating catalase activities, meanwhile RsmA affected catalase activities slightly negatively.
With respect to sterigmatocystin production, the highest level was determined in the *ΔrsmAΔnapA* double deletion mutant but increased sterigmatocystin production was also found in the OE*rsmA*OE*napA* strain. Our results indicate that NapA coordinated sterigmatocystin production *via* regulating oxidative species level while RsmA modulated toxin production independently of the redox regulation of the cells.
We also examined the effect of *rsmA* on the expression of wild type *napA* and *vice versa*. The pairwise comparison of *ΔrsmA*, control and OE*rsmA* strains showed that overexpression of *rsmA* increased the transcription of *napA* while overexpression of *napA* resulted in increased *rsmA* expression. Deletion of either *rsmA* or *napA* had no significant effect on the transcription of the other gene.
Considering the complex regulatory network of NapA and RsmA on the oxidative stress response and secondary metabolite production of *A. nidulans* as well as their observed influence on each other’s expressions we can assume that NapA and RsmA may interact with each other either genetically or even physically to orchestrate ST production.

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277F Heterotrimeric G-Protein Signaling and Carbon Catabolite Repression in *Neurospora crassa* *Yagna Anandkumar Oza*[1], Logan A Collier[2], Katherine A Borkovich[1] 1) Department of Microbiology and Plant Pathology, University of California, Riverside, CA; 2) Department of Biological Sciences, Biola University, La Mirada, CA.

Filamentous fungi utilize heterotrimeric G-protein signaling for sensing and responding to the environment. Growing on lignocellulolytic biomass, filamentous fungi secrete cellulases to decompose cellulose into simpler molecules such as cellobiose and glucose that can be directly transported into the cells. Upon sensing glucose in the environment, fungi induce carbon catabolite repression (CCR) by activating the CRE-1 transcription factor which represses the expression of cellulase genes. Our lab has recently shown the crucial role of G-protein signaling in cellulose degradation in *Neurospora crassa*. Single gene deletion mutants for 5 out of 6 G-protein subunits in *N. crassa* show no detectable secreted cellulase activity when pre-grown in glucose for 16 hours and then transferred to cellulose for 2-3 days. Interestingly, some of these mutant strains devoid of cellulase activity were still able to grow well after direct inoculation in cellulose liquid cultures. To elucidate the regulation of cellulase activity in these mutants, we are investigating the potential dysregulation of CCR. In *N. crassa*, CRE-1 is known to negatively regulate the expression of enzymes needed for the utilization of alternative carbon sources, including major cellulases, alcohol dehydrogenases (ADH), etc. In the absence of CRE-1, ADH is expressed and converts allyl alcohol into the toxic compound acrolein that is lethal to the cells. Results will be presented from testing the effects of allyl alcohol in G-protein mutants during growth on glucose and cellulose using experimental techniques, including total cell mass protein measurements and RT-qPCR analysis of transcription factor and cellulase genes.

278W Tryptophan biosynthesis genes in the mushroom *Coprinopsis cinerea* *Ursula Kües*[1], Zemin Fang[2], Xianhua Wang[2], Shanta Subba[1], Kiran Lakkireddy[1] 1) Molecular Wood Biotechnology and Technical Mycology, University of Goettingen, Goettingen, Germany; 2) School of Life Sciences, Anhui University, Anhui, China.

Trp is an amino acid underrepresented in protein sequences and it is often confined to catalytic centers of enzymes. This non-polar aromatic residue is the most costly amino acid in terms of its biosynthesis. Basal lines to fungi (Cryptomycota, Microsporidia) seem not possess *trp* biosynthesis genes, but younger fungal lines obtained the ability of Trp biosynthesis, probably by horizontal gene transfer (HGT) from photosynthetic Chloroflexi. A common bacterial operon structure is *trpEGDFCBA*, which follows roughly the order of the enzymatic actions in the Trp biosynthesis pathway. In the fungi, different genes in the biosynthesis pathway have been fused. Trp biosynthesis in *Coprinopsis cinerea* is covered by four genes, termed *trp1*$^+$ to *trp4*$^+$. Anthranilate synthase subunit Trp3 in combination with the anthranilate synthase subdomain of the trifunctional Trp2 protein undertakes the first step in the tryptophan biosynthesis in order to transform chorismate as end-product of the shikimate pathway into anthranilate. Next, the phosphoribosyl transferase Trp4 produces N-(5´-phosphoribosyl)-anthranilic acid (PRA) as substrate for Trp2 which in two further steps gives rise to 1-(2-carboxyphenylamino)-1-deoxy-D-ribulose 5-phosphate (CDRP) by phosphoribosyl anthranilate isomerase activity and then indole-3-glycerol-phosphate (InGP) by indole-3-glycerol phosphate synthase activity. InGP is substrate for the bifunctional Trp1 which with its N-terminal TrpA half converts InGP into indole and with the C-terminal TrpB half the indole with serine into tryptophan. A defect in any of the steps prevents the completion of the tryptophan biosynthesis process. Mutations in *trp1* and *trp3* are available in laboratory strains and are used in DNA transformation selection marker complementation. A paradoxical phenomenon occurs in transformations of *trp1* strains with the selectable *trp1*$^+$ wild-type marker gene. It leads in single *trp1*$^+$ vector transformation to half as much or less clone numbers as compared to parallel conducted cotransformations with a *trp1*$^+$ vector and an additional non-*trp1*$^+$vector. The results indicate that the difference in observed clone numbers base on loss of *trp1*$^+$ transformants in single vector transformations by stronger feedback control by Trp as the end product of the biosynthesis pathway. Transformation in *C. cinerea* is typically ectopic at several genomic sites. More genomic integration sites will thus be occupied in single vector transformation by the *trp1*$^+$ gene for potential expression. The *trp1*$^+$ gene studied in more detail. It has been split into two individual functions (*trpA* and *trpB*), various *trp1* mutants have been characterized and complementation tests were performed. Similary, the *trp3*$^+$ wildtype and *trp3* mutant are under study to define their actions and regulation in the Trp biosynthesis and effects on transformations.

279V The Crz1 transcription factor of *Fusarium verticillioides* is required for lipid metabolism regulation and fumonisin production. Marzia Beccacioli [1], *Andrea Cacciotti*[1], Stefania Vitale[2], Antonio di Pietro[3], David Turrà[4], Valeria Scala [5], Massimo Reverberi[1] 1) Department of Environmental Biology, “Sapienza” University of Rome, Rome, Italy; 2) National Research Council, Institute for Sustainable Plant Protection, Portici, Italy; 3) Department of Genetics, Campus de Excelencia Internacional Agroalimentario ceiA3, Universidad de Córdoba, Córdoba, Spain ; 4) Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy; 5) CREA-DC, Roma, Italy.

Calmodulin (CaM), the main calcium binding protein in eukaryotes, act as part of calcium signal transduction pathway in fungi, involved also in colony growth, stress response and pathogenicity regulation. Here we investigated the role of the fungal protein Crz1, a downstream transcription factor of the CaM pathway, in the fumonisin-producing fungus *Fusarium verticillioides*.
Previous studies have shown that the production of fumonisins in *Fusarium* is related to the presence of specific fatty acids (FAs) and oxylipins (e.g. oxidized FAs) in the growth medium. These molecules can act as intra/extracellular signals exchanged by the pathogen and its host during the infection.
In this study, we report the involvement of Crz1 in the regulation of both lipid metabolism and fumonisin production during the *F. verticillioides-Zea mays* interaction. *F. verticillioides crz1Δ* strains showed higher membrane permeability and susceptibility to ionic stress when compared to the wild type or the *crz1Δ+crz1* complemented strains, suggesting an involvement of Crz1 in structural lipid biosynthesis. Moreover, mass spectrometry revealed that loss of *crz1* was consistently associated with an overall reduction in oxylipin, FA and mycotoxin concentration in kernels infected with *F. verticillioides*. Finally, transcriptome profiling show that lack of Crz1 led to changes in the expression of the fumonisin genes, suggesting a key role in the regulation of this complex and interconnected pathways, particularly during the host-pathogen interaction. We postulate that Crz1 functions upstream of the lipid pathway to control FA synthesis, thereby promoting the production of oxylipins and indirectly modulating FB synthesis. Thus, Crz1 acts as a major switch of fatty acid-mediated responses such as mycotoxin synthesis during *Zea mays* infection.

280V Beyond the symbiosis: Novel modulating roles of lipochitooligosaccharides and chitooligosaccharides in the development of fungi and nearby microbes. *Tomas Rush*[1], Ivan Villalobos Solis[1], Joanna Tannous[1], Muralikrishnan Gopalakrishnan Meena[1], Matthew Lane[2], Nancy Engle[1], Alyssa Carrell[1], Margaret Spangler[1], Jean-Michel Ané[3], Nancy Keller[3], Sylvain Cottaz[4], Sébastien Fort[4], Daniel Jacobson[1], David Kainer[1], Dale Pelletier[1], Timothy Tschaplinski[1], Robert Hettich[1], Richard Giannone[1], Paul Abraham[1], Jesse Labbé[1] 1) Oak Ridge National Laboratory, Oak Ridge, TN, USA; 2) University of Tennessee, Knoxville, TN, USA; 3) University of Wisconsin-Madison, Madison, WI, USA; 4) Université Grenoble Alpes, CNRS, CERMAV, Grenoble, France .

Lipochitooligosaccharides (LCOs) and chitooligosaccharides (COs) are chemical signaling molecules produced by rhizobia bacteria and fungi. Both signals have been well characterized for their role as symbiotic molecules and their interactions with host plants. They are perceived by a lysin motif receptor-like kinases in various plants, which can elicit the Common Symbiosis Pathway. Some observed phenotypes are oscillations in nuclear calcium or, as shown in some legumes, will prompt root hair branching or root hair curling, observable phenotypes in developing roots. However, the role of LCOs and COs outside of symbiosis is still in its infancy. Recently, LCOs and COs were shown to be produced in most fungi in the absence of a host, regulating the fungal physiology and transcriptomic. However, it remains unknown why LCO- and CO- producing organisms use these molecules. We test for alternative roles of LCOs and COs, outside symbiosis with a host plant, to address this question. We first determined that these molecules could control secreted metabolites to modulate nearby microbial growth. In addition, we demonstrate that observed fungal physiological changes may be attributed to differences in proteomic and metabolomic outputs. Our results showed that various types of LCOs and COs would differentially regulate the production of known secondary metabolites in *Aspergillus fumigatus* and *Laccaria bicolor*. Moreover, through network analysis, we determined specific types of LCOs, or COs to promote the production of several unknown analytes. These metabolites negatively or positively influenced the growth behavior of soilborne bacteria across five different phyla. Finally, we provide evidence that LCOs produced in abundance by a fungus, will have in return, the most significant effect on its behavior. Overall, we speculate that LCOs could be a fungistatic compound produced and used by the fungus to organize microbial communities.

281V Electrophysiological characterization of a diverse group of sugar transporters from *Trichoderma reesei* *Sami Havukainen*[1], Jonai Pujol-Giménez[2,3], Mari Valkonen[1], Ann Westerholm-Parvinen[1], Matthias A. Hediger[2,3], Christopher P. Landowski[1] 1) Protein Production Team, VTT Technical Research Center of Finland Ltd, Espoo, Finland; 2) Membrane Transport Discovery Lab, Department of Nephrology and Hypertension, University of Bern, Bern, Switzerland.; 3) Department of Biomedical Research, Inselspital, University of Bern, Bern, Switzerland.

Trichoderma reesei is an ascomycete fungus known for its capability to secrete high amounts of extracellular cellulose- and hemicellulose-degrading enzymes. These enzymes are utilized in the production of second-generation biofuels and *T. reesei* is a well-established host for their production. Although this species has gained considerable interest in the scientific literature, the sugar transportome of *T. reesei* remains poorly characterized. Better understanding of the proteins involved in the transport of different sugars could be utilized for engineering better enzyme production strains. In this study we aimed to shed light on this matter by characterizing multiple *T. reesei* transporters capable of transporting various types of sugars. We used phylogenetics to select transporters for expression in *Xenopus laevis* oocytes to screen for transport activities. Of the 18 tested transporters, 8 were found to be functional in oocytes. 10 transporters in total were investigated in oocytes and in yeast, and for 3 of them no transport function had been described in literature. This comprehensive analysis provides a large body of new knowledge about *T. reesei* sugar transporters, and further establishes *X. laevis* oocytes as a valuable tool for studying fungal sugar transporters.

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282V Functional characterization of a highly specific Larabinose transporter from *Trichoderma reesei* *Sami Havukainen*[1], Jonai Pujol-Giménez[2,3], Mari Valkonen[1], Matthias A. Hediger[2,3], Christopher P. Landowski[1] 1) Protein Production Team, VTT Technical Research Center of Finland Ltd, Espoo, Finland; 2) Membrane Transport Discovery Lab, Department of Nephrology and Hypertension, University of Bern, Bern, Switzerland.; 3) Department of Biomedical Research, Inselspital, University of Bern, Bern, Switzerland.

Lignocellulose biomass has been investigated as a feedstock for second generation biofuels and other value-added products. Some of the processes for biofuel production utilize cellulases and hemicellulases to convert the lignocellulosic biomass into a range of soluble sugars before fermentation with microorganisms such as yeast *Saccharomyces cerevisiae*. One of these sugars is L-arabinose, which cannot be utilized naturally by yeast. The first step in L-arabinose catabolism is its transport into the cells, and yeast lacks a specific transporter, which could perform this task.

We identified Trire2_104072 of *Trichoderma reesei* as a potential L-arabinose transporter based on its expression profile. This transporter was described already in 2007 as D-xylose transporter XLT1. Electrophysiology experiments with *Xenopus laevis* oocytes and heterologous expression in yeast revealed that Trire2_104072 is a high-affinity L-arabinose symporter with a K_m value in the range of ~0.1–0.2 mM. It can also transport D-xylose but with low affinity (K_m ~9 mM). In yeast, L-arabinose transport was inhibited slightly by D-xylose but not by D-glucose in an assay with fivefold excess of the inhibiting sugar. Comparison with known L-arabinose transporters revealed that the expression of Trire2_104072 enabled yeast to uptake L-arabinose at the highest rate in conditions with low extracellular L-arabinose concentration. Despite the high specificity of Trire2_104072 for L-arabinose, the growth of its *T. reesei* deletion mutant was only affected at low L-arabinose concentrations.

Due to its high affinity for L-arabinose and low inhibition by D-glucose or D-xylose, Trire2_104072 could serve as a good candidate for improving the existing pentose-utilizing yeast strains. The discovery of a highly specific L-arabinose transporter also adds to our knowledge of the primary metabolism of *T. reesei*. The phenotype of the deletion strain suggests the involvement of other transporters in L-arabinose transport in this species.

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283V Sirtuins are involved in cell wall integrity, secondary metabolites production and virulence in Aspergillus fumigatus Natália Wassano[1], Jaqueline Gerhardt[1], Everton Antoniel[1], Gabriela da Silva[2], Daniel Akiyama[3], Leandro Neves[4], Bianca Oliveira[5], Adriana Leme[4], Fausto Almeida[5], Taicia Fill[3], Nilmar Moretti[2], *André Damasio*[1] 1) Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas (UNICAMP); 2) Department of Microbiology, Immunology and Parasitology, Paulista School of Medicine, Federal University of São Paulo; 3) Department of Organic Chemistry, Institute of Chemistry, University of Campinas (UNICAMP); 4) Brazilian Bioscience National Laboratory (LNBio), Brazilian Center for Research in Energy and Materials (CNPEM); 5) Department of Biochemistry and Immunology, Faculty of Medicine from Ribeirão Preto, University of São Paulo.

The incidence of invasive infections in humans caused by opportunistic fungi has increased dramatically due to the rise of immunosuppressive therapies and recently an increasing number of COVID-19-associated pulmonary aspergillosis. Aspergillus fumigatus is an opportunist filamentous fungus and the main agent of Invasive Pulmonary Aspergillosis (IPA), its pathogenicity depends on multiple factors from both pathogen and host. Recently, protein acetylation dynamics has been correlated to pathogenesis in some fungi, moreover, lysine deacetylase (class I and II) inhibitors have been proposed as potential antifungal agents. Sirtuins are NAD+-dependent lysine deacetylases (class III) that participate in the regulation of the acetylation status of many proteins. The influence of sirtuin in virulence has been described for Candida albicans and Cryptococcus neoformans, however, its biological role in A. fumigatus has not been reported. In silico analysis indicates that A. fumigatus harbors six putative sirtuins. We report here the acetylome of an A. fumigatus strain deleted for the six predicted sirtuins (SIRTko), in addition to the phenotypic profile of single knockout strains (ΔAfSirA, ΔAfSirB, ΔAfSirC, ΔAfSirD, ΔAfSirE and ΔAfHstA). Our results indicate that A. fumigatus sirtuins are not essential for cell viability, but they are involved in the cell wall structure and/or composition, protease secretion, secondary metabolite production and virulence in an invertebrate model. In addition, the analysis of the SIRTko strain acetylome revealed that sirtuins might regulate the acetylation of at least 205 proteins. Based on Gene Orthology Enrichment analysis, the proteins abundantly acetylated found in the SIRTko strain are potentially involved in growth regulation, histone acetylation, asexual reproduction, regulation of gene expression, response to stress, cellular localization, developmental process, and other biological processes. Here we show that sirtuins are involved in various biological processes by maintaining protein acetylation balance. In that way, understanding the biology of these enzymes in A. fumigatus and their potential inhibitors may open perspectives for the development of new molecules to combine with antifungal drugs.

284V Four cell surface phosphate transporters in *Candida albicans* contribute to homeostasis at distinct ambient pH and phosphate concentrations *Maikel Acosta Zaldivar*[1,2], Wanjun Qi[1,2], William King[3], Jana Vogt[3], Julia Köhler[1,2] 1) Boston Children›s Hospital; 2) Harvard Medical School; 3) Department of Biological Sciences, Duquesne University.

Phosphate is an essential macronutrient, needed for cells to initiate glycolysis, conduct oxidative phosphorylation, replicate DNA, produce the translational machinery and produce membranes. We previously found that in *Candida albicans*, the high-affinity inorganic phosphate (Pi) transporter Pho84 activates TORC1 signaling in response to Pi, and is required in oxidative stress responses and cell wall integrity. We here characterize *C. albicans'* 4 known or predicted cell surface Pi transporters, encoded by *PHO84*, *PHO89*, *PHO87* and *FGR2*, using single and triple mutants in these transporters. Pho84 is the most important, as its presence alone sustains cellular growth and Pi uptake over the widest range of pH and Pi concentrations. The other predicted high-affinity Pi transporter, Pho89, supports cellular growth in low ambient Pi in alkaline conditions, and in high Pi under all conditions. A triple mutant with intact *PHO89* efficiently takes up Pi between pH 6 and 9, while its uptake velocity abruptly slows at pH≤5. The predicted low affinity transporters Pho87 and Fgr2 sustain growth well at acidic pH≤6 in high ambient Pi. Pho84 alone among the studied transporters signals to TORC1; it is also most important for tolerance of fluconazole and amphotericin. A quadruple null mutant of these 4 transporters can grow in medium with inorganic phosphate as a sole source of Pi under acidic conditions, but not at alkaline pH. Glycerophosphocholine (GPC) can compete out Pi uptake in *pho84-/- pho89-/- pho87-/- fgr2-/-* quadruple mutants in acidic conditions, suggesting that the residual Pi uptake in these mutants is provided by GPC transporters. *C. albicans* may be better adapted to Pi acquisition at low pH, a condition rarely encountered during host invasion, but perhaps prevalent in microenvironments shaped by bacterial flora of the GI tract.

285V Endocytosis of the tetraspan eisosome-resident proteins, a developmentally regulated membrane-remodeling mechanism Spiros Gaitanos[1], *Ada Biratsi*[1], Alexandros Athanasopoulos[1], Vicky Sophianopoulou[1] 1) Institute of Biosciences and Applications, NCSR Demokritos.

In eukaryotic microorganisms, endocytosis is an efficient mechanism for adapting the PM to the needs of a rapidly changing environment [1]. Most membrane proteins in *A. nidulans* are endocytosed by addition of ubiquitin (Ub), mediated by the single member of the Nedd4 Ub-ligase family, Rsp5/HulA [2, p. 1]. Rsp5/HulA recognizes its substrates using a set of adaptors, the α-arrestin proteins [3]–[5]. We have shown that SurG and AnNce102 eisosome (MCC)-resident transmembrane proteins of *A. nidulans* are targeted to the vacuole for degradation during MCC disassembly, induced either genetically or upon sphingolipid depletion [6], [7] . While it is known that sphingolipid depletion induces TORC2 [8] no α-arrestin has up to date been shown to be regulated by the TORC2 signaling pathway, related to both eisosomes and the integrity and stress of the plasma membrane [9]. Thus, AnNce102 and SurG of *A. nidulans* appear to be ideal model proteins to dissect a novel molecular mechanism by which TORC2 regulates the abundance of membrane proteins. Our primary approach towards this was the identification of the pathway(s) governing the degradation of the MCC-resident transmembrane proteins Nce102 and SurG. Our results showed that degradation of AnNce102 and SurG is developmentally regulated. In quiescent conidia is carried out through a clathrin-dependent, (SagA-dependent), ubiquitin-independent (C2-independent) mechanism. On the contrary, in developed mycelia our results strongly evidenced that SurG and AnNce102 degradation is both clathrin- and ubiquitination-independent as well as is mediated through direct sorting from the Golgi apparatus without the participation of the plasma membrane.

286V Functional analysis of the Cwh43p ortholog CwhA of *Fusarium fujikuroi*. *Marta Franco-Losilla*[1], Rasha Khaddaj[2], Javier Avalos[1], M. Carmen Limón[1], Roger Schneiter[2] 1) University of Seville, Seville; 2) University of Fribourg, Fribourg.

Fusarium fujikuroi is a model for studying production of secondary metabolites, which include carotenoids. Protein CarS, a negative

regulator of carotenogenesis has two RING finger domains, characteristic of proteins with E3 ubiquitin ligase activity. A previous yeast-two hybrid assay showed that CarS interacts with different proteins, including CwhA, orthologous *of Saccharomyces cerevisiae* Cwh43p. Furthermore, a previous transcriptomic analysis in the *carS* mutant showed higher levels of *cwhA*, indicating that CarS could control *cwhA* transcription.
Cwh43p is a conserved transmembrane protein that plays a role in lipid homeostasis and in remodeling of GPI lipids to ceramide in glycosylphosphatidylinositol (GPI)-anchored proteins. To understand the role of CwhA in *F. fujikuroi* and its possible relation with CarS regulation, we deleted the *cwhA* gene in the wild type strain and in a *carS* mutant. A first phenotypic study showed no changes in the morphology of the colonies compared to control strains, but lower levels of conidia germination were detected in the *ΔcwhA* mutant, both in wild type and *carS* mutant backgrounds. To examine a possible role of CwhA in membrane integrity, susceptibility to different stresses are being investigated. No differences have been observed under osmotic stress or in the presence of CalcoFluor White. In addition, ubiquitination assays are in progress to study the possible role of CarS in the function of CwhA at the protein level.
Due to its similarity to Cwh43, we have examined the ability of CwhA to remodel GPI lipids to ceramide in glycosylphosphatidylinositol (GPI)-anchored proteins. CwhA was expressed under the *ADH* promoter in a *S. cerevisiae* Δ*CWH43* mutant and ceramides were analyzed. For this, GPI proteins were labeled in vivo with myo-[3H]-inositol and lipid moieties were isolated from radiolabeled GPI proteins. The different lipid moieties, phosphatidylinositol (containing a C26 fatty acid) and inositolphosphorylceramides, were analyzed by thin layer chromatography. *CwhA* restored the ability to remodel GPI lipids to ceramide in GPI-anchored proteins in *ΔCWH43 S. cerevisiae*, suggesting a similar role of this protein in *F. fujikuroi*.
To better understand the role of CwhA in lipid metabolism of F. fujikuroi, the lipids of the mutants ΔcwhA and carS-ΔcwhA will be analyzed biochemically and compared to those of control strains.

287V The emerging role of a cyclase gene in the biosynthesis of ochratoxin A: The case study of *Aspergillus carbonarius* *Massimo Ferrara*[1], Antonia Gallo[2], Carla Cervini[3], Lucia Gambacorta[1], Michele Solfrizzo[1], Scott Baker[4], Giancarlo Perrone[1] 1) Institute of Sciences of Food Production (ISPA) National Research Council (CNR), Bari, Italy; 2) Institute of Sciences of Food Production (ISPA) National Research Council (CNR), Lecce, Italy; 3) Applied Mycology Group, Environment and AgriFood Theme, Cranfield University, Cranfield, UK; 4) Pacific Northwest National Laboratory, Richland, WA, US.

Ochratoxin A (OTA) is a well-known mycotoxin with wide distribution in food and feed, including cereal products, grapes and by-products, coffee, beverages, cocoa, nuts, dried fruits, and cured meat. Ochratoxin A is produced by many species of the genus *Aspergillus* and *Penicillium*. One of the main OTA producing species is *Aspergillus carbonarius*, known not only for its high capacity for producing OTA and its high percentage of toxigenic strains but also because it is considered the main species for OTA contamination of grapes in the vineyard worldwide. Fungal genome sequencing has great utility for identifying secondary metabolites gene clusters for known and novel compounds. A comparative genomic analysis of the OTA-biosynthetic cluster in 21 OTA-producing species has revealed a high synteny in OTA cluster organization in five structural genes (*otaA*, *otaB, ota*, *otaR1*, and *otaD*). Moreover, a cyclase gene, *otaY*, located in the OTA cluster between the *otaA* and *otaB* genes, was identified. This gene encodes for a predicted protein with high similarity to SnoaLs domain containing proteins. Similar proteins have been shown in *Streptomyces* to catalyze cyclization steps in the biosynthesis of polyketide antibiotics and we hypothesized it to be involved also in OTA biosynthesis. The expression level of the cyclase gene has been investigated to demonstrate its correlation to the kinetics of OTA accumulation and the expression profile of the other OTA biosynthetic genes under permissive OTA conditions. We demonstrated the role of the *otaY* gene by complete gene deletion using the CRISPR/Cas9 approach. The deletion of *otaY* gene stopped the biosynthesis of OTA and giving the first functional evidence of the involvement of a new gene in the biosynthetic pathway of OTA in *A. carbonarius.* Our findings represent a knowledge advancement in the molecular basis of OTA biosynthesis.

288V A novel trichothecene toxin phenotype associated with horizontal transfer and altered gene function in the *Fusarium buharicum* species complex *Robert Proctor*[1], Guixia Hao[1], Hye-Seon Kim[1], Martha Vaughan[1], Susan McCormick[1] 1) USDA Agriculture Research Service, National Center for Agricultural Utilization Research, Peoria, Illinois, USA.

Contamination of food and feed crops with trichothecene toxins is attributed to species in two closely related lineages of *Fusarium*: the *Fusarium incarnatum-equiseti* (FIESC) and *F. sambucinum* (FSAMSC) species complexes. In these fungi, trichothecene production is conferred by the 10 – 14-gene trichothecene biosynthetic gene (*TRI*) cluster. Here, we identified and characterized a *TRI* cluster homolog in an undescribed *Fusarium* species, represented by strain NRRL 66739, in the *F. buharicum* species complex, which is distantly related to FIESC and FSAMSC. The *TRI* cluster in NRRL 66739 consisted of only eight genes, and chemical analysis revealed that the fungus produces high levels of two structurally simple trichothecene analogs, 7-hydroxyisotrichodermol and 7-hydroxyisotrichodermin, that have been reported only as minor metabolites in other *Fusarium* species. Despite this production phenotype, NRRL 66739 lacks *TRI1*, the gene that confers trichothecene 7-hydroxylation in other *Fusarium* species. Heterologous expression analysis revealed that the NRRL 66739 *TRI*-cluster gene *TRI13* confers 7-hydroxylation. This contrasts other *Fusarium* species in which *TRI13* confers trichothecene 4-hydroxylation. Results of phylogenetic analyses suggest horizontal transfer of *TRI* genes from a common ancestor of FIESC and FSAMSC to an ancestor of NRRL 66739. Based on these data, we propose that the novel trichothecene phenotype of NRRL 66739 has resulted from horizontal transfer of part of the *TRI* cluster between distantly related lineages of *Fusarium* as well as a change in *TRI13* function.

289V Evolution of secondary metabolite gene clusters: what is the role of fungal interactions in driving metabolic diversification? *Mario Franco*[1], Roxanne Bantay[1], Malak Tfaily[2], Lillian Moore[1], Megan Nickerson[1], Roya AminiTabrizi[2], Christian Ayala-Ortiz[2], Katherine Louie[3], Trent Northen[3], Jana U'Ren[1] 1) BIO5 Institute and Department of Biosystems Engineering, The University of Arizona, Tucson, AZ; 2) Department of Environmental Science, The University of Arizona, Tucson, AZ; 3) Department of Energy, The Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA.

The Xylariaceae (Sordariomycetes) comprise one of the largest and most diverse families of Ascomycota. Xylarialean fungi are common wood- or litter-degrading saprotrophs or woody pathogens in temperate or tropical ecosystems. In addition, many xylarialean fungi appear to have an endophytic life stage, whereby they asymptomatically inhabit the healthy, photosynthetic tissues of diverse lineages

of land plants and lichens. Our recent genomic analyses suggest that diversification of metabolite gene clusters (SMGCs), particularly those involved in the production of secondary metabolites, is driven by horizontal gene transfer (HGT). In particular, the genomes of *Xylaria flabelliformis* harbor the greatest number of SMGCs (i.e., >100 per genome) and have experienced the highest number of putative HGT events. We hypothesized that the high diversity of SMGCs in genomes of *X. flabelliformis* is due to selection for secondary metabolites that increase competitive abilities in complex microbial communities. Here, we used untargeted metabolomics to assess the metabolic activity of *X. flabelliformis* when grown alone or in co-culture with conspecific isolates, as well as more phylogenetically distant fungi. Overall, we observed that most co-cultures resulted in a deadlock between isolates, indicative of the production of inhibitory secondary metabolites. Statistical analyses revealed that 1,370 features were significantly up-regulated in all co-culture treatments compared to isolates grown alone or media controls. Our ongoing analyses will characterize the core set of metabolites that *X. flabelliformis* uses against all fungal competitors, as well as secondary metabolites employed against more specific antagonists. In doing so, we will assess the role of fungal interactions to potentially select diverse secondary metabolite gene clusters in xylarialean fungi.

290V The genetic basis of oligopeptide biosynthesis in the early diverging fungus *Mortierella alpina* *Jacob Martin Wurlitzer*[1], Aleksa Stanišić[2], Hajo Kries[2], Markus Gressler[1] 1) Department of Pharmaceutical Microbiology, Friedrich Schiller University, Jena, Germany; 2) Junior Research Group Biosynthetic Design of Natural Products, Leibniz Institute for Natural Product Research and Infection Biology (Hans Knöll Institute), Jena, Germany.

The Asco- and the Basidiomycota have been recognized as prolific producers of bioactive natural compounds while the early diverging fungi, specifically the “zygomycetes”, have traditionally been dismissed as poor producers of secondary metabolites. In fact, only a small number of compounds has previously been identified, among them the terpenoid signaling compound trisporic acid from *Blakeslea trispora* and antimitotic agent rhizoxin from *Rhizopus microsporus*, produced by its bacterial endosymbiont.
Relying solely on analytical chemistry, may have severely underestimated the true metabolic diversity of basal fungi, as genomic sequencing of the oleaginous fungus *Mortierella alpina,* used in food industry to produce polyunsaturated fatty acids, identified as many as 21 genes encoding nonribosomal peptide synthetases (NRPSs).
This work aimed at resolving the discrepancy between genetic and chemical data. We performed next generation sequencing, qRT-PCR, heterologous gene expression of adenylation domains and state-of-the-art LC-MS/MS based specificity profiling *in vitro.* This combined approach, in parallel with biological assays, identified three series of bioactive oligopeptide secondary metabolites from *M. alpina*: the malpibaldins, the malpicyclins[1] (both cyclic pentapeptides), and the malpinins[2], i.e., linear hexapeptides. For all three series of compounds, the respective biosynthetic genes were identified in the genome and functionally characterized.
From the genetic perspective, these oligopeptides are intriguing as a phylogenetic analysis suggested a bacterial origin, which is supported by the occurrence of bacterial dual epimerase/condensation domains. Still, conserved intron positions in the NRPS genes unambiguously identified these NRPSs as eukaryotic. Remarkable (bio-)chemical features include mostly D-configured amino acids, N-acetylation, and non-proteinogenic amino acids, such as dehydrobutyrine, broad A-domain specificity, and module skipping. These and other findings based on the unusual properties of the genes and metabolites will be part of the presentation.

[1] Wurlitzer et al. 2021 Bacterial-Like Nonribosomal Peptide Synthetases Produce Cyclopeptides in the Zygomycetous Fungus *Mortierella alpina*. AEM 87(3), e02051-20
[2] Baldeweg et. al. 2019. Fungal Biosurfactants from Mortierella alpina. Organic letters 21 (5), 1444-1448

291V New approaches to understand the regulation of neurosporaxanthin biosynthesis in *Fusarium fujikuroi* and its relationship with other biological processes. *Julia Marente* [1], Laura Perez-Fons[2], Javier Avalos[1], M Carmen Limón-Mirón[1], Paul Fraser[2] 1) University of Sevilla, Sevilla, Spain; 2) Royal Holloway University of London, Egham, UK.

Neurosporaxanthin is a particular xanthophyll with a high antioxidant potential and promising perspectives for industry. The filamentous fungus *F. fujikuroi* is a natural source of this carotenoid, and the regulation of its biosynthetic pathway has been studied for years. At the moment, the most studied regulator is the CarS protein that has been confirmed as a negative regulator of the structural genes involved in the pathway, which are called *car* genes. Mutations in the *carS* gene results in carotenoid overproducing strains in all culture conditions tested. However, the effect of *carS* mutation is not limited to the up-regulation of the *car* genes, as transcriptomic studies have revealed it is affecting the expression of a high number of genes, this suggests a functional role of CarS beyond the carotenoids pathway regulation. So, with the purpose to better understand the putative effect of CarS on other biological processes, the metabolic profiling of wild strain and some carotenoids regulator mutants have been analysed by GC-MS and HPLC-PDA, to establish new regulatory hubs associated with carotenoid formation.

New regulatory targets need to be found in order to better understand the molecular mechanisms underlying the regulation of this pathway. As a promising candidate, a gene for a putative zinc-finger protein transcription factor has been found next to the structural car genes. Preliminary data based on the deletion mutant of this gene is also presented.

292V Unprecedented polyketide synthase genes are associated with (pre-)anthraquinone biosynthesis in *Cortinarius* mushrooms *Nikolai Löhr*[1], Jonas Motter[1], Markus Gressler[1], Dirk Hoffmeister[1] 1) Friedrich Schiller University, Department Pharmaceutical Microbiology at the Hans-Knöll-Institute.

(Pre-)Anthraquinones (PAs) are a diverse class of polyketide natural products produced by both Asco- and Basidiomycota. In Ascomycota, polyketide synthase genes and enzymes that mediate PA-biosynthesis are well characterized, as shown, e.g., by *Aspergillus nidulans* enzymes AptA and MdpG.[1] Although chemical analyses showed that *Cortinarius* and numerous other basidiomycete species produce PAs and their derivatives, the corresponding biosynthetic genes and enzymes remained unknown.[2] Surprisingly, genomic data, e.g., for *Cortinarius* species, did not indicate the presence of any *aptA/mdpG* homolog, but revealed unprecedented PKS genes that fall into an evolutionarily distinct clade. Therefore, we hypothesized that *Cortinarius* and other Basidiomycota produce PAs via this new class of PKSs. To test this hypothesis, two near-identical candidate genes (*copks1* and *copks4*) were cloned and heterolo-

gously expressed in *Aspergillus niger*. Combined *in vivo* and *in vitro* product formation assays established CoPKS1 and CoPKS4 as active synthases producing the octaketide atrochrysone carboxylic acid (ACC), the universal precursor of PAs. Thus, these enzymes represent both the first mushroom octaketide PKSs and first characterized members of the entire evolutionary clade to which they belong. Strikingly, CoPKS1 exclusively produces ACC, while CoPKS4 is intrinsically flexible to simultaneously biosynthesize hepta- and octaketides. Therefore, these PKS twins represent an ideal system to study chain length control in fungal PKSs.

[1] L. K. Caesar, N. L. Kelleher, N. P. Keller, *Fungal Genet. Biol.* 2020, **144**, 103477.
[2] M. Gressler, N. A. Löhr, T. Schäfer, S. Lawrinowitz, P. S. Seibold, D. Hoffmeister, *Nat. Prod. Rep.* 2021, **38**, 702–722.

293W Heterochromatin protein 1 (HP1) knock-out mutants exhibit cellulolytic enzyme cocktail alterations in *Trichoderma reesei* Sarah Fajon[1], Jean Lagarde[1], Thiziri Aouam[1], Etienne Jourdier[1], Sophie Lemoine[3], Jade Guglieri[3], Aurélie Pirayre[1], Fabienne Malagnac[2], *Frederique Bidard*[1] 1) IFP Energies nouvelles, Département Biotechnologie, 1 et 4 avenue de Bois-Préau, 92852 Rueil-Malmaison, France; 2) Institute for Integrative Biology of the Cell, CEA, CNRS, Univ. Paris-Sud, Universite Paris-Saclay, 91198, Gif-sur-Yvette cedex, France; 3) Ecole normale superieure, PLS Research University, CNRS, Inserm, Institut de Biologie de l'Ecole normale supérieure (IBENS), Plateforme Genomique, 75005 Paris, France..

The filamentous fungus *Trichoderma reesei* can secrete high amounts of cellulolytic enzymes which are used at an industrial scale to produce fermentable sugars from lignocellulosic biomass. In fungi, various biological processes, such as a rapid response to environmental changes, the production of secondary metabolites or industrial enzymes, involve epigenetic control mechanisms via chromatin status changes. The acquisition of such knowledge is crucial for the definition of strategies aiming at efficiently controlling fungal productions.

Recently, Zhang et al. (2016) have investigated the contribution of HP1 to the regulation of cellulolytic enzymes in the filamentous fungus *Penicillium oxalicum.* The *hp1* gene encodes the H3K9me3 reader protein and is responsible for the recruitment of the DIM2 protein, which affixes methylations to DNA cytosines (5mC). The authors observed a down regulation of the main cellulases genes in the mutant strain whereas HP1 overexpression results in an up regulation.

To investigate the role of HP1 in cellulases production in *T. reesei, hp1* has been deleted in two strains: the wild type QM6a and the hyperproducer RutC30. In batch culture on cellulose – lactose mixture, no differences in protein secretion amount is observed for both strains but a lower enzymatic activity is measured for QM6a indicating an alteration of the secretome. In lactose fed-batch growth, a reduced protein production is obtained for RutC30 but not for QM6a. On the other hand, a transcriptomic study shows that cellulases genes are down regulated in both mutant strains and several secondary metabolite genes clusters upregulated. Genes differentially regulated between parental and mutant strains are not randomly distributed as genes close to AT-rich region are more likely to be less expressed in mutants.

Zhang X, Qu Y, Qin Y. (2016) Biotechnol Biofuels. 9:206.

294T Quantifying fungal pellets during submerged cultivation: from 3D X-ray microtomography imaging to diffusive mass transport *Lars Barthel*[1], Stefan Schmideder[2], Henri Müller[2], Heiko Briesen[2], Vera Meyer[1] 1) Technische Universität Berlin; 2) Technical University of Munich.

Filamentous fungal cell factories are of great importance in modern biotechnology and for the creation of a sustainable circular bioeconomy. They are broadly applied for the industrial production of many crucial biomolecules of everyday life, including enzymes or organic acids. Fungal (macro)morphology is strongly interlinked with product titers. In this study, X-ray microcomputed tomography (μCT) was proven to be a powerful tool for non-destructive, three-dimensional structural analysis of pellet-forming filamentous fungi in utmost detail on the example of *Aspergillus niger*. The morphological data obtained from μCT measurements of individual pellets were used to calculate the effective diffusion factor (k_{eff}) in representative cubic subvolumes and subsequently correlate them to the hyphal fraction (c_h, equal to solid fraction) of these cubes. This analysis revealed the relation $k_{eff} = (1- c_h)^a$, with only one fitting parameter *a*. To prove the universal validity of this law for diffusive mass transport through fungal mycelium, four morphologically different fungal strains as well as a total number of 3125 diverse simulated fungal structures, covering a very broad field of theoretically possible morphological properties of filamentous fungi, were analysed. This investigation validated the universality of the discovered law and determined the fitting parameter $a = 1.76$. Being able to calculate mass transport in fungal structures based on the profile of hyphal fraction constitutes a significant step towards the prediction of metabolite and nutrient concentration inside pellets.

295F Synthetic tools to regulate unconventional secretion for production of heterologous proteins in *Ustilago maydis* *Kai Hussnaetter*[1], Magnus Philipp[1], Michael Feldbrügge[1], Kerstin Schipper[1] 1) Heinrich Heine University.

Biotechnological production and secretion of heterologous proteins puts high demands on chassis and production conditions. Especially secretion of the product to the culture broth is advantageous because it drastically reduces downstream processing costs. We exploit unconventional secretion for heterologous protein expression in the fungal model microorganism *Ustilago maydis*. Hitchhiking of the endogenous unconventional secretory machinery of the chitinase Cts1 via an alternative export route allows secretion of unglycosylated proteins, which can be advantageous for example for distinct pharmaceutical or bacterial targets. Proteins of interest are fused to carrier chitinase Cts1 for export via the fragmentation zone of dividing yeast cells in a lock-type mechanism. The kinase Don3 is essential for assembly of the functional fragmentation zone and therefore acts as a gatekeeper for unconventional secretion of Cts1 fusion proteins Here, we developed a novel regulatory system for unconventional protein secretion using Don3 to regulate export. This enables uncoupling the accumulation of biomass and protein synthesis of a product of choice from its release. Regulation was successfully established at two different levels using transcriptional and post-translational induction strategies, based on diauxic switches of the carbon source or chemical genetics, respectively. Different cultivation strategies comprise tailored solutions for differentially products and thus constitute another important step towards a competitive protein production platform.

296W Novel Technologies For Investigating Biological Rhythms In Budding Yeast *Tom Mickleburgh*[1], Nicolas Buchler[1] 1) North Carolina State University.

Fungi secrete biomolecules and metabolites to modify their external environment and extract nutrients for growth. This shared growth environment makes a fungal population especially sensitive to metabolic synchronization. We study metabolic synchronization in budding yeast, which exhibit synchronous and robust oscillations in global metabolism when grown in bioreactors. This phenomenon, called the yeast metabolic cycle, is accompanied by the periodic expression of genes and metabolites, which interact with, but can be independent of, the cell division cycle. Current tools for studying the yeast metabolic cycle involve large, expensive bioreactors and manual extraction of samples for downstream analysis with biochemical or genomic assays. These bulk assays are population averages and do not provide any insights into metabolic and cell cycle dynamics within a single cell. To overcome these limitations, we developed and combined two technologies.

The first is a cost-effective miniature (20-mL) bioreactor array. Its manageable size increases throughput, and autonomous computer control permits continuous, real-time fluorescence and luminescence measurements for multiple strains. By measuring cell cycle events (i.e., Cln3 translation or Clb2 transcription) or metabolic states (i.e., NAD(P)H or storage carbohydrates) within these small reactors, we show how key metabolic and cell-cycle processes are compartmentalized relative to dissolved oxygen consumption. Surprisingly, we find that S288c and CEN.PK (the two most commonly used strains for the yeast metabolic cycle) exhibit opposite timing of key processes relative to oxygen utilization.

This bioreactor array, although useful, is still limited to measuring population-averaged, bulk signals. This inspired our second technology, a microfluidic device that images cell cycle and metabolic states of trapped single cells that are responding to the real-time, continuous flow of bioreactor-conditioned media. For the first time, we track single-cells through the yeast metabolic cycle and we identify temporally distinct sub-populations: (1) Mothers that enter the cell cycle every metabolic oscillation and (2) their daughters that delay cell cycle entry before producing a granddaughter during the next metabolic cycle.

While we have showcased our technologies in the context of measuring biological rhythms in budding yeast, it represents only a fraction of their potential uses. Chemostats are employed for a variety of fungal cell types and experimental applications (i.e., mutagenesis and evolution), and our bioreactor-microfluidic device could help accelerate advances in these parallel fields.

297T Insertional mutagenesis using *TC1-mariner* transposon *impala* in the wheat fungal pathogen *Zymoseptoria tritici* *Yohann Petit*[1], Anaïs Pitarch[1], Camille Delude[1,3], Marie Dufresne[2], Gabriel Scalliet[3], Marc-Henri Lebrun[1] 1) Université Paris-Saclay, INRAE, UR1290 BIOGER, 78850, Thiverval-Grignon, France; 2) Université Paris-Saclay, CNRS, INRAE, University of Evry, Institute of Plant Sciences Paris-Saclay, Orsay, France; 3) Syngenta Crop Protection AG, CH-4332 Stein, Switzerland.

Zymoseptoria tritici is a fungal pathogen causing one of the worst diseases on wheat, Septoria Tritici Blotch (STB). One way to decipher molecular interactions between such a fungus and its host plant is loss-of-function mutagenesis and the generation of a mutant library. Impala is a fungal Class II DNA transposon of the TC1-mariner family already used for such studies in several fungi. This transposon is composed of two Terminal Inverted Repeats (TIR) and a transposase coding sequence, the transposase being necessary to excise the transposon at the TIR sequences and to transpose it through a cut and paste mechanism. In this study we propose a transposon-based loss-of-function tool in *Z. tritici* using the fungal transposable element *impala*. Excision vectors containing an autonomous copy of *impala* inserted in the *A. nidulans* nitrate reductase gene were used to select *impala* excision events, allowing us to generate a library of 224 mutants for which the *impala* insertion point is known. *Impala* presents very good rates of excision (85%) and re-insertion (90%), and inserts mostly in genes (93%), more precisely upstream of the transcription starting site (70%). Those mutants have been tested for their ability to infect wheat, and some of them show a defect in pathogeny. Our loss-of-function mutagenesis tool is also amenable to perform gain-of-function screens by replacing the *impala* transposase coding sequence by a strong promotor in order to perform activation tagging. The chimeric *impala*:*Gpd* transposon was able to excise and re-insert in the *Z. tritici* genome, and characterization of a few insertion sites showed that *impala*:*Gpd* inserts mainly in 5'UTRs and promoters of *Z. tritici* genes, as with native *impala*. Our study provides a new tool to perform mutagenesis in *Z. tritici* with the final goal to identify candidate genes involved in infectious process. This tool also paves the way for the development of an even more efficient tool using an hyperactive version of the *impala* transposase, or a transposon with a higher transposition frequency. We have been able to identify such transposon, and preliminary results suggests that it will soon be possible to perform saturation mutagenesis in *Z. tritici*.

298F Identification and characterization of an intergenic "safe haven" region in human fungal pathogen *Cryptococcus gattii* *Yeqi Li*[1], Tuyetnhu Pham[1], Xiaofeng Xie[1], Xiaorong Lin[1] 1) University of Georgia.

Cryptococcus gattii is a primary fungal pathogen which causes pulmonary and brain infections in healthy as well as immunocompromised individuals. Genetic manipulations in this pathogen, such as gene deletion, gene complementation and gene overexpression, are widely employed to study its biology and pathogenesis. These genetic manipulations require integration of foreign DNA fragment into the genome. Currently, this is often achieved by ectopic integration, which could potentially disrupt other genes or cause unintended changes in fungal biology due to position effects. Thus, identification of gene free regions where integrated foreign DNA can be expressed without influencing or being influenced by nearby genes would be extremely valuable. To achieve that goal, we examined the publicly available genomes and transcriptomes of *C. gattii* and identified two intergenic regions in the reference strain R265 as potential "safe haven" region, named as CgSH1 and CgSH2. We found that insertion of a fluorescent reporter gene and a selection marker at these two intergenic regions did not affect the expression of the neighboring genes. The inserted genes were also expressed efficiently as expected for the constitutively active promoter used. Furthermore, the integrated DNA at CgSH1 or CgSH2 had no apparent effect on the growth of *C. gattii* or its response to various stresses. Compared to the parental strain, the transformants with DNA integrated at CgSH1 or CgSH2 also showed similar phagocytosis by murine macrophages. Importantly, a similar intergenic region of CgSH1 exists in all six species within the *C. gattii* species complex. Taken together, the identified safe haven regions in *C. gattii* provide an effective tool for researchers to reduce variations and increase reproducibility in genetic experiments.

299W Repair of CRISPR-Cas12a induced DNA double-strand breaks in *Magnaporthe oryzae* generates locus-dependent mutation profiles *Jun Huang*[1], David Rowe[1], Pratima Subedi[1], Wei Zhang[1], Tyler Suelter[1], Barbara Valent[1], David Cook[1] 1) Department of Plant Pathology, Kansas State University.

CRISPR-Cas mediated genome engineering has revolutionized functional genomics. However, basic questions remain regarding the mechanisms of DNA repair following Cas-mediated DNA cleavage. We developed CRISPR-Cas12a ribonucleoprotein genome editing in the fungal plant pathogen, *Magnaporthe oryzae*, and found frequent donor DNA integration despite the absence of long sequence homology. Interestingly, genotyping from hundreds of transformants showed that frequent non-canonical DNA repair outcomes predominated the recovered genome edited strains. Detailed analysis using sanger and nanopore long-read sequencing revealed five classes of DNA repair mutations, including simple donor DNA insertions, concatemer donor DNA insertions, large DNA deletions, deletions plus donor DNA insertions, and, infrequently, INDELs. Our results indicate that different DNA repair pathways resolved the Cas12a-mediated double-strand breaks (DSBs) based on the DNA sequence of edited strains. Furthermore, we found that the frequency of the different DNA repair outcomes varied across five tested loci, and repair at some loci resulted in more frequent large-scale mutations. To further show that multiple DNA repair pathways are active in *M. oryzae*, and that they may differentially contribute to DNA repair outcomes, we deleted a key component required for canonical non-homologous end-joining (NHEJ). In this mutant background, frequent edited transformants were recovered that had simple or large donor DNA insertions. This shows that there is both NHEJ-dependent and -independent pathways active in *M. oryzae*, and suggests the different repair pathways may result in different DNA repair mutations. While this research is still on-going, these results provide evidence that DNA repair pathways may provide preferential repair across the genome that could create biased genome variation, which has significant implications for genome engineering and genome evolution in *M. oryzae* populations.

300T Use of a cell-free expression (CFE) to fast characterize fungal enzymes in the wood decomposer *Rhodonia placenta* *Jesus Castano Uruena*[1], Joshua Goering[1], Irina Novikova[2], James E. Evans[2], Jiwei Zhang[1] 1) Department of Bioproducts and Biosystems Engineering, University of Minnesota, Twin Cities, MN; 2) Pacific Northwest National Laboratory, Richland, WA.

Brown rot fungi are efficient wood degraders that rely on ROS (Reactive Oxygen Species; e.g., ·OH) generated by the Fenton reaction to break down the lignin barrier. This system adapted the mechanisms used by white rotters for lignocellulose hydrolysis, but it may have also developed unique enzymes to tolerate ROS. Using crude enzymes, our previous work indicated that glycosyl hydrolases and pectinases are tolerant of ROS in the brown-rot fungus *Rhodonia placenta*, relative to white-rot and soft-rot fungi. However, this hasn't been validated using purified enzymes, and these brown rot enzymes with promising application potential haven't been fully characterized. A high-throughput method to produce high quality/quantity proteins for enzyme characterization is essential for this research. In our work, we targeted 14 key glycosyl hydrolases and oxidoreductases that are important for early wood depolymerization in *R. placenta*, and used a wheat germ-based CFE system to synthesize these enzymes. Using predicted coding sequences of *R. placenta* from the public JGI-MycoCosm genomic annotation database, we successfully synthesized all of the 14 enzymes. Four of them (~30% success rate) were characterized with the expected enzyme activities, namely an α-L-arabinofuranosidase, a benzoquinone reductase (BQR), a ferric reductase, and a heme-thiolate peroxidase (HTP). Further characterization of the optimal pH (4-6) and temperature (35°C-45°C) indicated that they were typical fungal enzymes. Among them, the α-L-arabinofuranosidase was remarkably stable at a broad range of pH values (3-8) and showed high stability to temperature (60°C), whereas the other enzymes were relatively sensitive. This α-L-arabinofuranosidase also showed clear resistance to potential inhibitors such as EDTA, NaN_3, or metal ions. More importantly, it was remarkably tolerant of ROS generated by the Fenton reaction compared to the other enzymes that were highly damaged by the oxidative treatment. These results suggest that brown-rot enzymes like α-L-arabinofuranosidases may withstand the pressure of a harsh environment characterized by the presence of ROS in fungi, and this implies a potential synergistic effect between oxidative and hydrolytic degradation during brown-rot. The CFE method and distinctive features revealed by this work certainly render brown-rot enzymes promising tools for new biotechnological applications.

301F Understanding DNA Uptake by Anaerobic Fungi *Tejas Navaratna*[1], Jessy Gonzalez[1], Michelle O'Malley[1] 1) UC Santa Barbara.

Anaerobic fungi, found in the rumen of herbivores, are powerful degraders of lignocellulosic biomass and are equipped with a broad suite of enzymes to accomplish this goal. These organisms are found in diverse multi-kingdom communities and have been shown to produce a range of biotechnologically valuable products, including short- and medium-chain fatty acids and bioactive small molecules. However, a major bottleneck remains in the translation of anaerobic fungi to the industrial setting as there is a lack of robust genetic engineering strategies to generate protein diversity, introduce heterologous genes, and create specific knockouts for functional enhancement.

A necessary first step for genetic manipulation is the introduction of foreign DNA and/or nucleoprotein complexes. The life cycle of anaerobic fungi involves production of motile, unicellular zoospores that due to their thinner cell wall, are potentially more amenable to genetic manipulation than mature life stages. We show that zoospores can be analyzed and sorted by fluorescence-activated cell sorting (FACS) to gain insights into the size-dependence of DNA entry. We constructed a library of fluorescent dsDNA probes and interrogated uptake by natural competence and electroporation across a range of parameters. Combined with insights into survival post-transformation, these data show robust zoospore entry across the length range. Furthermore, we tested cell entry and nuclear localization of a GFP-Cas9 ribonucleoprotein complex by FACS and fluorescence microscopy, and all together, these findings inform a systematic approach for transformation of anaerobic fungi.

302W Fungi to the rescue – revolutionizing food production through biotechnology Britta Winterberg[1], *Bastian Joehnk*[1] 1) Formo Bio GmbH.

The current food system is causing colossal problems. From climate change to food security and public health, the issues are manifold. Over 4% of global greenhouse gas (GHG) emissions come from dairy cattle alone. That's as much as the GHG as is produced by all airplanes and ships combined.worldwide shipping and aviation combined. The UN Food and Agriculture Organization predicts there'll

be a 50-100% increase in demand for animal protein by 2050. If we source this through traditional methods, we will continue causing serious environmental damage and resource scarcity. Animal agriculture is the number one source of zoonotic disease outbreaks. Almost 90% of the world's animal species will lose their habitat to agriculture by 2050 if we continue on the current path. It's also a key contributor to antibiotic resistance. We need a safer, more sustainable, and more efficient way of producing food.
Formo's approach of combining biotechnology and traditional cheese production is revolutionizing the food sector. To this end, Formo uses various microbial hosts and precision fermentation to recombinantly express the milk proteins. These form the basis for dairy products such as animal-free cheese. Caseins, the most prominent proteins in bovine milk, are responsible for texture and organoleptic properties of cheese. These proteins exhibit no secondary structure and form large protein aggregates (micelles) in milk. Expressing these proteins in microbial cell factories poses a major challenge due to their molecular structure. Fungi are ideal hosts due to their ability to secrete recombinant proteins with high yield and capability for post-translational modification.
Life-cycle assessments have shown that milk protein production through precision fermentation causes 93% less GHG emission, uses 98% less water and 84% less energy.
Microorganisms, especially fungi, carry enormous potential to help us achieve the UN Sustainability Development Goals. They have a long history in the production of enzymes and food additives. Producing the main ingredient of food through precision fermentation is the next logical step on the path to a more sustainable food system.

303T Development of fungal-based biomaterials using the tinder fungus *Fomes fomentarius* *Bertram Schmidt*[1], Carsten Pohl[1], Sophie Klemm[1], Ulla Simon[1], Isabel Regeler[1], Tamara Núñez Guitar[1], Aleksander Gurlo[1], Claudia Fleck[1], Vera Meyer[1] 1) Technische Universität Berlin.

Fungal-based materials could become the next generation of construction materials which are sustainable and suitable for a circular economy. Starting with a bioprospecting approach, we collected and isolated local wood-degrading fungi that were able to grow on local lignocellulosic residual streams from agriculture and forestry. One of the fast-growing species was the medicinal fungus *Fomes fomentarius* that grew well on rapeseed straw and hemp shives. To consider intraspecific diversity, eight isolates from different trees were analyzed regarding their growth rate and the best-performing strain PaPf11 was selected for biomaterial development.
We used fine, medium and coarse particle fractions of hemp shives and rapeseed straw to produce a set of diverse composite materials with *F. fomentarius* and show here that the mechanical materials properties are dependent on the nature and particle size of the substrates. Data from compression tests and scanning electron microscopy were used to characterize composite material properties that were similar to expanded polystyrene (EPS), a petroleum-based foam used for thermal isolation in the construction industry. We will furthermore show that pure mycelium of *F. fomentarius* can be used to produce stable pastes together with alginate to produce another class of composite materials by extrusion-based 3D printing. Interestingly, a combination of freeze-drying and calcium-crosslinking processes allowed the printed shapes to remain stable even in the presence of water. Our data demonstrate that *F. fomentarius* can be used for the production of composite materials including additive manufacturing to fabricate customized light-weight 3D objects.

304F Targeting *Aspergillus fumigatus* hypoxia response pathways to potentiate contemporary antifungal therapies *Cecilia Gutierrez Perez*[1], Sourabh Dhingra[1,2], Steven M Kwansy[3], Timothy J Opperman[3], Robert A Cramer[1] 1) Dartmouth College, Hanover NH, USA; 2) Clemson University, Clemson, SC, USA; 3) Microbiotix Inc, Worcester, MA, USA.

Aspergillus fumigatus is a ubiquitous airborne filamentous fungus that is estimated to contribute to 600,000 deaths each year. There are currently only three contemporary antifungal therapies to treat invasive *Aspergillus* infections. Rapidly increasing resistance to first line therapy voriconazole highlights a significant need to develop novel antifungals with innovative mechanisms of action. Research from our lab has shown that the hypoxia response, mediated by the transcriptional regulator SrbA, is necessary for virulence and azole resistance in *Aspergillus fumigatus*. Therefore, identifying a compound that inhibits the SrbA mediated hypoxia response pathway would introduce a potentially novel antifungal that could potentiate azoles activity *in vivo*. We designed and performed a high-throughput screen by adapting a gpdA-luciferase reporter system to screen over 200,000 small molecule compounds for antifungal activity in the presence of fluconazole or hypoxic conditions. Using a secondary screen measuring enhanced fluconazole sensitivity and hypoxia specificity, we confirmed 50 compounds that fit all parameters to date. We then prioritized compounds that show limited human toxicity and an MIC≤10 μM for further investigation. Increased SrbA expression through expression of the N terminus bHLH transcription reduces susceptibility to several of these molecules. These data suggest that the compounds may act on the SrbA dependent hypoxia response. Since SrbA pathway inhibition increases azole sensitivity, we next tested the compounds against voriconazole-resistant clinical isolates and determined that combination therapy increases voriconazole efficacy. Using this high-throughput screen and follow up secondary screens we have identified compounds that are hypoxia specific and potentiate azole therapy with minimal human toxicity. Additionally, preliminary data suggests that several compounds are acting through the SrbA dependent hypoxia response pathway, a well characterized virulence factor. Through this work we are finding that we can identify novel antifungal compounds that act through innovative and well characterized biological mechanisms. One potential application of these findings is that these compounds can be used in combination therapy to potentiate azoles and combat antifungal resistance.

305W Discovery and characterization of a potent antifungal peptide through OBOC combinatorial library screening. Shivani Bansal[1], *Kiem Vu*[2], Ruiwu Liu[1], Yousif Ajena[1], Wenwu Xiao[1], Angie Gelli[2], Kit Lam[1] 1) University of California, Department of Biochemistry and Molecular Medicine, SOM, Davis; 2) University of California, Department of Pharmacology, SOM, Davis.

Diseases caused by fungal pathogens affect over a billion people and kill approximately 1.7 million annually; however, these estimates are likely understated because of the lack of mandatory public health surveillance of fungal diseases. The dire need for novel antifungal drugs and the lack of antifungal therapies that act through novel mechanisms has prompted a greater interest in peptides as antifungal agents. Antifungal peptides (AFP) are bioactive molecules with broad spectrum activity and multiple mechanisms of action that have been used as template molecules for new drug design strategies with greater potency. The one-bead one-compound (OBOC) combinatorial chemistry technology generates unbiased bead-peptide libraries composed of discrete numbers of random natural and unnatural (i.e., non-coded) amino acids that can be rapidly screened. Using this innovative approach along with a unique screening platform, we discovered a bead-bound membrane active peptide (MAP), LBF127, that selectively binds fungal giant unilamellar vesicles (GUVs)

over mammalian GUVs. LBF127 was re-synthesized in solution form and demonstrated to have antifungal activity with limited hemolytic activity and cytotoxicity against mammalian cells. Through systematic structure-activity relationship (SARs) studies, including N- and C-terminal truncation, alanine walk, and D-amino acid substitution, an optimized peptide – K-oLBF127 – with higher potency, less hemolytic activity and cytotoxicity emerged. Compared to the parent peptide, K-oLBF127 is shorter by 3 amino acids and has a lysine at the N-terminus to confer an additional positive charge. K-oLBF127 was found to have improved selectivity towards the fungal membrane over mammalian membranes by 2-fold compared to LBF127. Further characterization revealed that while K-oLBF127 exhibits similar spectrum of antifungal activity compared to the original peptide, it had lower hemolytic activity and cytotoxicity against mammalian cells. Mice infected with *Cryptococcus neoformans* and treated with K-oLBF127 (16mg/kg) for 48 h had significantly lower lung fungal burden compared to untreated animals, consistent with K-oLBF127 being active *in vivo*. Our study demonstrates the success of the one-bead one-compound (OBOC) high-throughput strategy and sequential screening at identifying membrane active peptides with potent antifungal activities.

306T Copper bioleaching from treated wood waste and biosorption by *Phanerochaete chrysosporium* *Kevin Claudien*[1], Arnaud Besserer[2], Aurelie Deveau[3], Philippe Gerardin[2], Gaurav Pandharikar[3], Rodnay Sormani[1], Melanie Morel-Rouhier[1] 1) Université de Lorraine, INRAE, UMR1136, IAM, F-54000 Nancy, France; 2) Université de Lorraine, LERMAB, EA4370, F-54000 Nancy, France; 3) Université de Lorraine, INRAE, UMR1136, IAM, Champenoux, F-54280, France.

Wood can be used as a common building material thanks to the use of chemical preservation products that prevent degradation by ligninolytic fungi. The wood wastes generated by the wood building industry can reach 1.4 million tons per year in France. However, because of the high toxicity of the chemical compounds found in the preservative products, these wastes are not valorized and rather incinerated or buried. Based on the fact that some fungal strains are naturally resistant to fungicides used in such preservatives, we hypothesized that they could be efficient biocatalysts for wood waste decontamination. An *in vitro* set-up using the white-rot fungus *Phanerochaete chrysosporium* as a fungal model and copper-azole treated wood was used to follow copper bioleaching from treated sawdust. By combining X-Ray fluorescence spectroscopy (XRF) and scanning electron microscopy (SEM) coupled to microanalyses, we managed to demonstrate that *P. chrysosporium* is capable of copper bioleaching from the treated sawdust. Moreover, copper quantification by SEM microanalyses showed that the bioleached copper is efficiently sequestrated by the fungus, likely by biosorption within the hyphae cell wall. Further molecular analyses will help understanding the underlying mechanisms that explain the high resistance of the fungus to the preservative, the bioleaching of copper and the biosorption within hyphae.

307F Development of Broad-Spectrum Natural Antimicrobials using *Aspergillus oryzae* *Dasol Choi*[1], Ahmad Alshannaq[1], Emily Eix[1], Jeniel Nett[1], Jae-Hyuk Yu[1] 1) University of Wisconsin-Madison.

Despite tremendous efforts in the food and pharmaceutical industries, microbial foodborne illnesses and infectious diseases remain significant public health concerns resulting from antibiotic resistance. Over 48 million people get sick and 128,000 are hospitalized each year after eating food contaminated with pathogens. One of the best candidates to address these needs and challenges appears to be natural resources such as microbial, plant or animal origin. Regarding antimicrobials from microbes, filamentous fungi (molds) have a long history of being an excellent source of potent antimicrobials. We have developed two broad-spectrum antimicrobials called “NP1” and “NP2” by culturing the Generally Recognized As Safe (GRAS) fungus *Aspergillus oryzae* in proprietary food-grade media. Ethyl acetate extracts of NP1 and NP2 have shown strong antibacterial activity against food-borne pathogens: *Staphylococcus aureus, Listeria monocytogenes, Salmonella typhimurium*, and *Escherichia coli*. NP1 shows almost 3 log reduction and NP2 shows 2 log reduction of lag phase *L. monocytogenes* and *S. aureus* in 3 hours. Moreover, NP2 acts as a bactericidal agent whereas NP1 as a bacteriostatic agent against *E. coli* and *S. typhimurium.* Compared to stationary phase of bacterial cells, NP1 kills all initial log phase of gram-positive bacteria within 1 to 2 hours. NP2 works as a bacteriostatic agent against *S. aureus* and bactericidal agent against *L. monocytogenes*. Moreover, both NP1 and NP2 show bacteriostatic activity against log phase of *E. coli* and *S. typhimurium*. Working mechanism and effects appear to be dependent on the cells’ phase. Especially, the antimicrobial compound in NP2 found to be a heat-stable and non-protein-based substance, as unlike NP1, heat-treated NP2 still have same efficacy of antimicrobial activity. Moreover, NP2 exhibits antifungal activity against the opportunistic human pathogenic fungi *Aspergillus fumigatus* and *Candida* species, and the main food spoilage fungus *Penicillium roqueforti*. Ethyl acetate extract of NP2 shows fungicidal activity against *A. fumigatus* with almost 3 log reductions in 8 hours and up to 6% NP2 extract can inhibit the growth of *Candida auris*. Importantly, non-concentrated NP2 still prevents the growth of *A. fumigatus and Candida albicans.* The mode of action of both “NP1” and “NP2” appears to be by disrupting the bacterial and fungal cell membrane. In summary, proprietary *A. oryzae* culture fermentates can be used as broad-spectrum antimicrobial agents in biotechnology: natural alternatives to existing antibiotics and/or food antimicrobial preservatives.

308W High-Throughput Screening Platform for Novel Antifungals to Address Antimicrobial Resistance *S. Earl Kang Jr*[1], Michael Taylor[1], Sanjiv Shah[1] 1) Ginkgo Bioworks.

Antimicrobial resistance (AMR) is a severe emerging threat to the effective prevention and treatment of infections caused by bacteria, viruses, fungi, and parasites. Drug-resistant microbes are responsible for an estimated 700,000 deaths today, and future projections for the impact of unresolved AMR are as high as 10 million deaths per year by 2050. Synthetic chemical collection screens for antimicrobial discovery efforts have proven less than optimal for discovering new antimicrobials with a novel mechanism of action to address AMR.

Alternatively, recent advances in genome mining have confirmed the richness of biologically derived compounds from microbial sources as a genomics-based discovery approach to screen for an untapped source of various chemicals and peptides with unique structures possessing potent biological activities.

To examine the products of some of these biologically derived compounds for novel antimicrobial activity against fungal pathogens, we have developed a high-throughput, miniaturized whole-cell screen that is lower volume and more sensitive than the traditional absorbance-based or visual-based growth inhibition assay. Ginkgo Bioworks’ mission is to make biology easier to engineer. Utilizing a combination of Ginkgo’s strengths across sequencing, metagenomic mining, gene synthesis and expression, transcriptomics, mass spectrom-

etry, and high-throughput screening, we present this screening platform to discover novel classes of antimicrobials to address AMR.

309T Fungal Degradation Behavior of a High-biomass Content, Mixed Pressure-Sensitive Adhesive *Jesus D. Castano*[1], Drew A. Hauge[1], Steven J. Severtson[1], Jiwei Zhang[1] 1) Department of Bioproducts and Biosystems Engineering, University of Minnesota, Twin cities, MN.

Pressure-sensitive adhesives (PSAs) are widely used in everyday tasks commonly found on tapes and labels, but are also utilized in the packaging, construction, automotive, and medical industries. Unfortunately, they are mainly derived from fossil sources, and as such present low biodegradability rates, which creates an environmental problem. To solve this, biobased polymers emerged as a plausible alternative that both reduces the dependency on fossil fuels and increases their biodegradability. Through the incorporation of lactide-based macromonomers (MM) (US Patent 9,469,797) which contain ester linkages and acrylate functionality, a hybrid biobased PSA was synthesized with 50% MM and 50% traditional acrylate monomers. Biodegradability of the hybrid PSA was then evaluated using 58 different fungi, including several white-rot, brown-rot, and soft-rot fungal species. The initial screening was carried out in agar-PSA plates (1%), where clearance zones around the colonies were interpreted as a positive result for PSA degradation. Using this initial screening, we were able to identify 24 different species with the capability of degrading the PSA. To obtain more quantitative data, we prepared liquid cultures containing a typical nutrient solution, and the water-based latex PSA at the concentration of 1% (based on latex solids content). The Erlenmeyer flasks were incubated for 60 days, and the reduction in turbidity with respect to a uninoculated control was taken as the PSA degradation rate. We found outstanding turbidity reduction by some fungi from the genus *Trametes, Pestalotiopsis, Aspergillus*, *Ganoderma,* and *Lenzites* (50-96%). Furthermore, we used SEM to characterize PSA films after degradation by some of these fungi, and we found clear features of disintegration and degradation on the film surface, which confirmed the biodegradability of our polymer formulation. Collectively, these results showed high biodegradability of the tested PSA formulation, alleviating environmental concerns associated with typical PSA products with low biodegradation rates, and reducing reliance on petroleum-derived raw materials used in the production of PSAs. Future studies will include solid state fermentation experiments and composability assays.

310F Heterologous expression of a *Trichoderma longibrachiatum* xyloglucanase GH74 in *Aspergillus nidulans* with potential applications in biotechnology *Alex Contato*[1,2], Nelciele Guimarães[1,3], Rebekkah Friske[1], Guilherme Aranha[4], Tássio Oliveira[4], Roberto Silva[2], Maria de Lourdes Polizeli[4], Rolf Prade[1] 1) Department of Microbiology and Molecular Genetics, Oklahoma State University, OSU, Stillwater, OK, USA; 2) Department of Biochemistry and Immunology, University of Sao Paulo, USP, Ribeirão Preto, SP, Brazil; 3) Institute of Biosciences, Federal University of Mato Grosso do Sul, UFMS, Campo Grande, MS, Brazil ; 4) Department of Biology, University of Sao Paulo, USP, Ribeirão Preto, SP, Brazil.

The xyloglucan (XG) is the predominant hemicellulose at the primary cell wall of the superior plants. This includes all the dicotyledonous and non-gramineous monocotyledonous plants. It is usually found strongly associated to the cellulose through hydrogen bonds, forming a tridimensional network of cellulose and xyloglucan. It is probably the second most abundant polymer in nature, after cellulose. It is highly soluble in water, preventing it from forming crystalline microfibrils like the cellulose. It has a fundamental role in the stretching and expansion of the plant cell wall. There are five types of enzymes known for being able of cleaving the linear chain of the xyloglucan, the most famous of them being the xyloglucanase (XEG). The enzymes that cleave this polymer present great utility at the degradation and conversion of the lignocellulosic biomass. This report describes *Aspergillus nidulans* strain A773 recombinant secretion of a xyloglucanase (XegAC) cloned from *Trichoderma longibrachiatum*, a microorganism which was isolated at the *campus* of USP at Ribeirão Preto, Brazil. The ORF of the *T. longibrachiatum* xegAC gene is comprised of 2582 nucleotides and encodes an 860 amino acid protein. The deduced amino acid sequence was highly homologous with the xyloglucanase belonging to CAZY family 74 of the glycoside hydrolases.

311W Filamentous fungal cell factory for producing 7-aminocephalosporanic acid by engineering cephalosporin C producing fungus *Acremonium chrysogenum* *Xuemei Lin*[1], Jan Lambertz[2], Marc Nowaczyk[2], Ulrich Kueck[1] 1) General and Molecular Botany, Ruhr-University Bochum, Bochum, Germany; 2) Plant Biochemistry, Ruhr-University Bochum, Bochum, Germany.

Cephalosporins are widely prescribed β-lactam antibiotics with a broad spectrum against both Gram-positive and Gram-negative bacteria. Most of the highly effective semi-synthetic cephalosporins are produced by modifying the side chains of the core molecule 7-aminocephalosporanic acid (7-ACA), which is derived from cephalosporin C (CephC) by deacylation. In nature, CephC is exclusively produced by biosynthesis from the filamentous fungus *Acremonium chrysogenum* [1] [2]. In the pharmaceutical industry, CephC is isolated from fungal culture broth and converted to 7-ACA *in vitro* through two- or three-step enzymatic reactions. However, the high cost of the complex enzymatic processes has encouraged researchers to exploit bacterial CephC acylases for a one-step deacylation. Nevertheless, the industrial applications are still limited to *in vitro* production. Therefore, the objective of this project is to construct a filamentous fungal cell factory by introducing the bacterial CephC acylase into the CephC producer *A. chrysogenum,* for an efficient *in vivo* one-step conversion from CephC to 7-ACA.
Several acylase from various *Pseudomonas* species were discovered to be highly active to glutaryl-7-ACA, but they have rather low activities to CephC. In previous studies, some of these acylases have been optimized through site-directed mutagenesis aiming to obtain higher substrate specificities towards CephC. Three of optimized CephC acylase genes were synthesized using the codon usage of *A. chrysogenum.* The recombinant genes were designed to generate CephC acylases with an N-terminal His6-tag and a C-terminal HA-tag, and they were integrated into a fungal gene expression vector. DNA-mediated transformations were followed using conventional transformation protocol. The results of western blot analyses have shown that the CephC acylase genes were successfully expressed in *A. chrysogenum* transformants. Furthermore, autocatalytic cleavages of precursor enzymes occurred accurately in the fungal cells, which are crucial steps for activating the CephC acylases. Supportive analyses were performed by protein LC-MS to confirm the enzyme expression in full-length. Most importantly, using HPLC, we could detect significant amounts of 7-ACA from fungal culture supernatants. Moreover, the optimal conditions of enzymatic conversion were studied in terms of pH, temperature and the incubation time of fungal culture supernatants.

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312T Characterization and engineering of non-model microorganisms for biotechnological applications *Hugh Purdy*[1], Michelle O'Malley[1] 1) University of California, Santa Barbara, Santa Barbara, CA.

Microorganisms hold great promise for applications in biotechnology, including the renewable production of fuels and chemicals, the biosynthesis of therapeutics, and even the creation of novel materials. However, it is well known that only a relatively small percentage of microbial species are isolated and characterized sufficiently to be amenable for engineering purposes, thereby limiting our repertoire of available biological tools. This issue is particularly true for hard-to-culture organisms. One such group of organisms, the anaerobic gut fungi, hold significant, largely untapped biotechnological potential. These fungi, found predominantly in the digestive tracts of herbivores, possess an expansive array of uncharacterized carbohydrate-active enzymes, indicating a high-degree of potential for applications involving the processing and conversion of lignocellulosic material. Furthermore, as these fungi natively exist in a competitive, crowded microbial environment, they are believed to possess diverse secondary metabolites with potential for therapeutic applications. We are working to isolate and characterize these anaerobic gut fungi in order to leverage their unique catabolic and biosynthetic capabilities. In addition to the isolated characterization of these fungi, we are studying the behavior of broader anaerobic consortia derived from herbivore digestive systems and other relevant sites (e.g. landfills and commercial digesters). An improved understanding of the biological principles underpinning these consortia will allow us to develop biotechnologies that take advantage of the enhanced metabolic capabilities exclusive to natural microbial communities. In the same vein of searching for untapped biological potential, we have recently expanded our work into the study and engineering of diatoms for the production of silica-based materials. This group of microalgae is relatively understudied from an engineering perspective considering their biosynthetic capabilities as the primary producers of biogenic silica on Earth. We are beginning investigations into the genetic mechanisms underpinning diatom silicification with the ultimate goal of developing novel siliceous materials. Overall, the O'Malley Lab at UC Santa Barbara is working to expand the range of useful biological tools by studying and engineering these and other non-model microbial systems.

313F Chance favours the prepared spore – how to jumpstart cellulase production *Wolfgang Hinterdobler*[1], Miriam Schalamun[1], Monika Schmoll[1,2] 1) AIT Austrian Institute Of Technology, Tulln, Austria; 2) University of Vienna, Vienna, Austria.

Asexual propagation in filamentous fungi is tightly regulated as many resources are needed for spore production. In *T. reesei*, the cellobiohydrolase CBH2/Cel6a is deposited on the spore surface and sporulation correlates with CAZyme expression. Recently, it was shown that the conditions of spore production are relevant for physiology and stress resistance of the fungi growing from these spores. These findings suggest that the protein composition on the spore surface may be important for the ability of fungi to efficiently colonize a habitat or to initiate growth in a fermenter.

We investigated the surface proteome isolated from spores grown under different light regimes (constant light, constant darkness) and on different carbon sources (cellulose, glucose, malt extract). Indeed, we detected significant differences in these proteomes, indicating an altered preparation for germination conditions. Using these conditions in liquid cultivations on cellulose showed considerable differences in secreted cellulase activity early after germination (48 hours). Transcriptome analysis of these conditions was performed to evaluate cellulose sensing and regulation mechanisms differentially modulated by those spore proteins. Among others, *cbh1*, *cre1* and *xyr1* are characteristically regulated depending on the conditions of inoculum production.

Consequently, *T. reesei* deposits a protein mixture adjusted to the conditions to be expected for germination on its spores. These proteins are involved in sensing and cellulase regulation after germination, reflecting a potential to improve fermentations.

314W Heterologous expression of biosynthetic gene clusters from lichen-forming fungi *Riccardo Iacovelli*[1], Kristina Haslinger[1] 1) University of Groningen, Groningen, NL.

Lichens are complex organisms that arise from the symbiotic association between a (lichen-forming) fungus and a photosynthetic partner—microalgae or cyanobacteria. Historically, they have been exploited by humans for the production of natural dyes and scents, and as powerful bio-indicators to assess environmental pollution. Lichens are also very prolific producers of secondary metabolites (SMs): up to date, approximately 1000 compounds have been isolated from lichens, many of which have shown interesting pharmaceutical activities such as antimicrobial and antioxidant. Despite the undoubted potential, the secondary metabolism of lichens is largely understudied due to the challenges that working with these organisms entails. For instance, they are notoriously slow growers (< 1 mm/year) and it is often not possible to grow them in laboratory conditions. Because of this, little is known about how lichen SMs are biosynthesized and how many there are left to discover. Our research aims at characterizing unknown biosynthetic gene clusters (BGCs) from lichen-forming fungi by means of heterologous expression, with the goal of identifying new biosynthetic pathways and their products. For that, we use a targeted genome mining approach to identify and select BGCs of interest based on criteria such as complexity of the cluster, presence of a self-resistance gene, and expected chemistry of the produced metabolite. Subsequently, we employ a stepwise approach to reconstitute the target BGCs in fungal hosts for overexpression, fermentation, and metabolite analysis via LC-MS.

315T The fungal battery: A redox flow battery containing the biosynthesised quinone phoenicin from *Penicillium astrosanguineum* Charlotte Overgaard Wilhelmsen[1], Sebastian Birkedal Kristensen[1], Oliver Nolte[2], Ivan Volodin[2], Johan Vormborg Christiansen[3], Thomas Isbrandt[3], Trine Sørensen[4], Celine Petersen[4], Thomas Ostenfeld Larsen[3], Jens Christian Frisvad[3], Martin Hager[2], Ulrich S. Schubert[2], Kåre Lehmann Nielsen[4], Teis Esben Sondergaard[4], Jens Muff[1], *Jens Laurids Sørensen*[1] 1) Aalborg University, Esbjerg; 2) Friedrich-Schiller-University, Jena; 3) Technical University of Denmark; 4) Aalborg University, Aalborg.

Filamentous fungi display a wide palette of colorful quinone pigments, which can provide protection against oxidative stress and act as antimicrobial agents. The biosynthetic pathways for quinone pigments in fungi are initiated by non-reducing polyketide synthases (NR-PKSs) to produce entry compounds that undergo various modifications (e.g. oxidation, methylation, ammonia incorporation and dimerization) resulting in huge structural variation. Besides their natural biological role, quinones are gaining increased interest as prom-

ising electrolytes in organic redox flow batteries (RFBs) that can be used to store energy from solar and wind power plants. However, so far the quinones used in RFBs have been chemically synthesized from crude oil, which is not aligned with the sustainable thinking behind renewable energy.
To determine the electrochemical potential of fungal quinones, we initially performed computational analyses of all known compounds and identified several promising candidates. In order to be able to identify the responsible gene clusters for biosynthesis of these candidate compounds, we have genome sequenced >150 fungal strains. Tapping into this dataset, we decided to develop a RFB based on the bibenzoquinone phoenicin, which is produced by several *Penicillium* species. Through bioinformatic analyses of known phoenicin producers in our sequence database, we identified a target NR-PKS in several strains. We then developed a CRISPR-Cas9 system to knockout the NR-PKS in *P. astrosanguineum*, which subsequently lost the ability to biosynthesize phoenicin. In addition to the NR-PKS, the gene cluster contains a hydroxylase, a laccase and a transcription factor. The following analyses showed that the entry compound orsellinic acid is hydroxylated to form the intermediate 6-methyl-1,2,4-benzenetriol, which is oxidized and dimerized to phoenicin. Through cultivation optimization, we were able to achieve a production of >3 g/L phoenicin in a week, which was extracted through liquid-liquid extraction to obtain a purity of >95%. The extracted phoenicin was then used as negolyte together with ferrocyanide as posolyte to generate a RFB with a cell voltage of 0.86 V and an initial capacity of 11.75 Ah/L. The electrochemical properties of phoenicin are similar to the published petro-quinones, which demonstrates that fungal biosynthesized quinones provide a sustainable solution for energy storage.

316F Computer-Aided, Resistance-Gene-Assisted Genome Mining for Proteasome and HMG-CoA Reductase Inhibitors Cory Jenkinson[1], Adam Podgorny[2], Cooncong Zhong[2], *Berl Oakley*[1] 1) Department of Molecular Biosciences, University of Kansas, Lawrence, KS; 2) Department of Electrical Engineering and Computer Science, University of Kansas, Lawrence, KS.

Fungi produce a plethora of biologically active small molecules, called secondary metabolites (SMs), many of which are medically valuable. The genes that encode particular SM biosynthetic pathways are usually clustered together in the genome, forming biosynthetic gene clusters (BGCs). Genome sequencing reveals that the number of SM BGCs vastly exceeds the number of known SMs, and, thus, that huge numbers of potentially valuable SMs are yet to be discovered. Resistance-gene-assisted genome mining is a strategy to exploit the greater fungal secondary metabolome efficiently, by identifying SM BGCs that are likely to make useful products. It takes advantage of the fact that some SM BGCs contain a gene encoding a resistant version of the protein targeted by the compound produced by the BGC. This allows the producing organism to survive while its competitors are inhibited. The bioinformatic signature of such SM BGCs is that they contain an allele of an essential gene with no SM biosynthetic function, and there is a second allele elsewhere in the genome. Manually applying this approach to thousands of sequenced genomes is daunting, so we have developed a computer-assisted approach that allows users to query large databases for SM BGCs that putatively make compounds that have particular targets of therapeutic interest. Working with the MycoCosm genome database, we create databases of the genomic coordinates of all core SM biosynthetic genes. We next generate genomic coordinates for all alleles of our target gene. A computer script then determines if an allele of our target gene is located within a user-defined distance of a core SM biosynthetic gene AND at least one other allele of the target gene is present elsewhere in the genome. We have applied this approach to look for SM BGCs that target the proteasome β6 subunit, the target of the proteasome inhibitor fellutamide B, or HMG-CoA reductase (HMGCR), a key enzyme in sterol biosynthesis and the target of cholesterol reducing therapeutics such as lovastatin. Our approach proved effective, finding known fellutamide and lovastatin SM BGCs as well as fellutamide- and lovastatin-related BGCs with variations in the SM genes that suggest they may produce structural variants of fellutamides and lovastatin. Gratifyingly, we also found SM BGCs that are not closely related to lovastatin BGCs but putatively produce novel HMGCR inhibitors. Supported by the KU Endowment and NIAID Grant R21AI156320.

317W The *Aspergillus oryzae* Fermentate D-Tox Effectively Degrades Aflatoxins and Patulin *Dasol Choi*[1], Ahmad Alshannaq[1], Jae-Hyuk Yu[1] 1) University of Wisconsin-Madison.

Mycotoxins are toxic secondary metabolites produced by certain filamentous fungi (molds), threatening the world food supply by contaminating 25% of the world's crops. Over 4.5 billion people in the world are exposed to unmonitored levels of the most potent carcinogen found in nature, aflatoxin B1 (AFB1), one of the most concerning mycotoxins produced mainly by the ubiquitous soil fungus *Aspergillus flavus*. AFB1 is chemically and thermally stable during food processing; therefore, those are not easily eliminated or degraded. Trace levels, e.g., 20 ppb (U.S. FDA action level), of aflatoxins (AFs) can be dangerous, and foods contaminated with higher amounts of AFs are not fit for human consumption. Despite 60 years of endeavors, no practical methods have been developed to remove mycotoxins in food, particularly AFB1. We have developed a proprietary aflatoxin-degrading natural fermentate called "D-Tox" by growing the food-grade fungus *Aspergillus oryzae* in a novel food-grade medium under specific culture conditions. When treated with D-Tox, 89% and over 90% of 5 ppm AFB1 were degraded after 48 hours at room temperature and after 24 hours at 50°C, respectively. Importantly, boiling the sample in D-Tox greatly enhanced AFB1 degradation: over 97% of lethal level of AFB1 (100 ppm) was degraded in 30 min of boiling. Moreover, near 100% of AFB1 in D-Tox was eliminated by autoclaving at 121°C for 15 minutes. Additionally, D-Tox can degrade aflatoxin M1, B2, G1, G2, and patulin. Unlike other methods used for AF detoxification, e.g., nixtamalization of corn, D-Tox is stable, and the degradation process is irreversible. In addition, we have identified AFD1 as a key degradation intermediate product, leading to the proposed mechanism that D-Tox causes hydrolysis of the AFB1 lactone ring followed by decarboxylation. As AFD1 is also completed degraded by D-Tox, we envision the AFB1→AFD1→unknown fragmented product process. To validate D-Tox's real world applications, corn, wheat, and barley were inoculated with highly toxigenic *A. flavus* NRRL3357 spores and the final concentrations of AFB1 were 2.6 ppm, 1.2 ppm, and 7.0 ppm, respectively. When boiled with D-Tox for 60 min, over 70% of AFB1 was degraded in each sample. Moreover, D-Tox can effectively degrade AFB1 in ginseng and other herb-based medicines. In summary, we believe that the D-Tox technology would be a major game-changer in our fight against aflatoxins and patulin.

318T Heterologous expression of pheichrome, a photosensitizer used for photo dynamic therapy using the co-expression system of *Saccharomyces cerevisiae* Heewon Seo[1], Kum-Kang So[1], Jeesun Chun[1], *Dae-Hyuk Kim*[1] 1) Department of Bioactive Material Sciences, Department of Molecular Biology, Institute for Molecular Biology and Genetics, Jeonbuk National University, Jeonju, Jeonbuk, South Korea.

Phleichrome is a secondary metabolite produced by *Cladosporium phlei* and is a perylenquinone-based substance. Phleichrome is a photosensitizer and may be used for photo dynamic therapy. When a photosensitizer is exposed to light or laser of a specific wavelength, it emits singlet oxygen and necrotizes surrounding cells. Using this, photosensitizers are accumulated in tumors such as cancer, and then light or laser of a specific wavelength is applied to necrotize the tumor. Producing a large amount of phleichrome using *Saccharomyces cerevisiae*'s expression system is expected to be of great industrial value. Through previous studies, it was confirmed that phleichrome is synthesized through the polyketide synthase pathway, and the polyketide synthase (PKS) gene responsible for the biosynthesis of phleichrome was identified. PKS gene is produced with the help of a coenzyme called PPTase. PPTase makes the precursor in the inactive state of PKS gene active so that secondary metabolism can occur successfully. However, this PPTase function does not exist in S. cerevisiae. So, *S. cerevisiae*'s The Co-expression system strategy was used. The fungal PPTase gene was cloned into a yeast integrative vector, and the PKS gene was cloned into a yeast episomal vector, respectively. Co-transformation was performed on S. cerevisiae using these two vectors, and cultured on leucine-deficient selective media and uracil-deficient selective media. Co-transformants were selected through PCR using the DNA of colonies. In future work, we will isolate RNA from co-transformant to check whether transcription of fungal PPTase gene and PKS gene occurs. And we will check the expression of phleichrome through thin layer chromatography.

319F MY-CO SPACE: An artistic-scientific vision on how to build with fungi *Vera Meyer*[1], Bertram Schmidt[1], Carsten Pohl[1], Christian Schmidts[2], Sven Pfeiffer[3] 1) Technische Universität Berlin, Germany; 2) University of the Arts Berlin, Germany; 3) Bochum University of Applied Sciences, Germany.

MY-CO SPACE is a collaborative work of the interdisciplinary ArtSci collective MY-CO-X, that enables an artistic-scientific discussion about a future social significance of fungi for the creation of places and spaces. MY-CO SPACE is a wooden fungal sculpture that makes it possible to experience living in a space capsule as well as in a fungal fruiting body. The wooden construction with fungal panels was built from biological materials and is biodegradable. The living space of approx. 20 sqm, can be divided by the guests themselves into sleeping, reading and working areas. It is not a completely enclosed space but a retreat and study space that lives from and deals with the contact with the outside world. The habitable sculpture is reminiscent of a space station and transports the work of Galina Balashova (born 1931), the architect of the Soviet space programme, into the 21st century. Galina Balashova was responsible for the interior design of the manned spacecraft Soyuz and the Mir space station. Her central design question in the 1960s-1980s was: "How can physical-technical structures and essential living functions be integrated in the smallest space in such a way that people can live and work under conditions of weightlessness and extreme physical stress?"
MY-CO SPACE as a sculptural habitat translates this question to today's challenges: „How can biological-technical structures and essential living functions be integrated in the smallest possible space in such a way that people can still live and work light-hearted under conditions of limited resources?" This architectural artwork thus strives for a different point of view and a process of interaction in which humans are involved in a conscious as well as unconscious conversation with their environment, a point of view that tacitly implies a flattening of hierarchies between the different agents and authors - human as well as non-human. MY-CO SPACE is therefore a built reflection on a cooperation with biological systems that store, transform and recycle organic matter and energy, and an exploration of fungi as a future lightweight building material resistant to fire, shock and water, and whose modification through biotechnology is possible. It is the urgency of the planetary situation and the issues we now face that require a holistic approach and close collaboration between art and science.

320W Laccase expression in the dung fungus *Coprinopsis cinerea* with 17 natural laccase genes Chelsea Cumagun[1], Zemin Fang[2], Shanta Subba[1], Michael Unger[1], *Ursula Kües*[1] 1) Molecular Wood Biotechnology and Technical Mycology, University of Goettingen, Goettingen, Germany; 2) School of Life Sciences, Anhui University, Anhui, China.

Laccases are phenoloxidases that can oxidize phenolic and aromatic compounds and occur in bacteria, fungi, insects, and plants. Among fungi, wood-rotting and litter-decaying Basidiomycetes are considered to be main producers of laccase in nature. Under different environmental conditions, such fungi secrete various forms of this enzyme, being either laccase isoforms encoded by the same gene, isoenzymes encoded by different laccase genes, or allozymes encoded by different alleles of a gene. The ink-cap mushroom *Coprinopsis cinerea* for example has 17 different laccase genes divided into 2 subfamilies. Different monokaryotic strains of the fungus can have inactivated copies of some of these genes. Most often, laccase gene *lcc15* is affected from gene inactivations, but *lcc4* or *lcc7* may also be inactivated. Functionally, the different laccases in the species are only at the beginning to be understood. Laccase genes in *C. cinerea* are differentially expressed during growth on distinct media and at different temperature regimes, during fungal differentiation and as defense in confrontation with other microbes. Monokaryotic strains usually express Lcc1 and Lcc5 as main laccases, under stress at lower temperature of 25-28 °C much higher than at 37 °C as the best growth temperature. Lcc9 is active at neutral pH and can also be expressed in cultures in traces while full expression is encountered as response in presence of competitors. By special features such as unusual pH preferences, laccases of *C. cinerea* are of interest for biotechnological applications. This requires good production rates of properly glycosylated enzymes. Enzymes can be overexpressed in *C. cinerea* upon cloning their genes behind highly active promoters, transformation of the constructs into suitable monokaryotic strains and cultivation of transformants under favorable environmental conditions, with yields up to 30 U/ml culture supernatant depending on the gene used for cloning. Crossing of transformants can further enhance laccase yields with dikaryons expressing a single enzyme or mixtures of laccases when transformants of different laccase genes were mated.

321T Expression of an immunocomplex for dengue virus in *Saccharomyces cerevisiae* Kum-Kang So[1], Heewon Seo[1], Ngoc My Tieu Le[1], Yo-Han Ko[1], Jeesun Chun[1], *Dae-Hyuk Kim*[1] 1) Institute for Molecular Biology and Genetics, Department of Molecular Biology, Department of Bioactive Material Sciences, Jeonbuk National University, Jeonju, Jeonbuk, South Korea.

Dengue virus (DENV) infection, a mosquito-transmitted viral disease, causes a life-threatening Dengue Hemorrhagic Fever and Dengue Shock Syndrome. In a previous study, we reported the synthetic consensus dengue envelope domain III (scEDIII) from all four DENV serotypes specific to all four serotypes and induced a balanced tetravalent immune response. In this study, Immunoglobulin complex method was applied for delivery of the dengue scEDIII in *S. cerevisiae* to enhance vaccine uptake. The antigen replaced the

variable region of IgG chain molecule. IgM-derived small piece was added on the C terminal of IgG to produce polymeric forms. To enhance mucosal immunization, a M-cell targeting ligand was fused with scEDIII. The gene encoding the ligand-scEDIII-IgM fused IgG single chain protein was cloned into yeast episomal vector and confirmed using PCR. Northern blot analysis showed the presence of transcripts of target gene. Western blot analysis indicated the expression of target gene including predicted polymeric forms, confirmed by IgG purification using Protein A agarose resin. Immune response was assessed to check antigen efficacy *in vitro* and *in vivo*. Keywords: Dengue virus, Saccharomyces cerevisiae, Domain III of the dengue envelope protein (EDIII).

322V Deciphering new compound pathways in non-engineered Aspergilli using a CRISPR toolbox: Aspergillus californicus as a starting point *Fabiano Contesini*[1], Yaojie Guo[1], Xinhui Wang[1], Simone Ghidinelli[2], Ditte Tornby[3], Thomas Andersen[3], Uffe Mortensen[1], Thomas Larsen[1] 1) Department of Biotechnology and Biomedicine, Technical University of Denmark, Søltofts Plads, Kgs, Lyngby, Denmark; 2) Department of Molecular and Translational Medicine, University of Brescia, Viale Europa 11, Brescia, Italy ; 3) Department of Clinical Microbiology, University of Southern Denmark and Odense University Hospital, Odense, Denmark.

The growing number of filamentous fungi genome sequences published since 2000s shows a myriad of gene clusters that have not been investigated so far, potentially hiding new bioactive compounds. One important reason for this is the lack of genetic tools for pathway investigation. However, with the successful implementation and evolution of CRISPR technologies for fungal genetic engineering, metabolic pathways can now be elucidated in quite exotic fungi never engineered previously. Nevertheless, since the complete elucidation often requires both deletion and overexpression of gene(s) of interest, the construction of a platform strain for heterologous production is necessary. To complement genetic pathway elucidation in the natural host, we have recently developed a gene-expression platform, DIVERSIFY, including in an *Aspergillus oryzae* strain, which can be used for heterologous production of secondary metabolites. *A. oryzae* is an excellent host for pathway elucidation as it has a very low background of endogenous secondary metabolite production. Our *A. oryzae* strain contains a target expression site that harbors an *uidA* marker gene. Insertion of novel genes into the target site eliminates the marker gene and correct transformants containing the relevant gene at the target expression site can therefore be easily identified. To use our setup, we chose the fungus *Aspergillus californicus* that has never been engineered previously to investigate the pathways behind production of the newly identified compounds. After a chemical investigation, we isolated a polyketide-nonribosomal peptide hybrid calipyridone A. Its biosynthetic gene cluster *cpd* was next discovered by genome mining. Initially a CRISPR/Cas9 multiplex technology was applied to generate a non-homologous end joining and *pyrG* deficient strain by using four different guides to cleave DNA using a silent 4 kb cassette for DNA repair. The compound biosynthesis was elucidated by multiple gene deletion experiments in the host strain as well as the heterologous expression of the hybrid gene *cpdA* in *A. oryzae*. The results indicate that the 2-pyridone moiety of calipyridone A is formed directly from the nucleophilic attack of the amide nitrogen to the carbonyl group, which is different from the generation of other fungal 2-pyridone products via P450-catalyzed ring expansions. These findings open door for elucidation of new metabolic pathways in non-engineered fungi.

323V Identification of transcription factors involved in *Aspergillus nidulans* adaptation to recombinant protein production *Everton Paschoal Antoniel*[1], Jaqueline Aline Gerhardt [1], Natália Sayuri Wassano[1], Michelle Alexandrino de Assis[1], Fernanda Lopes de Figueiredo[1], André Damasio[1] 1) Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas, Campinas, Brazil .

Filamentous fungi, such as *Aspergillus* spp, can secrete significant amounts of proteins and are used as a platform for the production of industrially important enzymes, such as hydrolytic enzymes used in the biorefinery industry. However, in terms of recombinant protein production, particularly of non-fungal protein, there is still potential for improvement. In this regard, many strategies have been explored to increase the capacity of fungal cell factories production; however, as consumer demand grows and productivity remains low, identifying new genetic targets to promote increased enzyme secretion becomes increasingly critical. Thus, the rational use of transcription factors has emerged as an interesting and promising approach. Therefore, our aim was to evaluate the production/secretion of enzymes in strains of *Aspergillus nidulans* in which target transcription factors have been genetically manipulated. To identify these transcription factors, we used RNA-seq data from *A. nidulans* strains overexpressing three heterologous enzymes and we performed differential expression and GO enrichment analysis. Next, using CRISPR-Cas9, we knock out these transcription factors in *A. nidulans* and analyze their phenotype to determine if they have any effect on enzyme secretion. Based on RNA-seq data, we identified six transcription factors candidates – AN8772, AN9373, AN7913, AN3420, AN0094 and AN7592 – with predict function in golgi vesicle transport, protein refolding, cell redox homeostasis and proteolysis. We found that the strain ΔAN7592 had decreased sporulation and slight resistance to osmotic stress, while the strains ΔAN9373 and ΔAN7913 had increased protease secretion. Taken together, our results suggest that these transcription factors may be involved in a variety of biological processes associated with the protein secretion pathway. We concluded that transcriptomic data could be used to identify transcription factors with possible involvement in the protein secretion pathway of *A. nidulans*, and we intend to expand our research by conducting additional experiments to determine the role of these transcription factors in the secretion of biotechnologically relevant recombinant enzymes.

324V *Agrobacterium tumefaciens*-mediated transformation of *Aspergillus nidulans*: an efficient tool for targeted gene recombination using selectable nutritional markers *Virginia Casado del Castillo*[1], Andrew P. MacCabe[2], Margarita Orejas[2] 1) Instituto de Investigación en Agrobiotecnología (CIALE), University of Salamanca; 2) Instituto de Agroquímica y Tecnología de Alimentos (IATA), Consejo Superior de Investigaciones Científicas (CSIC).

Protoplast transformation for the introduction of recombinant DNA into *Aspergillus nidulans* is technically demanding and dependant on the availability and batch variability of commercial enzyme preparations. Given the success of *Agrobacterium tumefaciens*-mediated transformation (ATMT) in diverse pathogenic fungi, we have adapted this method to facilitate transformation of *A. nidulans*. Using suitably engineered binary vectors, gene-targeted ATMT of *A. nidulans* non-homologous end-joining (NHEJ) mutant conidia has been carried out for the first time by complementation of a nutritional requirement (uridine/uracil auxotrophy). Site-specific integration in the Δ*nkuA* host genome occurred at high efficiency. Unlike other transformation techniques, however, cross-feeding of certain nutritional requirements from the bacterium to the fungus was found to occur, thus limiting the choice of auxotrophies available for ATMT. In complementation tests and also for comparative purposes, integration of recombinant cassettes at a specific locus could provide a

means to reduce the influence of position effects (chromatin structure) on transgene expression. In this regard, targeted disruption of the *wA* locus permitted visual identification of transformants carrying site-specific integration events by conidial colour (white), even when auxotrophy selection was compromised due to cross-feeding. The protocol described offers an attractive alternative to the protoplast procedure for obtaining locus-targeted *A. nidulans* transformants.

325V Editing the *Trichoderma reesei* genome using *in-vitro* assembled MAD7/gRNA ribonucleoprotein *Sandra Merino*[1], Romil Benyamino[1], Barbara Cherry[1], Doreen Bohan[1], Jeff Shasky[1], Randy Berka[1] 1) ADM, Davis, CA.

Use of CRISPR-Cas9 to create genome modifications in a variety of filamentous fungi, including *Trichoderma reesei*, has been published. Originally, expression of Cas9 along with the guide RNA, was carried out *in vivo*. However, in recent publications *in vitro* assembled Cas9 and guide RNA complexes have been used for genome editing in filamentous fungi. In this study, a codon-optimized version of the *E. rectale* MAD7 (Inscripta) nuclease was expressed in the prokaryotic host *Bacillus subtilis* under transcriptional control of an IPTG-inducible promoter. In addition, an 8X-His affinity tag was added to the N-terminus for subsequent protein purification, and a codon-optimized SV40 nuclear localization signal was added to the C-terminus. Initially, the functionality of the MAD7 ribonucleoprotein was demonstrated by digesting DNA *in vitro*. To evaluate its use *in vivo* a plasmid containing an mCherry expression cassette flanked by regions homologous to the *T. reesei egl1* locus was used in co-transformations of *T. reesei* protoplasts with various concentrations of MAD7 nuclease complexed with a guide RNA targeting the *egl1* locus. After a week on selective medium, transformants were counted and evaluated by PCR. Results showed increasing transformant number with increasing MAD7 ribonuclease concentration and targeting to the *egl1* locus. More recently, we have used *in vitro* assembled MAD7/gRNA ribonucleoprotein for gene deletions and replacements in *T. reesei* and *Aspergillus oryzae*. The advantage of using a pre-assembled ribonucleoprotein complex for genome editing is simple; the method provides a faster means to edit a genome requiring neither prior modification to the host strain, nor extensive cloning efforts.

326V A library of *Aspergillus niger* chassis strains for morphology engineering connects strain fitness and filamentous growth with submerged macromorphology *Timothy Cairns*[1], Xiaomei Zheng[2,3,4,5], Claudia Feurstein[1], Ping Zheng[2,3,4,5], Jibin Sun[2,3,4,5], Vera Meyer[1] 1) Technische Universität Berlin; 2) Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin; 3) Key Laboratory of Systems Microbial Biotechnology, Chinese Academy of Sciences, Tianjin; 4) University of Chinese Academy of Sciences, Beijing; 5) College of Biotechnology, Tianjin University of Science & Technology, Tianjin.

Submerged fermentation using filamentous fungal cell factories is used to produce a diverse portfolio of useful molecules, including food, medicines, enzymes, and platform chemicals. Depending on strain background and abiotic culture conditions, different macromorphologies are formed during fermentation, varying from dispersed hyphal cells to approximately spherical pellets several millimetres in diameter. These macromorphologies have a critical impact on product titres and rheological performance of the bioreactor. Pilot productivity screens in different macromorphological contexts is technically challenging, time consuming, and thus a significant limitation to achieving maximum product titres. We therefore developed a library of conditional expression mutants in the organic, protein, and secondary metabolite cell factory *Aspergillus niger*. Thirteen genes transcribed during fermentation were placed via CRISPR-Cas9 under control of a synthetic Tet-on gene switch. These genes were selected for functional analysis based on (i) their robust coexpression with the citric acid synthase encoding gene *citA* from 283 microarrays and (ii) their predicted function in morphology associated processes. Quantitative analysis of submerged growth reveals that these strains have distinct and titratable macromorphologies for use as chassis during strain engineering programs. We also used this library as a tool to quantify how pellet formation is connected with strain fitness and filamentous growth. Using multiple linear regression modelling, we predict that pellet formation is dependent largely on strain fitness, whereas pellet Euclidian parameters depend on fitness and hyphal branching. Finally, we have shown that conditional expression of the putative kinase encoding gene *pkh2* can decouple fitness, dry weight, pellet macromorphology, and culture heterogeneity. We hypothesize that further analysis of this gene product and the cell wall integrity pathway in which it is embedded will enable more precise engineering of *A. niger* macromorphology in future.

327V Decaying hardwood associated lignolytic enzyme producing fungi as mediators in low density polyethylene deterioration and the draft genome sequence of *Phlebiopsis flavidolba* *Prameesha Perera*[1], Harshini Herath[1], Gayathri Senanayake[1], Priyanga Wijesinghe[2], Renuka Attanayake[1] 1) Department of Plant and Molecular Biology, University of Kelaniya, Kelaniya, Sri Lanka; 2) Department of Botany, University of Peradeniya, Peradeniya, Sri Lanka.

Sri Lankan dry zone forests are rich in economically important hardwood-bearing plant species. Fungi associated with the decaying hardwoods are rich in lignolytic enzymes; laccases, lignin peroxidases (LiP), and manganese peroxidases (MnP). Objectives of the present study were to; report the diversity of the hardwood-associated culturable fungal species in a dry zone forest reserve, Sri Lanka, determine the variation in lignolytic enzyme production, asses their abilities to deteriorate low density polyethylene (LDPE), and to present the draft genome sequence of one of the best LDPE degrader. Ninety fungal isolates were obtained and 76 were identified using rDNA-ITS sequencing. Shannon-Wiener diversity index was determined for ascomycetes and basidiomycetes separately. Based on qualitative assays of laccase production and lignin degradation, laccase, LiP and MnP activities were determined for 22 isolates. Fungi were incubated in wood enriched media containing 37.5 μm LDPE sheets at room temperature for 45 days. Deterioration was assessed by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), percent reductions of tensile properties, weight and contact angles. Ascomycetes were represented by 63% and showed higher diversity with a Shannon-Wiener index of 2.93 than the basidiomycetes, 2.59. *Xylaria feejeensis* showed the highest LiP activity (105.05±5.59 mU/mL) followed by *Fusarium decemcellulare* and *Phlebiopsis flavidolba. Perenniporia tephrophora* showed the highest laccase activity (60.34±5.74 mU/mL) followed by *X. feejeensis.* Both *Fusarium pseudensiforme* and *X. feejeensis* showed the highest MnP activity (39.81±2.3 and 38.71±5.67 mU/mL). Samples showed 3.1% - 26.4% reduction in tensile stress, 4.2% - 12.8% reduction in weight loss and 19% - 11% reduction in contact angles. Based on the overall performances, *P. flavidolba, P. tephrophora*, *X. feejeensis*, and *Schizophyllum commune* were among the best LDPE degraders. Even though the exact mechanism is not clear, it is clear that lignolytic enzyme producing fungi play a role in LDPE deterioration. Draft genome of one of the best polyethylene degraders, *P. flavidolba,* was obtained with a genome length of 38 Mbp and 53.1% GC content. The largest contig contained 3,279,816 bp and N50 was 528,493bp. In conclusion, Sri Lankan dry zone

forests are rich in enzymatically fascinating and versatile fungal species with potential applications in LDPE deterioration.

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328V *In vitro* polyethylene degradation ability of *Schizophyllum commune* is supported by the presence of the laccases with polyethylene binding sites as reveled by *in silico* molecular docking analysis *Hasni Dharmasiri*[1], Prameesha Perera[1], Anupama Halmillawewa[2], Renuka Attanayake[1] 1) Department of Plant and Molecular Biology, University of Kelaniya, Kelaniya; 2) Department of Microbiology, University of Kelaniya, Kelaniya.

Polyethylene accumulation has become a serious global environmental concern. However, polyethylene is susceptible to hydrolytic cleavage by several lignolytic enzymes and laccase is one of them. Objectives of this study are to evaluate the ability to degrade low-density polyethylene (LDPE) by hardwood decay fungi originated from a dry-zone forest reserve of Sri Lanka and to evaluate LDPE binding affinities to laccases of one of the potential LEDP degrader, *Schizophyllum commune,* using *in silico* homology modeling and molecular docking. Initially 76 isolates were obtained and identified using ITS-rDNA sequencing. Twenty-two isolates were incubated in wood-enriched medium containing 37.5-micron LDPE sheets at room temperature for 45 days. Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), percent reduction of tensile properties and weight loss and hydrophilicity were determined to assess the biodegradation. Of the species showing changes in FTIR spectra and holes/cracks in SEM, *S. commune* showed 15.17% reduction of tensile stress, 5.76% ± 0.43 weight loss and 87.80 ± 4.39° of contact angle indicating hydrophilicity compared to the controls. Since *S. commune* showed *in-vitro* laccase production and the protein sequence was publicly available, it was subjected to homology modeling. Identification of homologous templates for the target was performed using BLASTp algorithm of NCBI. Laccase from *Cerrena* sp., which had 61.24% percentage identity and 1.38 Å resolution was selected as the template for 3D model building of the enzyme using Modeller10.1. The quality of resulted 3D model was verified by its energy and stereochemical properties using SAVES v6.0 server. ModLoop web server was used to remodel the unstable regions by loop modeling. Stereochemical quality and energy of the remodeled protein structure showed that predicted model was of good quality due to the presence of 90.2% residues in the most favored region. ERRAT and VERIFY3D resulted 82.17 of overall quality factor and 96.17% of the residues have averaged 3D-1D score >= 0.2. CB-dock tool was used for molecular docking studies using LDPE, specifically dodecane. The amino acid residues of modeled enzyme in contact with the LDPE were identified as Val162, Trp456, His457 and Ile460 with -4.8 kcal/mol affinity. These findings help explaining the potential involvement of laccases of *S. commune* in LDPE degradation process.
Acknowledgements: ICGEB research grant (CRP/LKA18-03) and NSF Sri Lanka (RG/2019/BT/03).

329V Sexual crossing, chromosome-level genome sequences, and comparative genomic analyses for the medicinal mushroom *Antrodia cinnamomea* Chia-Ling Chen[1], Wan-Chen Li[1], Yu-Chien Chuang[1], Hoy-Cheng Liu[1], Chien-Hao Huang[1], Ko-Yun Lo[1], Chung-Yu Chen[2], Fang-Mo Chang[3], Guo-An Chang[4], YuLing Lin[5], Wen-Der Yang[5], Ching-H Su[6], Tsung-Ming Yeh[2], *Ting-Fang Wang*[1] 1) Institute of Molecular Biology, Academia Sinica, Taipei 115, Taiwan; 2) Shen Nong Fungal Biotechnology Co. Ltd., Taoyuan City, Taiwan ; 3) School of Dentistry, College of Oral Medicine, Taipei Medical University, Taipei, Taiwan; 4) KFK Biotech Co. Ltd., 29 Haibian Rd., Ling-Ya Dist., Kaohsiung, Taiwan; 5) HIMA Foundation, Taipei, Taiwan; 6) Department of Microbiology and Immunology, Taipei Medical University, Taipei, Taiwan.

Antrodia cinnamomea (syn. *Antrodia camphorate* or *Taiwanofungus campgoratus*) mushrooms are a complementary and alternative *medicine* for hangover, cancer, hypertension, obesity, diabetes and inflammation. Though *A. cinnamomea* has attracted considerable biotechnological and pharmacological *attention*, neither classical genetic nor genomic approaches have been properly established for it. We isolated four sexually competent monokaryons from two *Ac* dikaryons used for the commercial cultivation of orange-red (HC1) and milky-white (SN1) mushrooms, respectively. We also sequenced, annotated and comparatively analyzed high-quality and chromosome-level genome sequences of these four monokaryons.These genomic resources represent a valuable basis for understanding biology, evolution and secondary metabolite biosynthesis of this economically important mushrooms. We demonstrate that *A. cinnamomea* has a tetrapolar mating system and that HC1 and SN1 represent two intraspecies isolates displaying karyotypic variation. Compared to several edible mushroom model organisms, *A. cinnamomea* underwent a significant contraction in gene family and individual gene numbers, most notably for plant, fungal and bacterial cell wall-degrading enzymes, explaining why *Ac* mushrooms are rare in natural environments, are difficult and time-consuming to artificially cultivate, and are susceptible to fungal and bacterial infections. Our results lay the foundation for in-depth *A. cinnamomea* study, including precise genetic manipulation, improvements to mushroom fruiting, and synthetic biology applications for producing natural medicinal products.

330V Fungal Batteries: Production Of Fungal Quinones To Be Used As Electrolytes In Redox Flow Batteries *Johan Christiansen*[1], Thomas Isbrandt[1], Thomas Larsen[1], Jens Frisvad[1] 1) Technical University of Denmark.

As society aims at reducing its climate impact, it is evident that the development of technologies suited for storage of sustainable energy is needed. Conventional rare-metal batteries, are generally unsuited for this task due to their high cost, combustibility, usage of rare metals as well as the challenges related to scalability. In contrast, quinone redox flow batteries have been proposed as ideal candidates. Currently, industrially used quinones are synthesized from fossil fuels, but are also produced by living organisms, such as plants, bacteria and fungi. Additionally, filamentous fungi have long been used as industrial workhorses for metabolite production, due to their fast growth and their ability to grow in industrial scale, independent from seasonal variability.
Therefore, we have explored the potential of using quinones from filamentous fungi as electrolytes in redox flow batteries. We investigated several known quinones produced by fungi of the genera *Penicillium*, *Aspergillus* and *Taleromyces* and found that the dibenzoquinone phoenicin, a deep purple exo-metabolite produced by certain *Penicillium* species, was very promising. We discovered that its production was highly variable on different growth media but can be controlled by manipulating the amount of carbon in the medium. By tripling sucrose concentration in Czapek-yeast broth medium we observed a change in phoenicin production from no production to over 5 grams per liter. We found that this triggering mechanism is conserved through the different other *Penicillium* species tested.
Results so far show that only few known metabolites besides phoenicin are present in the supernatant after cultivation, and that phoe-

nicin constitutes over 70 % of the crude medium extract determined by LC-DAD-MS. Importantly, the target compound can easily be precipitated out of solution. Studies performed by our collaborators at Aalborg University showed that phoenicin is a well performing electrolyte in redox flow batteries compared to academic standards. Conclusively, we showed that fungal produced quinones, with the specific example of phoenicin, is well suited for further testing and development of a fungal quinone based redox flow battery.
Main methods used include UHPLC-DAD-QTOF mass spectrometry for detection and quantification of quinones and other metabolites, as well as feature based molecular networking and multivariate statistics for general metabolomics analysis.

331V Predicting production of known, putative, and unknown microbial metabolites through network analysis *Muralikrishnan Gopalakrishnan Meena*[1], Matthew Lane[1,2], Joanna Tannous[1], Tomás Rush[1] 1) Oak Ridge National Laboratory, Oak Ridge, TN, USA; 2) University of Tennessee, Knoxville, TN, USA.

We introduce various network analysis routes to predict the interaction of known, putative, and unknown secondary metabolites (SMs) with respect to various sources responsible for their production and regulation. Understanding and predicting the production of SMs is of utmost importance for developing enhanced natural products. Due to the abundance of SMs produced by microbes, targeted analysis to identify new SMs from both putative and unknown analytes is extremely difficult. Moreover, even though various state-of-the-art techniques help identify new SMs through AI and data-driven approaches, they provide limited information on the origin of the SMs. We use the mathematical platform of network analysis to quantify the interaction of SMs with their source of production. Broadly, these relationships could be of 2 forms: 1) interactions with the various species that produce SMs and 2) influence of various chemical treatments which regulate SMs. We demonstrate the capability of our framework to quantify these 2 forms of relationships on 1) the influence of various *Trichoderma* species on putative metabolite production and 2) influence of chemical treatments, like Lipochitooligosaccharides (LCOs), COs, and lipids, on the regulation of known, putative, and unknown SMs in the opportunistic human pathogen *Aspergillus fumigatus*.

As a general route, we build the so-called bipartite networks quantifying the relationship between metabolites and the source of their production (various species of microbes and chemical treatments). The networks provide in-depth quantification of the influence of microbe species and chemical treatments on SM production. Using the bipartite network formulation, we provide 2 routes to predict SM production: 1) directed route to predict known and putative SMs and 2) discovery route to predict unknown SMs. In the directed route, once the network of SMs is built, we use network centrality metrics to rank the SMs and the source of their production. The sources are ranked based on their capability to produce SMs and the SMs are ranked based on their popularity in being produced by various sources. In the discovery route, we use novel analyte peaks extracted from processed spectral data to build the bipartite network and analyze edges and neighborhoods to identify key analytes among the total pool. The insights about the most influential sources and most influenced SMs are extremely valuable for 1) validating known SMs through chemical analysis and 2) identifying new SMs from putative and unknown metabolites by genetic knockouts to characterize their gene clusters. Furthermore, inferences obtained from our framework can be used as a guide for industry partners to concentrate efforts on natural product discovery for the most influential microbe species and chemical treatments.

332V A genetic platform to produce secondary metabolites of non-Dikarya fungi Leo Kirchgaessner[1], Jacob Wurlitzer[1,2], Malik Rakhmanov[1], *Markus Gressler*[1,2] 1) Pharmaceutical Microbiology, Institute of Pharmacy, Friedrich Schiller University, Jena, Germany; 2) Pharmaceutical Microbiology, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Jena, Germany.

Aspergilli have become a versatile platform to produce secondary metabolites (SM) from various biological sources. Several classes of SM enzymes were successfully heterologously produced - among them polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS). However, natural product research has so far mainly focused on biosynthetic genes of manageable and cloneable length up to 12 kb. In contrast, longer genes are preferably studied by either knock-out or promoter replacement strategies. Both strategies require cultivable and transformable fungi which is rarely applicable to Basidiomycota or non-Dikarya fungi which have recently discovered as a novel source of natural products [1]. Hence, current knowledge on the secondary metabolism is mostly based on works on ascomycetes, whilst basal fungi are still underrepresented.
Here, a novel strategy to heterologously express long natural product biosynthetic genes >12 kb is presented. The system is based on the tetracycline-inducible ATNT expression platform in *Aspergillus niger* [2] and does not require cloning of the full-length gene. Instead, it is amplified in up to five DNA fragments and is accurately assembled by homologous recombination in the host in one single step. On-site integration into the *fwnA* locus responsible for conidial pigmentation allows for a simple visual screening for the correct transformants.
The system was validated by the integration of two different SM biosynthetic genes from phylogenetically divergent fungi: As proof of concept, the 8.2 kb long *lpaA* gene from the basidiomycete *Laetiporus sulphureus* [3], encoding a highly reducing polyene PKS, was successfully assembled and integrated. The enzyme catalyzes the formation of chromophoric laetiporic acids of different main chain lengths ranging from C_{26} to C_{32}. In a similar approach, we succeeded to heterologously express a full-length SM biosynthetic gene from a non-Dikarya species for the first time: The expression of a 21 kb orphan NRPS gene from *Mortierella alpina* resulted in the production of an anti-mycobacterial peptide with an unusual C-terminal ε-caprolactam moiety.
The presented system (i) sets the stage to biotechnologically produce compounds of interest with a minimal cloning work load and (ii) might be pivotal to study extremely long biosynthesis genes from novel, yet underinvestigated secondary metabolite producers, such as the basal fungi.

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333V Enhanced production of Taxol® by elicitor-induced transcription factors: Two endophytic fungi from *Taxus wallichiana* Zucc. *Kamalraj Subban*[1], Frank Kempken[1] 1) Genetische Botanik und Molekularbiologie, Botanisches Institut und Botanischer Garten, Christian-Albrechts-Universität, Olshausenstraße 40, D-24098, Kiel, GERMANY..

Paclitaxel/Taxol is an essential anticancer drug and hundreds of reports have been shown for the production of taxol as well as their biosynthetic genes in endophytic fungi. Finding strategies for producing cost-effective microbial taxol is imperative to the demand. Elicitation is considered as a very important strategy towards improving the production of taxol from endophytic fungi. Preliminary reports indicated that elicitors such as salicylic acid, methyl jasmonate (MeJA), sodium benzoate etc., have effectively stimulated the production of fungal taxol. Moreover, till date, there is no report for the action of transcription factors (TFs) from taxol producing fungi.
We investigate whether TFs play an important role in production of endophytic fungal taxol while treated with elicitors in the submerged culture. In the present study, WRKY and other TFs were obtained from *Didymella glomerata* and *Pestalotiopsis microspora* fungus by genomic PCR methods. MeJA elicitor induced effects on the enhanced amount of Taxol production, which was analysed using various chromatography techniques. In addition, mycelial biomass increased and TF genes were up-regulated upon treatment of elicitor. RT-PCR analysis of key gene expression in the Taxol biosynthesis pathway is under progress. Our preliminary study demonstrates that the WRKY and other TF play significant roles in elicitor induced taxol biosynthesis from fungi. These findings will open novel studies for molecular mechanism and regulation of TFs in the fungal taxol biosynthesis.

334V *Aspergillus niger* customized enzymatic cocktail for cello-oligosaccharide production *Fernanda Lopes de Figueiredo*[1], Fabiano Fabiano Jares Contesini[2], Jaqueline Aline Gerhardt[1], César Rafael Fanchini Terrasan[1], Everton Paschoal Antoniel[1], Uffe H. Mortensen[2], André Damasio[1] 1) University of Campinas (UNICAMP); 2) Technical University of Denmark (DTU).

In the last decades, reducing the use of fossil fuels has been an urgent global concern. In this context, the biorefinery model is an excellent alternative, directing the production of biofuels (renewable energy) and high-added value products from lignocellulosic biomass. Obtaining a robust enzymatic cocktail capable of efficiently accessing and converting plant cell wall polysaccharides is therefore essential for biorefineries implementation. Traditionally, the biorefinery model was established for predominant fermentation of monosaccharides, however, oligosaccharides are also fermentable by yeasts adding advantage to the process by reducing the competition with contaminating microorganisms and consequently reducing the cost of the process. Here we demonstrate the design of genetically modified *Aspergillus niger* strains producing a tailor-made secretome that can be used for optimized production of cello-oligosaccharides (COS). The mutant strains were designed using the CRISPR/Cas9 technology and named *quadrupleΔ* harboring deletion of genes encoding key CAZymes, *i.e*, two cellobiohydrolases (CBHs), one β-glucosidase (BGL) and one β-xylosidase, and *quintupleΔ* that carries an additional deletion of the transcriptional regulator of extracellular proteases (prtT). The *quadrupleΔ* and *quintupleΔ* mutants were grown on solid minimal medium (MM) with glucose, sugarcane bagasse (SCB), carboxymethylcellulose (CMC) and xylan from beechwood as the sole carbon sources. Enzymatic activities of the *A. niger* secretome produced on SCB were measured on AZO and 4-nitrophenyl-derived substrates (pnP) and then applied for the saccharification of phosphoric acid swollen cellulose (PASC). The mutant strains showed reduced growth on xylan agar plates, whereas submerged cultivation revealed decreased protein secretion, and, specifically, reductions in cellulolytic and hemicellulolytic activities. Additionally, protease activity was highly reduced in the *quintupleΔ* mutant. Moreover, applying the optimized secretomes on PASC degradation resulted in the production of 0.4 and 0.2 g/L of cellobiose and cellotriose, respectively (products absent in the reference strain - *REF*), in addition to a 50% decrease in glucose concentration.The results show the optimization of the enzymatic production of COS, contributing to the still limited information about its application and large-scale production.

335V Polygalacturonases from *Aspergillus japonicus* and *Thermoascus aurantiacus*: Enzyme production using low-cost carbon source, biochemical properties and applications in clarification of fruit juices *Nelciele Cavalieri de Alencar Guimarães Oliveira*[1,2], Nathalia Nunes Glienke[1], Alex Graça Contato[2,3], Rebekkah Friske[2], Roberto Ruller[1], Douglas Chodi Masui[1], Fabiana Fonseca Zanoelo[1], Giovana Cristina Giannesi[1], Rolf Alexander Prade[2] 1) Oklahoma State University; 2) Federal University of Mato Grosso do Sul; 3) University of São Paulo.

Filamentous fungi are preferred sources for production of industrial enzymes due to fast growth and easy availability. They have the enzymatic repertoire necessary for the decomposition of the plant cell walls. Microbial enzymes can be used as an alternative to hazardous chemicals pollutant generally used by industries owing to their biodegradable and nontoxic nature, and because of this have a wide variety of applications in several industrial sectors, like in food, pharmaceutical, textile, paper, and other industries. In this study, polygalacturonases (PG) were produced using low-cost carbon sources (agro-industrial wastes/products) in submerged (SmF) and solid state (SSF) fermentation. Passion fruit peel was the best production inducer for Aspergillus japonicus (AJ) (120 ±0.3 U), and cassava flour from Rondonópolis/MS for Thermoascus aurantiacus (TA) (106.8 ±0.1 U), both in SSF. Crude extracts containing extracellular PGs were characterized in terms of growth time for production, and optimum pH and temperature for enzymatic activity. The best growth time for PG production by AJ was 48 hours (227.75 ± 13.1 U), and for enzymatic activity sodium acetate (100mM) buffer pH 4 and 60 °C were the optimal parameters. While for TA, 96 hours (114.5 ± 0.26 U), McIlvaine pH 4 and 70 °C were the optimal parameters. A. japonicus PG was semi-purified in two chromatography steps with a specific activity of 7.9 U/mg protein, 2.9 -fold purification, and 81% final yield, exhibiting a molecular weight about 40 kDa (SDS-PAGE). The optimum pH and temperature of the semi-purified PG from AJ were 4 and 55 °C, respectively. In the evaluation of pH and thermal stability, the PGAj enzyme retained up to 90% of its initial activity for 4 h at pH 4.0, 5.0 and 6.0, and the enzyme maintained 83% residual activity after 20 min at 50 °C, respectively. The best results for juice clarification using the semi-purified enzyme (3 U/mL) were obtained with the pulp of Palmer and Tommy mango varieties, white guava, banana, and apple, with increases of 65, 41, 40, 11, and 9.4% in transmittance values, respectively, compared to the control. This was superior to that obtained using commercial pectinase for some of the pulps, as 49% and 21% for Palmer and Tommy mango pulps, respectively. With these results we can conclude that the polygalacturonase from AJ may be suitable for application as a clarifying agent in the fruit juice industry.

336T Structural Insights of the Blue-Light Photosensor BcLOV4 from the Plant Pathogenic Fungi Botrytis cinerea *Matthew Cleere*[1] 1) CUNY.

Wavelengths of blue light can be interpreted through Light-Oxygen-Voltage or LOV photosensing domains and translated into biological processes. One such photoswitch found in the fungal plant pathogen Botrytis cinerea is BcLOV4, a Regulator of G-protein Signaling (RGS) domain paired with a LOV photosensor. When activated, BcLOV4 undergoes a conformational change and translocates to the plasma membrane where it is believed to regulate G-protein signaling. These RGS-LOV proteins are found almost exclusively in plant pathogenic fungi making them distinct. We performed an initial alanine screen of BcLOV4 using our current structural model to selectively target residues of hypothesized importance. These mutant proteins were successfully expressed in the budding yeast Saccharomyces cerevisiae where blue light activation can be induced under confocal microscopy. The C-terminal addition of mCherry fluorescent protein allows us to observe how BcLOV4 associates with the inner plasma membrane over time and can be quantified using imaging software. We continue to probe BcLOV4's function through intrusive glutamate mutations guided by video analysis of our initial screen. By integrating this information with our in vitro structural data, we can develop a more accurate model for BcLOV4 and help us to understand the mechanics of RGS-LOV proteins in plant pathogenic fungi.

337F FBAR proteins anchor the fission yeast contractile ring *Blake Commer*[1], Kimberly Bellingham-Johnstun[1], Caroline Laplante[1] 1) North Carolina State University, Raleigh NC.

The mechanism of cytokinesis evolved in a common ancestor to fungi, amoeba, and mammals, and the proteins involved in this process are highly conserved. Cytokinesis in yeast and mammals relies on the constriction of a contractile ring (CR) of actin and myosin, which must be tethered to the plasma membrane (PM) and the cell wall by an anchoring mechanism in order to successfully divide a cell. While it is critical for tension production and furrowing of the PM, the exact anchoring mechanism remains unknown due to the lack of information surrounding the molecular structure of the CR. Here, Single Molecule Localization Microscopy (SMLM) is applied in living cells of the fission yeast *Schizosaccharomyces pombe* in order to investigate the molecular organization of the CR. During the final stages of cell division in *S. pombe*, cytokinesis protein clusters called nodes serve as the basic units of constriction for the CR. Based on the molecular organization and preliminary mechanical measurements of cytokinesis nodes, it is likely that they also act as anchors. The F-BAR domain containing proteins Cdc15p and Imp2p are putative protein anchors. When these proteins are removed (or depleted in the case of Cdc15p), cells display a ring sliding phenotype consistent with a disruption in the CR anchoring mechanism. The molecular organization of Cdc15p has revealed that it is part of the nodes; however, there is far less information available on the molecular organization of Imp2p. Here, confocal microscopy shows similar distribution profiles of Imp2p-mEGFP and Cdc15p-mEGFP at the division plane. To determine the molecular organization of these putative anchors, we also use SMLM in live fission yeast cells. Based on reconstructed SMLM images, Imp2p-mEos3.2 appears as protein complexes in the constricting CR, which is also similar to Cdc15p-mEos3.2. In addition, quantitative measurements reveal that Cdc15p-mEos3.2 and Imp2p-mEos3.2 share a common molecular organization based on the radial density distributions of single molecule emissions and overall dimensions of protein clusters. Based on our results, Imp2 and Cdc15p may co-cluster to anchor the CR. We will use two-color SMLM to determine if Imp2p and Cdc15p colocalize at the nanoscale. Overall, this work has elucidated that each of these protein complexes is part of the mechanism which anchors the CR to the PM and cell wall.

338W Protein Secretion Requires Extracellular Vesicles *Rebekkah Friske*[1], Rolf Prade[1] 1) Oklahoma State Univeristy.

Fungi are known to adapt to environmental changes and communicate with other organisms via their secretome. Since fungi have a thick cell wall, this begs the question of how large biomolecules are able to transport through the cell wall to the extracellular medium. Many studies have supported the idea that a variety of molecules are transported out of the cell via extracellular vesicles (EVs). What remains unclear is how EVs originate in the cell, cross the cell wall, and their overall destination and purpose. This project tries to find these answers by studying the filamentous fungi, *Trichoderma reesei* and *Aspergillus nidulans*. We hypothesize that all biomolecules directed towards the extracellular environment are packaged into vesicles, then hauled to the hyphal tip (the Spitzenkörper) before secretion. A method was created to isolate and count the extracellular vesicles using ultrafiltration and flow cytometry with a fluorescent membrane stain. Growth kinetic measurements were taken during all phases of growth, lag, early-, mid-, late-exponential, and stationary in liquid media with a 1% carbon source. A 6-well microtiter dish and a 96 Well Microplate Reader (Tecan) were used to take multiple 600 nm absorbance measurements during growth. High vesicle counts (~2-5 million vesicles/mL) were consistently observed within the first 24 hours or 48 to 72 hours (early exponential phase) of growth in *T. reesei* and *A. nidulans*, respectively. In complex carbon sources (CMC, casein, and xylan) a second peak was noticed late in exponential phase, suggesting additional release of vesicles for further break down of these carbon sources. We measured mycelial dry weight, EV counts, total protein, and xylanase activity in the isolated vesicles and total medium in two strains of *A. nidulans*: A773 (wild type) and XLN7, a strain that over-secretes total protein to the medium. It was found that samples with a higher dry weight, also showed a higher count of EVs, suggesting a role in cell wall building. When both strains were required to use xylan as a carbon source, xylanase activity was detected in the vesicles. Moreover, XLN7 contained 3X more vesicles and 3X more xylanase when compared to the wild type; this suggests a relationship between amount of total protein secreted and number of EVs released. Quantifying fungal EVs, as well as total protein produced throughout growth, allows for determination of when EVs are released and their overall purpose for being released.

339T Phosphorylation-mediated Ccp1-Ndc80 switch at the N-terminus of CENP-T regulates kinetochore assembly in fission yeast Qianhua Dong[1], Xue-lei Liu[2], Xiao-hui Wang[2], Yu Zhao[3], Yu-hang Chen[2], *Fei Li*[1] 1) New York University; 2) Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China; 3) Institute for Systems Genetics and Department of Biochemistry and Molecular Pharmacology, NYU Langone Health, New York NY.

Kinetochores, a protein complex assembled on centromeres, mediate chromosome segregation. In most eukaryotes, centromeres are epigenetically specified by the histone H3 variant CENP-A. CENP-T, an inner kinetochore protein, serves as a platform for the assembly of the outer kinetochore Ndc80 complex during mitosis. It has been shown that the CENP-T complex directly associates with centromeric DNA. How CENP-T is regulated through the cell cycle remains unclear. Ccp1 (*c*ounteracter of *C*ENP-A loading *p*rotein 1)in fission yeast associates with centromeres during interphase, but delocalizes from centromeres during mitosis. Here we demonstrated that Ccp1 directly interacts with CENP-T. CENP-T is important for the association of Ccp1 with centromeres, whereas CENP-T centromeric localization depends on Mis16, a homolog of human RbAp48/46. We identified a conserved Ccp1 interaction motif (CIM) at the N-termi-

nus of CENP-T, which is adjacent to the Ndc80 receptor motif. The CIM domain is required for Ccp1 centromeric localization. The CIM domain-deleted CENP-T mutant phenocopie *ccp1*D. We further found that the CIM domain can be phosphorylated by CDK1 (Cyclin-Dependent Kinase 1). Phosphorylation of CIM weakens its interaction with Ccp1. Consistent with this, Ccp1 dissociates from centromeres through all stages of the cell cycle in the phosphomimetic mutant of the CIM domain, whereas in the phospho-null mutant of the domain, Ccp1 associates with centromeres during mitosis. We further show that the phospho-null mutant disrupts the positioning of the Ndc80 complex during mitosis, resulting in chromosome missegregation. Our results suggest that CDK1-mediated phosphorylation of the motif at the onset of mitosis promotes the switch of Ccp1 to Ndc80 at the N-terminus of CENP-T, resulting in dissociation of Ccp1 and proper assembly of Ndc80. At the end of mitosis, the domain is dephosphorylated, leading to reassociation of Ccp1 with CENP-T. Our results reveal a previously unrecognized mechanism underlying kinetochore assembly through the cell cycle, and also provide insights into how the homeostasis between CENP-A nucleosome and CENP-T-containing particles within centromeres is achieved.

340F Investigating heterochromatin-mediated anti-fungal resistance in *Cryptococcus neoformans* isolated from HIV patients in Tanzania *Becky Yeboah*[1], Manu Shukla[1], Neil Stone[2], Tihana Bicanic[2], Robin Allshire[1] 1) Wellcome Centre for Cell Biology, Institute of Cell Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, UK; 2) Centre for Global Health, Institute for Infection and Immunity, St. George›s University of London, United Kingdom.

Antifungal resistance in the human pathogenic fungus *Cryptococcus neoformans* commonly occurs via genetic mutations [1]. We recently showed that heterochromatin (H3K9 methylation) forms epimutations in fission yeast (*Schizosaccharomyces pombe*) that exhibit unstable resistance to caffeine and cross-resistance to various azole-based antifungals [2]. Thus the unstable fluconazole resistance exhibited by some heteroresistant clinical *C. neoformans* isolates [3] might in some cases be mediated by epimutations.

Here, we investigate the role of H3K9me-dependent heterochromatin in anti-fungal resistance in heteroresistant *C. neoformans* samples obtained from Tanzanian HIV patients. Using Chromatin immunoprecipitation followed by sequencing (ChIP-seq), novel heterochromatin islands were identified at distinct locations in several of these clinical isolates. Distinct H3K9me2 islands were detected over a locus containing a cluster of genes implicated in cellular response to iron starvation, mitochondrial function and the oxidative stress response. Based on these observations, we suggest that the repression of genes underlying such heterochromatin islands may trigger endogenous stress pathways that ultimately upregulate efflux pumps leading to resistance.

Intriguingly, heteroresistant clinical *C. neoformans* isolates lose both their novel heterochromatin islands and fluconazole resistance following continual passaging on non-selective media. This suggests that, as in fission yeast, heterochromatin-dependent epimutations may contribute to unstable fluconazole heteroresistance in *C. neoformans.* Our finding that heteroresistant clinical isolates also display cross-resistance to agritech azole-based anti-fungals is also relevant to the current debate concerning the possible link between excessive agricultural azole use driving antifungal resistance in human fungal pathogens.

In summary this study demonstrates that heterochromatin-mediated repression of specific genetic loci may impart transient resistance to fluconazole therapy in clinical isolates of *C. neoformans.*

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3. Stone, N. R., Rhodes, J., Fisher, M. C., Mfinanga, S., Kivuyo, S., Rugemalila, J., & Bicanic, T. (2019). Dynamic ploidy changes drive fluconazole resistance in human cryptococcal meningitis. *The Journal of clinical investigation*, *129*(3), 999-1014.

341W Localization of frequency mRNA by PERIOD-2 contributes to period length determination in the Neurospora crassa circadian clock. *Bradley Bartholomai*[1], Amy Gladfelter[2], Jennifer Loros[1], Jay Dunlap[1] 1) Geisel School of Medicine at Dartmouth, Hanover, NH, USA; 2) University of North Carolina - Chapel Hill, NC, USA.

Core molecular mechanisms of circadian rhythms have been elucidated in animals, plants, fungi, and some prokaryotes over the past several decades. Animal and fungal clocks are remarkably similar in their molecular architecture, and although much is understood about their central mechanism, little is known about the spatiotemporal dynamics of the gene products involved. Studies in the filamentous fungus, Neurospora crassa have revealed significant temporal delays between rhythmic accumulation of mRNAs and the proteins they give rise to, indicating a role for complex post-transcriptional regulation. We have employed single molecule RNA-FISH (smFISH) to show that the mRNA of frequency (frq), which encodes the primary component of the negative arm of the oscillator, cycles in abundance throughout the circadian day with a single digit ratio of transcripts to nuclei. Furthermore, the number of nuclei actively transcribing frq changes in a circadian manner and is also generally quite low compared with the total number of nuclei in each hyphal compartment. Using spatial point patterning statistics, we show that frq is spatially clustered near to nuclei in a time-of-day dependent manner. This clustering is abolished in the absence of the RNA-binding protein PRD-2 that was recently shown also to bind to mRNA encoding Casein Kinase 1. PRD-2 is an intrinsically disordered protein that displays cellular behavior and in vitro characteristics that are consistent with liquid-liquid phase separation. Taken together these data suggest that frq is sequestered in biomolecular condensates by PRD-2 near to nuclei, consistent with a role for RNA-binding proteins and phase-separated regions in spatiotemporally organizing clock mRNAs to facilitate local translation and assembly of clock protein complexes.

342T Circadian Oscillations in *Trichoderma atroviride* and the Role of Core Clock Components in Secondary Metabolism, Development, and Mycoparasitism Against the Phytopathogen *Botrytis cinerea* Marlene Henríquez-Urrutia[1,2], Aldo Seguel-Avello[1,2], Consuelo Olivares-Yánez [1,2,3], Héctor Guillén-Alonso[3], Robert Winkler[3], Alfredo Herrera-Estrella[4], Paulo Canessa[5], *Luis Larrondo*[1,2] 1) Millennium Institute for Integrative Biology (iBio), Santiago, Chile; 2) Pontificia Universidad Catolica de Chile, Santiago, Chile; 3) Department of Biotechnology and Biochemistry, Cinvestav Unidad Irapuato, 36824 Irapuato, Mexico; 4) Laboratorio de expresión

génica y desarrollo en hongos, Unidad de Genómica Avanzada-LANGEBIO, Cinvestav, 36824 Irapuato, Mexico. ; 5) Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello..

Circadian clocks are essential for individuals' fitness, and recent studies underline their role in the outcome of biological interactions. However, the relevance of circadian clocks in fungal-fungal interactions remains largely unexplored. We sought to characterize a functional clock in the biocontrol agent *Trichoderma atroviride* to assess its importance in the mycoparasitic interaction against the phytopathogen *Botrytis cinerea*. By utilizing luciferase reporters to monitor the *T. atroviride* core-clock, we confirmed the existence of circadian oscillations that are temperature compensated and modulated by environmental cues such as light and temperature. Confrontation assays between WT and clock mutant strains of *T. atroviride* and *B. cinerea*, in constant light or darkness, revealed an inhibitory effect of light on *Trichoderma's* mycoparasitic capabilities. Interestingly, when confrontation assays were performed under light/dark cycles, *Trichoderma's* overgrowth capacity was enhanced when inoculations were at dawn compared to dusk. Deleting the core-clock negative element FRQ in Botrytis, but not in Trichoderma, was vital for the daily differential phenotype, suggesting that the Botrytis clock has a more significant influence in this interaction.
Additionally, we observed that *T. atroviride* clock components modulate the development and secondary metabolism in this fungus, affecting the production of several molecules, including volatile compounds, such as 6-pentyl-α-pyrone (6-PP). Notably, we detected the rhythmic production of distinct *T. atroviride* volatile organic compounds (VOCs), which depended on its circadian clock. Thus, this study provides evidence on how clock components impact diverse aspects of *T. atroviride* lifestyle and how daily changes modulate fungal interactions and dynamics.

343F A versatile set of protein tags to improve purification of nuclei *Shan Hays*[1], Michael Freitag[2] 1) Western Colorado University; 2) Oregon State University.

The ALFA-tag is a short, versatile epitope tag that was developed in tandem with an ALFA-specific nanobody (NbALFA) (Götzke et al, 2019; doi: 10.1038/s41467-019-12301-7). We generated fluorescently-labeled ALFA- and NbALFA-tagged proteins to determine their usefulness as molecular biology tools *in vitro* and *in vivo* in fungi. We generated homokaryotic *Neurospora crassa* strains that expressed both tagged proteins, which appeared to localize to specific subcellular locations. Preliminary results suggest that NbALFA and ALFA-tag colocalize *in vivo,* providing a potential new system to drive protein-protein interactions. In parallel we developed a system to tag nuclear membranes by generating fusion proteins of ALFA, mCherry, and the two membrane-spanning domains of the Neurospora homologs of the yeast Brr6 and Spo7 genes. Preliminary results suggest that this may provide a system to enrich for nuclei by immunoprecipitation with affinity beads.

344W 4-color live-cell imaging and other novel microscopy tools reveal dynamic sub-cellular distributions of core clock components in *Neurospora crassa* *Ziyan Wang*[1], Bradley Bartholomai[1], Jennifer Loros[2], Jay Dunlap[1] 1) Department of Molecular & System Biology, Geisel School of Medicine at Dartmouth, Hanover, NH; 2) Department of Biochemistry & Cell Biology, Geisel School of Medicine at Dartmouth, Hanover, NH.

Circadian rhythms are endogenous daily oscillations driven by a molecular clock that help organisms better coordinate their metabolism and behaviors with the environment. Organisms from fungi to animals share a similar phosphorylation-driven transcription/translation negative feedback loop (TTFL) as the core clock mechanism, which describes an oscillator composed of positive and negative elements. Research on the filamentous fungus, *Neurospora crassa*, has provided answers to many fundamental clock-related questions. In *N. crassa*, the White Collar Complex (WCC) is a heterodimeric transcription factor and serves as the positive element. Frequency (FRQ) forms a complex with the FRQ-interacting RNA Helicase (FRH) and Casein Kinase-1 (CK-1a), known as the FFC complex, which is the negative element.
Molecular components of the circadian clock have been described over thirty years of genetic and molecular biological studies. However, little is known about their dynamics and regulation at the sub-cellular level. In recent years, live-cell imaging has become a new hotspot in circadian research because of its high spatiotemporal resolution. A few characteristics of *N. crassa,* such as difficulties in heterologous protein expression, rapid growth rate, and tendency to form large masses of fused hyphae, have made it a difficult organism to use for live-cell microscopy on a circadian time scale. We are developing strategies to overcome such difficulties in applying live-cell imaging to *Neurospora* circadian research.
We have now successfully developed and applied constitutive promoters at various expression levels for expression of heterologous proteins and cellular compartment markers, as well as bright fluorescent tags across the visible light spectrum in *N. crassa*. In addition, we have optimized and tested photoconvertible and photoswitchable fluorescent proteins for monitoring a subset of proteins in *N. crassa*. With mTagBFP2 tagged BML (cytoskeleton), mNeonGreen tagged Cdc11 (septa), mApple tagged WC-2 (nuclei), and iRFP670 tagged SON-1 (nuclear envelop), we can use 4-color living cell imaging in fungal system to look at different cellular compartment. Through multicolor live-cell imaging of core clock components (together with a nuclear marker) with these novel microscopy tools, we can potentially elucidate the dynamics of their subcellular localization and protein-protein interactions in high spatiotemporal resolution.

345T Novel GCaMP6 imaging of cytosolic free calcium dynamics reveals stress-specific signalling responses in the fungal pathogen, *Candida albicans* *Callum J Parkin*[1], Claudiu Giuraniuc [2], Mariana Almeida [2], Mark D Fricker [3], Srishti Chawla[2], Silvia Wehmeier[2], Tina Bedekovic[1,2], Neil Gow[1,2], Alexandra C Brand[1,2] 1) MRC Centre for Medical Mycology at the University of Exeter, Exeter, UK; 2) School of Medicine, Medical Sciences & Nutrition, University of Aberdeen, Aberdeen, UK; 3) School of Plant Sciences, University of Oxford, Oxford UK.

Calcium (Ca^{2+}) ions are an important second messenger for stress adaptation responses and growth in eukaryotic cells. In fungi, Ca^{2+}-signalling is poorly understood due to sequestration of Ca^{2+} reporter dyes into the vacuole but the availability of genetically encoded Ca^{2+} indicators (GECIs) offers new insights into Ca^{2+}-induced cell responses in real time. We have engineered the GCaMP6 construct for use in the fungal pathogen, *Candida albicans*, and used it to reveal dynamic cytoplasmic Ca^{2+} spiking in cell populations and individual yeast and hyphae for the first time. Growing yeast cells emit Ca^{2+} spikes of <10 s in duration at a rate dependent on extracellular [Ca^{2+}]. Ca^{2+} spikes are inhibited by chelation of extracellular Ca^{2+} with BAPTA, acidic pH and treatment with the Ca^{2+}-channel blocker, verapamil. GCaMP6 also revealed slower changes in the resting level of intracellular Ca^{2+} in a treatment-dependent manner.

Exposure of cells to stress compounds elicited differential GCaMP6 response signatures. The anionic surfactant, SDS, caused an instant, population-wide burst of spiking and a rise in resting levels. Cells did not adapt to repeated exposure to SDS, which led to ~10% cell death per treatment. Hyperosmotic shock (1 M NaCl) inhibited spiking and caused cell shrinkage on repeated exposure but cell viability was unaffected. Spiking ceased while resting levels rose in cells exposed to physiologically-relevant oxidative stress (5 mM H_2O_2) but this response diminished on repeated treatment, suggesting activation of an adaptive response. Adaptation was not seen in cells lacking the key antioxidant transcription factor, Cap1, or the phosphatase, calcineurin. Interestingly, the oxidative stress response was not seen in the *hog1Δ* mutant. These results are consistent with the role of the Cap1 and calcineurin signalling pathways in adaptation to oxidative stress and suggest that the *hog1Δ* mutant is pre-adapted to oxidative stress induced by 5 mM H_2O_2.

Live-cell imaging of GCaMP6 reporter activity is a useful tool for dissecting the role of Ca^{2+} in the response pathways of *C. albicans,* to diverse environmental challenges by observing stress-induced Ca^{2+} signatures in individual cells.

346F Hyphal growth rate correlates to the spatial distribution of the endocytic collar in three ascomycete species *Joseph Vasselli*[1], Ellen Kainer[1], Brian Shaw[1] 1) Texas A&M University.

Filamentous fungi produce hyphae as their basic cellular unit. Hyphae grow in a polarized manner that coordinates exocytosis at the cell apex and endocytosis in a collar just distal to the apex. These two processes are essential for maintenance of polarized growth. Understanding this means of growth regulation is both medically and agriculturally essential, as most fungal patho-systems require polarized growth to initiate disease. Building upon a prior study that utilized *Neurospora crassa* hyphae, we hypothesized that the spatio-temporal localization of endocytic activity, as well as the rate of endocytic activity, are directly associated with growth rate of hyphae. Using advanced microscopy techniques such as 4-dimensional imaging and FRAP we quantified a strong positive association between the distance the endocytic collar is positioned distal to the hyphal apex and the growth rate of hyphae. This association was consistent in three tested fungi including, including *Neurospora crassa*, *Aspergillus nidulans*, and *Colletotrichum graminicola*. Other tested variables, such as the size of the collar and the diameter of the hypha, were less strongly associated with growth rate.

347W Interspecies interactions of *Neurospora crassa* and *Botrytis cinerea* are mediated by a conserved cell-cell communication mechanism Hamzeh Hammadeh[1], Antonio Serrano[1], Natascha Stomberg[1], Ulrike Brandt[1], *Andre Fleissner*[1] 1) Technische Universitaet Braunschweig.

Cell fusion is essential for the development of most eukaryotic organisms, its molecular basis is, however, only poorly understood. An established model organism to study cell-cell-fusion is *Neurospora crassa*. Germinating spores of this fungus grow towards each other and fuse to form a supracellular network. This type of cell-cell fusion is common in many other filamentous ascomycete fungi. Fusion germlings of *N. crassa* employ an unusual signaling mechanism, in which the two fusion partners coordinately alternate between signal sending and signal receiving, thereby establishing a kind of cellular dialog. This process involves the alternating recruitment of the MAP kinase MAK-2 and the SO protein to the plasma membrane. To test if this mechanism is conserved in other fungi, we characterized the roles of the MAK-2 and SO homologs in the grey mold *Botrytis cinerea*. Comparable to *N. crassa*, both proteins are required for germling interactions. In addition, we observed an identical alternating membrane recruitment of the two proteins in interacting cell tips, suggesting that the “cell dialog” signaling mechanism is conserved. When *N. crassa* and *B. cinerea* spores are mixed, interactions between the two species are frequently observed, which result in mutual interspecies attraction and cell-cell contact. However, interspecies fusion was never observed. These findings suggest that the so far unknown signal and receptor that mediate cell-cell communication are also conserved, and that so far uncharacterized downstream mechanisms have evolved, that prevent interspecies fusion after cell-cell contact. In addition, we found that the presence of *N. crassa* can reprogram developmental decisions in *B. cinerea*. In the grey mold, cell fusion and pathogenic growth appear to be mutually exclusive. When confronted with *N. crassa*, however, *B. cinerea* also undergoes fusion under growth conditions, which usually trigger infectious growth. We hypothesize that the pathogenic development may be suppressed in the presence of the so far unknown fusion signals.

348T Functional Amyloids Are Widespread in Fungal Biofilm Adhesins *Peter Lipke*[1], Melissa Garcia-Sherman[1], Yves Dufrene[3], Stephen Klotz[2] 1) CUNY Brooklyn College; 2) U. Arizona, Tucson, AZ; 3) U. catholique de Louvain, Louvain-la-Neuve, BE.

Amyloid structures assemble through a periodic repeating type of bonding called “cross-β,” in which identical sequences in many protein molecules form β-sheets that interdigitate through side chain interactions. Cross-β bonding requires identical sequences on many molecules; therefore, the bonds form only when threshold concentrations of identical sequences are present. Cross-β bonds cluster adhesins on fungal cell surfaces into patches with extremely high ligand-binding avidity. The clusters on individual cells also interact *in trans* to form cell-cell adhesions. Therefore, compounds that inhibit amyloid formation also inhibit fungal adhesion, aggregation, and biofilm formation. We present a model for how amyloid-like cross-β bonds form *in trans* between adhering cells.
These cell-to-cell bonds are highly stable and are species- or strain-specific. A wide variety of fungal adhesins have evolved to form such cross-β bonds, which form in biofilms and stabilize them against flow. This property allows biofilms to include members with identical adhesins, and to marginalize heterologous strains and species (conforming to Dawkins’ “greenbeard” theory of altruism). Thus, amyloid-like bonds are widespread feature of abscesses in pathological mycoses and in environmental biofilms. The cross-β bonds are bound by the PRR Serum Amyloid P component, which skews macrophages toward non-inflammatory responses. This finding helps explain the lack of inflammation in deep mycoses. On the other hand, there are now reports of association of CNS fungal infections with Alzheimer’s disease. We speculate that fungal amyloids may help seed pathological amyloids as well.

349F Nuclear Competition and Coordination in *Neurospora crassa* Syncytia *Alexander Mela*[1], Louise Glass[1] 1) The University of California, Berkeley Campus.

The multinucleate syncytium is an important growth stage or strategy that allows for a wide range of adaptations to enable fungi to overcome mutations, coordinate growth and communication, as well as shuffle nuclear and cytoplasmic organelles between genetically distinct organisms. The underlying mechanistic details of how syncytia regulate cellular and molecular processes spatiotemporally is

largely unexplored. We have implemented a strategy, using Fluorescence Activated Cell-Sorting (FACS) to analyze dynamic mixing of tagged histone proteins in heterokaryons, which were derived from numerous combinations of auxotrophic and morphological mutants, as well as strains that were vegetatively incompatible. We observed that heterokaryons are largely permissive with regards to nuclear mixing, suggesting 'cheater' nuclei within heterokaryons readily persist, even those that are completely dependent upon other nuclei for survival, despite clear disadvantages to the syncytium. However, in strains that are vegetatively incompatible, we observed particular combinations that present clear barriers to nuclear mixing, suggesting that this process provides a distinct competitive advantage, in addition to mediating allorecognition.

350W Live cell imaging to understand fungicide mode of action *Gero Steinberg*[1], Martin Schuster[1], Sreedhar Kilaru[1] 1) Univ Exeter.

Cell biological analysis of genetically modified fungi is traditionally used to better understand the role of particular gene products in fungi. Considering the need for new fungicides against human and plant pathogens, we started to apply such combination of quantitative live cell imaging and molecular genetics to the study of fungicide mode of actions in various fungal pathogens. Here we will present unpublished results that demonstrate the power of this approach and reveal unexpected and new ways of fungicide activity in the fungal cell. This knowledge promises to help developing new strategies in pathogen control.

351T Antifungal Screening of 54 Single Plant Essential Oils Against *Aspergillus fumigatus* Matthew Swearingen[1], Elizabeth Myers[2], *Yainitza Hernandez-Rodriguez*[2] 1) Heartland Food Products Group; 2) Florida Gulf Coast University.

One bioprospective approach towards antimicrobial resistance is to source and research antimicrobial compounds from natural sources. Plant essential oils conceivably represent a safe and natural alternative to fight against microbial infections. These volatile oils have been used in medicine for years, and current research explores their possible efficacy towards diseases and infections that have been challenging to treat, or more significantly, in cases where current medicine is being challenged by antimicrobial resistance. For instance, some clinical isolates of *Aspergillus fumigatus,* an opportunistic pathogen of the respiratory system, have shown resistance to current azole treatments against aspergillosis. In addition, some studies have shown that prolonged azole treatments can result in negative implications for patients. In order to explore alternative methods for inhibiting the growth of *A. fumigatus*, we conducted a screen of 54 single plant essential oils (PEOs) from a single vendor and measured their inhibitory activity. We conducted disc diffusion assays of the PEOs and voriconazole against *A. fumigatus* (*Af293*) and measured zones of inhibition. We found that 21/54 PEOs had average ZOIs ranging from 10 – 30 mm, 16/54 with ≥ 30 mm, and cilantro, lemongrass, Melissa (lemon balm), thyme, and oregano were fully inhibitory. In addition, 30/54 PEOs efficacy was greater than the inhibitory effects of voriconazole (1μg/ml). The majority of the PEOs tested displayed some inhibitory effect against *A. fumigatus*. Interestingly, fully inhibitory PEOs contain a variation in their major constituents, with the presence of different monoterpenes being common for all. Lemongrass and Melissa (lemon balm) contain geranial and neral as common major constituents which are monoterpene aldehydes shown to work as antimicrobial, anti-inflammatory, analgesic, and chemo-preventives. In addition, thyme and oregano both contain thymol a monoterpene phenol with antimicrobial, antifungal, anti-inflammatory, and anesthetic activities. Moreover, cilantro contains linalool a monoterpene alcohol shown to have antibacterial, antifungal, anti-inflammatory, and anti-cancer activities. The present study shows that some PEOs may serve as an alternative for inhibiting fungal growth and raises the possibility for their use in combination with current treatments. In addition, PEOs efficacy variation may help indicate components in the oils that could be used as possible treatments against resistant microbes.

352F Identification of a protein-protein interaction site essential for mitotic entry to guide antifungal drug design in *Aspergillus fumigatus* *Isabelle Storer*[1], Benjamin Thornton [1], Lydia Tabernero[1], Michael Bromley[1] 1) University of Manchester, Manchester, UK.

Invasive fungal diseases have high associated mortality and there are limited antifungal agents to combat infections. Resistance mechanisms are rapidly emerging against current antifungal drugs that mainly target the cell wall and cell membrane; therefore, there is an urgent need to develop drugs with novel modes of action. Protein kinases and phosphatases regulate critical processes including metabolism, signalling, and the cell cycle, making them promising drug targets. However, high homology with human proteins causes issues with selectivity and specificity when considering them as antifungal targets. During the *Aspergillus fumigatus* cell cycle, a signalling cascade initiated by the protein phosphatase NimT mediates progression from G2 phase to mitosis. Although these processes are highly conserved, we hypothesise that drugs can be developed to selectively target the interaction of the fungal enzymes.
Here we characterise domains within the essential protein phosphatase NimT that mediate its interaction with its target, the protein kinase NimX. The interaction interface we describe is physically distant from the highly conserved catalytic domain of NimT and NimX and is poorly conserved in the orthologous human enzymes CDC25 and Cdk2. Using our virtual screening pipeline (VSpipe) we have identified a druggable pocket at the NimT-NimX interface. We hypothesise that drug-like molecules occupying this space will inhibit the interaction between NimT and NimX, arresting the cell before mitosis can occur.

353W Membrane integrity contributes to resistance of *Cryptococcus neoformans* to the cell wall inhibitor caspofungin *Maureen Donlin*[1], Brenda Moreira-Walsh[1], Woei Lam[2], Rajendra Upadhya[2], Jennifer Lodge[2] 1) Saint Louis University School of Medicine; 2) Washington University School of Medicine.

Cryptococcus neoformans is a fungal pathogen of immunocompromised people that causes up to 1 million infections each year globally, resulting in up to 250,000 deaths annually. Therapeutic options for treating *C. neoformans* infections are very limited. The echinocandin class of antifungals, *i.e.* caspofungin, are generally well tolerated by are clinically ineffective against *C. neoformans*. We are interested in understanding why *C. neoformans* is so resistant to this well-tolerated class of anti-fungals. Due to the inherent resistance of *C. neoformans* to caspofungin, identifying genes or pathways that can be targeted to render the cell more susceptible to echinocandins is a strategy for development of new combination therapies. Our motivation for this work was to expand the identification of genes and processes with a role in caspofungin resistance by systematically screening the libraries of gene deletions made in the KN99a background for caspofungin sensitivity. We adapted a fungal biofilm assay developed for screening *Candida albicans* to reflect the growth characteristics of *C. neoformans.* After calibrating and confirming the caspofungin concentration needed to reveal sensitive

phenotypes, we systematically screened 4030 individual gene deletions in triplicate plate assays and identified 25 strains that showed caspofungin sensitivity. We followed up with a dose-dependent assay and confirmed that 17 of the 25 remained sensitive. We tested all 17 strains on agar plate-based assays and found that only 5 strains showed sensitivity under those conditions. We made new deletions of four of the five genes: *CFT1*, an iron transporter; *ERG4*, a sterol desaturase; *MYO1*, encoding the myosin heavy chain and *YSP2*, a sterol transporter. We examined the sensitivity of these deletions to a variety of stress conditions and found that all were more sensitive to membrane stress and showed significantly increased sensitivity to caspofungin at higher temperatures. Surprisingly, none showed any obvious cell wall defects nor differences in their chitosan content as would be expected for caspofungin sensitive strains. Our microscopy analyses using various membrane targeting dyes support the hypothesis that loss of membrane integrity contributes to the caspofungin sensitivity, either by allowing more caspofungin to enter or remain in the cell or by altering the location or orientation of the β-1,3-glucan synthesis enzyme target to render it more susceptible to inhibition.

354T The *Aspergillus fumigatus* morphogenesis-related kinase, CotA, orchestrates hyphal growth in response to carbon source quality *Adela Martin-Vicente*[1], Xabier Guruceaga[1], Ashley V Nywening[1], Jinhong Xie[1], Harrison I Thorn[1], Wenbo Ge[1], Jarrod R Fortwendel[1] 1) University of Tennessee Health Science Center.

Fungal pathogens must exhibit strong nutritional plasticity, effectively sensing and utilizing varying nutrient sources, for development of invasive disease. How the molecular signals generated by nutritional sensing are efficiently translated to the cellular morphogenetic machinery for optimal orchestration of growth under nutritional stress remains incompletely understood. Here, we sought to identify and characterize protein kinases required for pathogenic growth in the opportunistic fungal pathogen, *Aspergillus fumigatus*. To identify molecular mechanisms supporting *A. fumigatus* growth in the mammalian lung environment, we first screened a protein kinase disruption library for growth on a mouse lung agar. This screen identified the disruption of only the conserved morphogenesis-related protein kinase gene, *cotA*, as essential. Further *in vitro* studies revealed that, although the *cotA* disruption mutant (*cotA*-1) displayed only a slight growth defect compared to the parental strain when grown on glucose, hyphal growth was almost completely abolished when grown on lung agar, as well as on lung explants. Employing a mouse model of invasive pulmonary aspergillosis, we also found that the *cotA*-1 mutant displayed reduced virulence, characterized by the inability to form tissue-invasive hyphae *in vivo*. Additional *in vitro* studies revealed that, unlike the parental and the complemented strains, the *cotA*-1 mutant formed only compact colonies when provided non-sugars as the sole carbon source. However, we show that the CotA protein does not regulate, nor is *cotA* gene expression regulated by, the carbon catabolite repression system. Using a novel CotA-specific antibody, we instead uncovered that wild type *A. fumigatus* produces two CotA protein isoforms, long and short, whereas the *cotA*-1 disruption mutant produces only the short isoform. Culture on non-sugar carbon sources caused complete loss of CotA protein in the *cotA*-1 mutant. Additional mutational analyses showed that conserved phospho-regulatory sites on the CotA protein were required for CotA support of hyphal growth, independent of carbon source quality. Taken together, our data show that the *A. fumigatus cotA* gene encodes a conserved morphogenesis-related kinase that is produced as two protein isoforms, long and short. The presence of the long isoform is required for growth under host mimicking *in vitro* conditions and for *in vivo* virulence in a manner dependent on carbon source quality.

355F The phosphatase Sac1 mediates capsule secretion in *Cryptococcus neoformans* *Elizabeth Gaylord*[1], Guohua Chen[1], Tamara Doering[1] 1) Washington University in St. Louis, St. Louis MO, USA.

Cryptococcus neoformans is an environmentally-acquired, opportunistic fungal pathogen that is a significant cause of death in individuals with HIV/AIDS. A major virulence factor of *C. neoformans* is its thick polysaccharide capsule, which is required for survival and proliferation in the human host. This capsule provides the dual benefit of masking the underlying immunogenic cell wall and increasing the size of the cells, both of which reduce uptake by host phagocytes. Despite the importance of the capsule for cryptococcal virulence, our understanding of its synthesis is limited. Various genes involved in the synthesis of capsule polysaccharides have been identified; however, the mechanisms of capsule secretion and attachment to the cell are not well understood.

The goal of this study is to identify novel genes with roles in capsule biosynthesis. We developed an automated, fluorescence microscopy-based screen to measure capsule thickness. The advantage of this approach is that it allows for the detection of subtle changes in capsule size, thus identifying hypocapsular or hypercapsular strains that may have been otherwise overlooked. We applied this approach to 400 deletion mutants with altered phagocyte interactions. We identified 87 mutants with altered capsule sizes, including the deletion mutant of *CNAG_06080*, which encodes the phosphatidylinositol-4-phosphate (PI4P) phosphatase Sac1. In *S. cerevisiae*, Sac1 maintains normal secretory traffic by dephosphorylating the lipid signaling molecule PI4P on intracellular membranes. This protein has not been studied in *C. neoformans*. s*ac1Δ* cells had smaller capsules, decreased fungal burden in mice and defective lipid trafficking in host-like conditions. Interestingly, the capsules of *sac1Δ* cells exhibited altered capsule fiber appearance using electron microscopy. Further compositional analysis of s*ac1Δ* capsular material by HPAEC-PAD demonstrated different ratios of the capsule components compared to wild-type capsule. We hypothesize that the loss of Sac1-mediated PI4P turnover in the secretory pathway results in reduced secretory capacity, leading to the smaller capsules of Sac1 mutants. Planned studies include localizing Sac1 and PI4P to interrogate the role of PI4P signaling under host-like conditions. Due to the importance of Sac1 in the secretory pathway in model yeasts, understanding this phosphatase in *C. neoformans* will further elucidate the process of capsule production.

356W Investigating temperature-induced transcriptional changes that underlie fungal morphogenesis in *Histoplasma capsulatum* *Anna Morrison*[1], Mark Voorhies[1], Anita Sil[1] 1) University of California San Francisco, San Francisco, CA.

Temperature plays a critical role in altering the developmental program and virulence of thermally dimorphic fungal pathogens, including *Histoplasma capsulatum*. In the environment, *H. capsulatum* grows as a multicellular hyphal form that produces vegetative spores. Upon inhalation by a mammalian host, spores undergo a developmental change to a parasitic yeast form that secretes virulence factors and causes disease. This morphological transition can be recapitulated in the laboratory, where temperature is a sufficient signal to shift cultures of *H. capsulatum* between hyphal and yeast forms, which grow at room temperature (RT) and 37°C, respectively. A longstanding question in the field is how *H. capsulatum* and other thermal dimorphs link temperature to changes in morphology and gene expression. Prior studies revealed that over a quarter of the *H. capsulatum* transcriptome is differentially expressed between

room temperature and 37°C; however, it is not understood how these transcripts are linked to morphogenesis and virulence. Here, we investigated changes in gene expression and morphology in response to intermediate temperatures to uncover gene networks that are associated with temperature-dependent regulation of cell fate and virulence in *H. capsulatum*. We performed RNA sequencing on samples grown for two hours at 33°C and 29°C, in addition to 37°C and RT, to capture differences in early transcriptional changes. We found that the hyphal gene expression program, which has been characterized at RT, is partially induced at 33°C, and more strongly induced at 29°C. Corresponding morphology studies revealed that growth for up to ten days at both 29°C and 33°C results in a mixed population of yeast and hyphae, indicating that maintenance of yeast-phase growth is disrupted at these temperatures. Finally, we identified sets of phase-specific transcripts whose expression is dependent on growth at specific temperatures. This work contributes to a wider effort to define temperature-responsive gene networks that control development and virulence in *H. capsulatum.*

357T Hyphal branch formation in the opportunistic human pathogen *Candida albicans* *Antonio Serrano*[1], Martine Bassilana[1], Robert Arkowitz[1] 1) University Cote d›Azur/CNRS/INSERM, Nice, France.

Candida albicans is a yeast that can cause superficial, as well as systemic infections, in immunocompromised individuals. Formation of invasive hyphal filaments contributes to fungal virulence and a single hypha has the capacity to generate multiple growth sites, in a defined and coordinated process known as filament branching. Interestingly, hyphal branching is observed in mice tissues infected with *C. albicans*, with an increase in branching frequency in necrotic zones (under nutrient limitation), suggesting that it is important for fungal virulence [1]. Additionally, in some fungi, branching appears to be activated in response to host immune responses [2].

In order to understand how branching is initiated and regulated in *C. albicans*, we have been quantitatively analyzing the frequency, distribution, and extension of branches on hyphal cells. Filament branching is observed in all hyphal compartments with a constant frequency between the formation of the adjacent septa and the branch, and with a fixed distance between the branch and the septa. The branching angle is not dramatically different from compartment to compartment, yet it appears to increase when branching is initiated from the sub-apical compartments, which tend to be larger Interestingly, we observe differences between the main growing hyphae and the branches, *i.e.* branches extend slower with a reduced diameter. Our preliminary analyses indicate that the localization of several cell growth reporters is somewhat altered in branches. All together, our initial observations suggest that the growth process is likely to be differently regulated between the main and the branching hyphae.

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358F The RAM signaling pathway links morphology, thermotolerance, and CO_2 tolerance in human fungal pathogen *Cryptococcus neoformans* *Benjamin Chadwick*[1], Tuyetnhu Pham[1], Xiaofeng Xie[1], Xiaorong Lin[1] 1) University of Georgia.

Cryptococcus neoformans is a basidiomycete with worldwide distribution. This fungus is also an opportunistic human pathogen that claims more than 180,000 lives per year. The pathogenicity of this environmental fungus relies on its adaptation to the host conditions. One important difference between its natural environment and the mammalian host is concentration of CO_2. CO_2 levels of ambient air are about .04%, and CO_2 levels in the host are over 100 times higher (~5%). We recently found that while clinical isolates are tolerant to host levels of CO_2, many environmental isolates are sensitive to host levels of CO_2 and are attenuated in animal models. Given that CO_2 tolerance is only a recently considered virulence trait, the genetic mechanism underlying this trait has not been investigated. Here, we screened cryptococcal gene knockout libraries constructed in the CO_2-tolerant clinical isolate H99 background to identify mutations that render the fungus CO_2-sensitive. Interestingly, we found that mutants in multiple pathways which are activated by heat stress are CO_2-sensitive, including the Calcineurin, Ras1-Cdc24, cell wall integrity, and RAM pathways. Furthermore, overexpression of Cbk1, the downstream kinase of the RAM pathway, was able to restore defects in thermotolerance and CO_2 tolerance of Calcineurin, Ras1-Cdc24, and cell wall integrity pathway mutants, demonstrating its pivotal role in integrating different upstream signaling pathways. In ascomycete fungi such as *Saccharomyces cerevisiae* and *Candida albicans*, Ace2 is the downstream transcription factor of Cbk1 and it activates genes responsible for cytokinesis. However, no Ace2 homolog or any downstream components of the RAM pathway have been identified in basidiomycetes. Through natural evolution, we isolated multiple suppressor strains of the *cbk1*D mutant. By whole genome sequencing and comparative genomics, we found multiple distinct mutations in the gene *SSD1* among the suppressor strains. Indeed, deletion of *SSD1* in *cbk1*D was able to partially restore CO_2 tolerance, thermotolerance, and morphological defects in vitro. Deletion of *SSD1* also partially restored *cbk1*D's defect in virulence in both inhalation and intravenous infection mouse models of cryptococcosis. The findings of this study suggest that CO_2 tolerance is co-regulated with other virulence factors such as morphology and thermotolerance, and highlights the essential role of the RAM pathway in adaptation to the host environment in *Cryptococcus*.

359W A role for the Rsp5 ubiquitin ligase and its interactome in the pathogenesis of *Cryptococcus neoformans* *Lukas du Plooy*[1,2], Calla Telzrow[1,2], Andrew Alspaugh[1,2] 1) Department of Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, NC; 2) Department of Medicine, Duke University School of Medicine, Durham, NC.

Ubiquitination, frequently described as the "molecular kiss of death", is an enzymatic post-translational modification known to be involved in proteasomal degradation of misfolded and spent proteins. The 76-amino acid ubiquitin polypeptide becomes covalently linked, most often via lysine residues, to target proteins by the three-component ubiquitination system consisting of the ubiquitin activating, conjugating and ligase enzymes. Studies have also elucidated a regulatory role for ubiquitination in addition to its role in protein degradation, including the regulation of the location, activity, and expression of proteins with the attachment of mono- or poly-ubiquitin tags. One such ubiquitin ligase, Rsp5, is known to be required for the survival of the human fungal pathogen, *Cryptococcus neoformans,* in a murine infection model and for growth at elevated temperatures. In our hands, an *rsp5*Δ mutant strain is also unable to grow in the presence of various cell surface stressors, such as Congo red and sodium chloride. We have employed both biased and unbiased

techniques, including a high-throughput differential ubiquitination screen and phenotypic screens, to identify targets of Rsp5 that are involved in stress response and, therefore, also potentially in pathogenesis. Several proteins known to be involved in pathogenesis were identified as Rsp5 targets through our screens, including proteins involved in biosynthesis of cell surface components, such as 1,3-beta-D-glucan. Additionally, Rsp5 is required for alkaline pH sensing through the Rim101 alkaline pH sensing pathway. This ubiquitin ligase is known to interact with adaptor proteins, most notably arrestin-like (or ART, arrestin-related trafficking adaptor) proteins to facilitate recognition of substrate proteins. We have previously identified four arrestin-like (Ali1-4) proteins in *C. neoformans*, and showed that Ali1 contributes to cytokinesis, likely through interactions with Rsp5. These observations demonstrate that Rsp5 and its adaptor network are likely involved in the regulation of multiple other pathogenesis-related pathways, which will form the basis of further studies.

360T The role of cytochrome c in leukocyte induced *Aspergillus fumigatus* cell death. *Matthew James*[1], Ko-Wei Liu[1], Elisa Vesley[1], Tobias Hohl[2], Robert Cramer[1] 1) Geisel School of Medicine at Dartmouth College, Hanover, NH; 2) Memorial Sloan Kettering Cancer Center, New York, New York.

Aspergillus fumigatus is a ubiquitous environmental mold that can cause a life-threatening infection known as invasive aspergillosis (IA). IA is caused by defects in innate immune system function that result in failed clearance of inhaled conidia from the lung. While it is known that innate immune function, particularly NADPH oxidase activity, is responsible for clearance of these conidia from the lung, the mechanism by which these conidia are killed by leukocytes remains unknown. While processes of cell death in have been identified in animals, these processes remain largely unknown in outside of metazoa. Contributing to this question regarding non-metazoan cell death, many canonical components of regulated cell death are not conserved outside of metazoa. Here we investigate the role of *A. fumigatus* cytochrome c (*cycA*) in both hydrogen peroxide-induced and leukocyte-induced fungal cell death. Cytochrome c is a canonical cell death effector in higher order metazoa that functions in programmed and regulated forms of cell death and is conserved across eukaryotes. Using a flow cytometry approach, we observe that a Δ*cycA* strain displays altered cell death phenotypes including reduced histone fragmentation, reduced caspase-like activity, and reduced sytox blue staining after 6hr and 8hr exposure to 10mM H2O2. However, using a germination assay to monitor growth 30hrs after acute 2.5hr exposure to 10mM H2O2, we observe that loss of *cycA* results in no observable growth after treatment, suggesting a loss of viability as compared to the WT and complement strains. Using in vivo FLuorescent *Aspergillus* REporter (FLARE) technology, we observe that loss of *cycA* results in lower in viability in leukocyte subsets including neutrophils and total macrophages as compared to WT and complement strains. However, the Δ*cycA* strain displayed higher viability in specifically the alveolar macrophage subset as compared to other leukocyte subsets. Taken together, these data suggest cytochrome c presence in *A. fumigatus* contributes to cell survival under death inducing conditions and future studies will seek to define the underlying mechanisms.

361F The *Cryptococcus neoformans* Flc1 homologue controls calcium homeostasis and survival in the infected host *Lukasz Kozubowski*[1], Piotr Stempinski[1], Kristie Goughenour[2,3], Marnus du Plooy[4], Michal Olszewski[2,3], Andrew Alspaugh[4] 1) Clemson University, Clemson SC, USA; 2) University of Michigan Medical School, Ann Arbor MI, USA; 3) LTC Charles S. Kettles VA Medical Center, Ann Arbor, MI, USA; 4) Duke University Medical Center, Durham, NC, USA.

The opportunistic human pathogen *Cryptococcus neoformans* relies on a complex network of stress response pathways critical for survival and proliferation in the host. Proteins containing a transient receptor potential (TRP) domain, including the flavin carrier protein 1 (Flc1) in *Saccharomyces cerevisiae*, are poorly characterized integral membrane transporters that regulate stress responses in fungi. While Flc1 homologue is expressed by *C. neoformans*, its role in stress response and cryptococcal virulence remains unknown. Here, we report that deletion of cryptococcal *FLC1* results in cytosolic calcium elevation and increased nuclear content of calcineurin-dependent transcription factor Crz1, which accounts for the aberrant cell-wall chitin over-accumulation observed in *flc1*Δ mutant. Absence of Flc1 or inhibition of calcineurin with cyclosporine A prevents vacuolar fusion under conditions of combined hyperosmotic and temperature stress, which is reversed by the inhibition of TORC1 kinase with rapamycin. A compromised vacuolar fusion in the *flc1*Δ mutant is also observed under starvation conditions, including conditions that stimulate formation of carbohydrate capsule. Consequently, the *flc1*Δ mutant fails to proliferate in the presence of 1 M sorbitol at 37°C or under low nutrient conditions and displays a defect in capsule formation. The *flc1*Δ mutant presents defective survival in J774A.1 macrophage cell-line and profound virulence defects in both the *Galleria mellonella* larvae and mouse pulmonary infection models, demonstrating that Flc1 is essential for pathogenicity of *C. neoformans*. Thus, cryptococcal Flc1 in a calcineurin-dependent manner contributes to cryptococcal stress response, required for pathogen's fitness and virulence. Importantly, Flc1 and its close homologues in other pathogenic fungi are sufficiently divergent from their mammalian functional homologues, indicating that fungal TRP domain proteins constitute promising new targets for broad spectrum/low toxicity antifungal drugs.

362W Elucidating a Novel Role for Septins During High Temperature Stress Response in *Cryptococcus neoformans* *Stephani Martinez Barrera*[1], Lukasz Kozubowski[1] 1) Department of Genetics and Biochemistry, Eukaryotic Pathogens Innovation Center, Clemson University, Clemson SC, USA.

The pathogenic yeast *Cryptococcus neoformans* needs to adapt to changes in temperature upon entering its human host. *C. neoformans* strains lacking septin proteins Cdc3 or Cdc12 are viable at 25°C but fail to proliferate at 37°C and are avirulent in the heterologous host infection model. Septins are a family of conserved filament-forming GTP-ases that bind to phosphoinositides and assemble as higher order complexes at the cell cortex to support cytokinesis and morphogenesis in fungal and animal cells. The exact contribution of septins to growth of *C. neoformans* at high temperature remains unclear and a similar putative stress-related function has not been investigated in any other organism. Current model assumes that *C. neoformans*septins contribute to growth at 37°C by localizing to the mother-bud neck and supporting cytokinesis and/or final cell separation. However, our recent findings suggest a novel role for septins in high temperature response. We find that upon temperature change to 37°C, septins Cdc12, Cdc10, and Cdc11 accumulate at the plasma membrane (PM) as puncta. The localization of Cdc10 to the PM is dependent on Cdc12 suggesting that septins associate with the PM as a complex. Mutants lacking septin Cdc3 or Cdc12 exhibit an increased internalization of propidium iodide and are hypersensitive to drugs that perturb PM lipid composition suggesting that absence of septin Cdc3 or Cdc12 causes an aberrant

bio-physical state of the PM that increases its permeability. Our study points to a novel function of septin proteins in the regulation of PM homeostasis that may be critical for high temperature stress response.

363T Determining the role of the spore-enriched protein Isp2 in the maintenance of dormancy *Anna Frerichs*[1], Mingwei Huang[1], Christina Hull[1] 1) University of Wisconsin Madison, Madison, WI.

Spores are vital for the long-term survival of many organisms due to their roles in reproduction and stress resistance. Among fungal pathogens of plants and animals, spores are infectious particles that are more resistant to environmental conditions than other cell types. In the human fungal pathogen *Cryptococcus*, spores cause disease only when they germinate into yeast, initiating vegetative growth in the host. Although little is known about the germination process, it holds the potential to reveal fungus-specific pathways or proteins that could serve as targets for germination inhibitors. We previously discovered a spore-enriched protein, Identified Spore Protein 2 (Isp2), which is required for normal spore biology. Strains lacking *ISP2* (*isp2Δ)* exhibit increased sporulation and initiate germination more quickly than wild type spores. This "jump start" is then followed by a stall later in germination, resulting in a net increase in total germination time. These findings suggest that Isp2 plays an active role in maintaining spore dormancy or a repressive role in germination initiation. Our hypothesis is that Isp2 controls a checkpoint that senses germination conditions in the environment and permits germination when growth conditions (e.g. nutrient requirements) are met. To test this hypothesis, we are using genetic, biochemical, and biological approaches to evaluate the function of Isp2. Understanding the full extent of Isp2 function promises to provide valuable insights into germination and how the process is initiated to ultimately cause disease.

364F Sterol homeostasis is critical for surface structure organization and virulence in *Cryptococcus neoformans* *Hau Lam Choy*[1], Tamara Doering[1] 1) Washington University in St. Louis, St. Louis MO, USA.

Cryptococcus neoformans is an opportunistic fungal pathogen that causes pulmonary and meningeal infections in immunocompromised hosts and is responsible for 15% of AIDS-related deaths. The standard treatment for Cryptococcosis comprises three antifungals, two of which, Fluconazole and Amphotericin B, target ergosterols. These lipids have essential cellular and biophysical functions in fungal cells. Ergosterol biosynthesis has been well-defined in *Saccharomyces cerevisiae.* However, our understanding of the relationship between sterol homeostasis and cryptococcal pathogenesis is lacking.

Our objective is to determine the function of the lipid transfer protein Ysp2 and its effect on cryptococcal pathogenesis. Ysp2 is homologous to an *S. cerevisiae* retrograde sterol transporter that moves sterols from the plasma membrane to the endoplasmic reticulum. There have been no previous reports about lipid transfer proteins in *C. neoformans*, making this protein and its roles in pathogenesis of great interest. We found that when *YSP2 (CNAG_00650)* was deleted, the mutant strain had reduced ability to survive within phagocytes and significantly attenuated virulence in a mouse infection model. Based on the known Ysp2 function, we hypothesized that the accumulation of sterols at the plasma membrane is detrimental to cryptococcal survival in host-like environments. Consistent with this hypothesis, we observed that when grown in host-like conditions, *ysp2Δ* cells had a significantly reduced growth rate and increased sterol levels at the surface. They also displayed invaginations of both the plasma membrane and cell wall. Strikingly, when ergosterol synthesis was reduced by fluconazole addition, all of these defects were rescued. To further test our hypothesis, we are currently isolating plasma membrane fractions to analyze sterol composition using GC-MS.

We are also interested in potential novel roles of the cryptococcal Ysp2. In *S. cerevisiae*, Ysp2 is one of six lipid transfer proteins with different functions. In *C. neoformans*, however, we find only one homolog, suggesting that cryptococcal Ysp2 acts at multiple sites.

Overall, we have shown that a lipid transfer protein is critical for the virulence of *C. neoformans*. By determining how Ysp2 affects sterol organization, we will elucidate the mechanisms of cryptococcal cellular distribution of ergosterol, an important drug target during human infection.

365W Two distinct lipid transporters together regulate invasive filamentous growth in *Candida albicans* Miguel Basante-Bedoya[1], Stephanie Bogliolo[1], Rocio Garcia-Rodas[1,2], Oscar Zaragoza[2], Robert Arkowitz[1], *Martine Bassilana*[1] 1) University Cote d›Azur/CNRS/INSERM, Nice, France; 2) Mycology Reference Laboratory, National Centre for Microbiology, Health Institute Carlos III, Madrid, Spain.

Flippases transport lipids across the membrane bilayer to generate and maintain asymmetry. The human fungal pathogen *Candida albicans* has 5 flippases, including Drs2, which is critical for filamentous growth and phosphatidylserine (PS) distribution (1). Furthermore, a *drs2* deletion mutant is hypersensitive to the antifungal drug fluconazole and to copper (1,2). We now show that such a mutant also has an altered distribution of phosphatidylinositol 4-phosphate, PI(4)P, and ergosterol, as well as reduced virulence in a murine model of systemic candidiasis. Analyses of additional lipid transporters, *i.e.* the flippases Dnf1-3, and oxysterol binding protein (Osh) family lipid transfer proteins, *i.e.* Osh2-4 and Osh7, indicate that they are not critical for filamentous growth. However, deletion of *OSH4*, which encodes a lipid transporter that exchanges PI(4)P for sterol, in a *drs2* mutant specifically bypass the requirement for Drs2 in invasive filamentous growth. In addition, removal of the lipid phosphatase Sac1, which dephosphorylates PI(4)P, in a *drs2* mutant results in a synthetic growth defect, suggesting that Sac1 and Drs2 function in parallel pathways. Together these data indicate that a balance between the activities of two different classes of lipid transporters regulates invasive filamentous growth, *via* PI(4)P. In contrast, deletion of *OSH4* in the *drs2* mutant does not restore growth on fluconazole, copper or papuamide A, a toxin that binds PS in the outer leaflet of the plasma membrane, suggesting that Drs2 has additional role(s) in plasma membrane organization, independent of Osh4.

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366T Live-Cell Imaging of Sexual Reproduction in *Podospora anserina*: the foreplay *Sylvain Brun*[1] 1) Institut Jacques Monod - UMR 7592, Universite de Paris, CNRS, Paris, France.

Sexual reproduction in fungi and fruiting body development have attracted interest of researchers for centuries [1]. Imaging these structures under non-live-cell imaging conditions, such as electronic microscopy, have highlighted the extraordinary complexity of fruiting body development [2–5]. However, lack of live-cell biology has been an obstacle to better understand sexual reproduction. Using live-cell imaging, for the first time we have observed live male and female nuclei during sexual reproduction in the model fungus *Neurospora crassa*. This study has revealed the specific behaviour of resident female nuclei within the trichogyne (the female organ) after fertilization and the extraordinary manner with which male nuclei migrate across the trichogyne [6]. To test whether this process is conserved in ascomycetes, I have started the live-cell imaging of fertilization in the model fungus *Podospora anserina* and first observations suggest overall conservation of the process between both species. These studies started in *P. anserina* will aim at answering important questions such as, which elements of cytoskeleton control nuclear movements during sexual reproduction in filamentous fungi, is there a system avoiding polyspermy in fungi, which determinants control male *vs.* female identity of nuclei, *etc*. These studies will also aim at investigating male and female nuclei in the next steps of sexual development by imaging nuclei in the core of the perithecium where they proliferate before entry into the ascogenous cell and karyogamy. These cell biology approaches will be expanded by the functional study of the genes involved through forward and reverse genetics strategies easily undertaken in this amenable model fungus.

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367F Regulatory role of VE-1 in transcription during sexual development in the fungus *Neurospora crassa* *Sara Cea-Sánchez*[1], Sara Martín-Villanueva[1], Gabriel Gutiérrez[1], David Cánovas[1], Luis M. Corrochano[1] 1) University of Seville.

Fungi can serve as highly valuable models for understanding the molecular mechanisms behind sexual development in multicellular organisms. Changes in gene expression and the analysis of regulatory networks have demonstrated that genes and regulatory factors work together to shape morphological development. The velvet complex is a fungal-specific protein complex that participates in the regulation of gene expression in response to environmental signals such as light, regulation of development, pathogenesis, and secondary metabolism. The velvet complex in the fungus *Neurospora crassa* is composed of three proteins (VE-1, VE-2, and LAE-1). Strains lacking VE-1 and/ or VE-2 display increased conidiation, as well as a markedly delayed and reduced sexual development with fewer fruiting bodies compared to the wild-type strain. Alterations in the development of female structures, protoperithecia, in the *ve-1* and *ve-2* mutants suggested that the VE-1/VE-2 complex should regulate transcription during sexual development. We have characterized the transcriptome of wild-type and D*ve-1* mutant strains over the time course of sexual development in both dark and light. We identified 2117 genes with different transcriptional profiles between the wild-type and the mutant strain in cultures kept in the dark, and 4364 genes when cultures were kept in the light with an overlap of 1648 genes. Among the missregulated genes, we detected genes that are known for their regulatory roles in vegetative growth and sexual / asexual processes, as well as genes in the mitogen-activated protein kinase (MAPK) signaling pathway, cell-cell fusion genes (*ham* genes) and cell-wall integrity genes (*wsc* genes). Furthermore, we detected transcription of *ve-1*, *ve-2,* and *lae-1* during all stages of sexual development, but the three proteins were not detected in the later stages of development (96 and 144 hours after fertilization), suggesting a major role for the velvet complex in the early stages of sexual development. Our results provide key insights into the control of the multistage development process of fruiting body formation and ascospore production by the regulatory velvet complex in the fungus *Neurospora crassa.*

368W The fungal sexual revolution continues: indications of sexuality in the citric acid producing fungus *Aspergillus niger.* *Valeria Ellena*[1,2], Matthias Steiger[1,2] 1) Austrian Centre of Industrial Biotechnology (ACIB GmbH), Vienna, Austria. ; 2) Institute of Chemical, Environmental and Bioscience Engineering, TU Wien, Vienna, Austria. .

Sexual reproduction is an important proliferation mechanism which provides advantages to a species by promoting genetic variation. Interestingly, for a large group of fungal species, including the industrially relevant *Aspergillus niger*, this mode of reproduction has not been described yet. In recent years, strong indications of the sexual potential of *A. niger* have accumulated [1]. These include the ability of certain strains to produce pre-mature sexual structures (sclerotia) and the presence of a mating type MAT1-1 locus in the commonly used *A. niger* strains, such as ATCC 1015 and CBS 513.88.

Here we show additional evidence of the sexual potential of *A. niger*. Formation of sclerotia could be induced in the progenitor of the industrial citric acid producing strains of *A. niger,* ATCC 1015, and in *pyrG* mutants derived from it [2]. The capability of ATCC 1015 to form sclerotia, known to act as sexual structures in related organisms, represents a step forward towards the discovery of a sexual cycle in this important industrial species. To find the second mating type locus, we performed genome sequencing of the *A. niger* neotype strain CBS 554.65 and identified the missing MAT1-2 locus [3]. A nucleotide alignment showed a different orientation of the MAT1-1 locus of ATCC 1015 compared to the MAT1-2 locus of CBS 554.65. While the genomic context of the MAT1-2 locus in CBS 554.65 is similar to the one of other MAT1-2 *A. niger* strains and other *Aspergillus* species, the region comprising the MAT1-1 locus is inverted in all sequenced strains of *A. niger*. These observations suggest the occurrence of genetic flipping or switching events at the MAT1-1 locus of *A. niger*, which might have a direct impact on its sexuality. These results provide new insights in the mating system of *A. niger* and pave the way for the discovery of a sexual cycle in a species long thought to be asexual.

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369T The Mating Transcriptome of *Phycomyces blakesleeanus* *Jesús F. Peña*[1], Jason E. Stajich[1] 1) University of California, Riverside.

The first description of sexual reproduction in a fungus was reported nearly two centuries ago in the Mucoromycotan *Syzygites megalocarpus*. Since then, efforts to understand sexual development in fungi have largely focused on dikaryan fungi, given the tractability of species in these groups as model organisms and their relevance to human health. Investigation of these model fungi has enabled the discovery of genes that are important for sexual reproduction and development in the Dikarya, yet less is known about these processes in early diverging fungal lineages like the Mucoromycota. In this study, we focus on *Phycomyces blakesleeanus,* a heterothallic mucoromycete notable for its large, phototropic and gravitropic sporangiophores. The sexual cycle of *P. blakesleeanus* is characterized by drastic changes in cell morphology. We used RNA sequencing to explore global gene expression of the *P. blakesleeanus* sexual cycle. We isolated RNA at three timepoints characterized by distinct cell types or sexual structures: before crossing, pre- cell fusion, and post- cell fusion. We identified four sets of differentially expressed genes with similar expression patterns. Of the four expression patterns, three harbored genes which had highest expression at one of the three timepoints. The fourth expression pattern had comparably high expression before crossing and at the pre-cell fusion stage. GO enrichment analysis of the four expression profiles suggests a dynamic transcriptional landscape. Our data also supports previous work that identified mating-induced carotenogenesis and trisporic acid synthesis in mucoromycetes. This study is among the first to look at global gene expression during sexual reproduction in a mucoromycete and provides novel insights about the genetic and molecular mechanisms underlying sexual reproduction in the Mucoromycota.

370F Diverse sexual strategies underpinned by the mating-type locus in the non-model fungal family *Ceratocystidaceae* *Markus Wilken*[1], Emma Steenkamp[1], Michael Wingfield[1], Brenda Wingfield[1] 1) Forestry and Agricultural Biotechnology Institute (FABI), Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria, South Africa.

The *Ceratocystidaceae* (*Microascales*) accommodates more than 120 species that include plant pathogens and insect-associated fungi. For many years, these species were grouped in the single genus *Ceratocystis*, even though species complexes defined by morphological and ecological differences were apparent. Subsequent taxonomic revisions, mainly based on molecular phylogeny, allowed resolution of this taxon and recognition of the *Ceratocystidaceae* that currently contains 15 well-defined genera, with a unique biology that often extends to idiosyncrasies regarding their sexual strategies. The latter includes heterothallism, both primary and secondary homothallism, as well as unisexual reproduction. Some species are also considered putatively asexual due to the lack of an observed sexual cycle. Using a large-scale targeted genome sequencing approach, we have systematically analysed the mating-type loci of selected species representing 14 genera in the *Ceratocystidaceae*. This has revealed conservation in the mating-type locus linked to sexual reproduction. This pattern is sufficiently robust to enable prediction of the reproductive mode in the apparently asexual species, based on the structure of the *MAT* locus alone. Some variation in the structure of the mating-type locus among species in a single genus was found, highlighting a complex evolutionary history for sexual reproduction in the group. This research has also led to the identification of *MAT1-2-7*, a lineage-specific mating-type gene unique to the *Microascales*. Functional studies of *MAT1-2-7* have also provided insights into its role in sexual development in these species. The foundation provided by these studies continues to drive ongoing functional studies aimed at understanding the molecular intricacies of atypical and unexplored sexual processes in the fungi.

371W MATch Maker: A curated web-portal and database for fungal mating-type sequences Stephanie van Wyk[1], *Markus Wilken*[1], Chris Harrison[2], Frances Lane[1], Daniella Krämer[1], Emma Steenkamp[1], Brenda Wingfield[1] 1) Forestry and Agricultural Biotechnology Institute (FABI), Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria, South Africa; 2) Hawk.Rocks Development, Pretoria, South Africa.

Fungi, especially those in the Pezizomycotina, have evolved a remarkably diverse range of reproductive strategies. This diversity extends to an equally diverse range of mating proteins, many of which that function as regulators of the mating system. However, inconsistent application of the nomenclature guidelines for fungal mating genes represents a major hurdle when studying the reproductive system of fungi, especially non-model species. This is because there is no centralized database of curated mating-type genes, which in turn makes comparative analyses of genes across genera challenging. Here we introduce “MATch Maker” (http://matchmaker@fabi.up.ac.za), which was developed as a user-centric, web-based portal that contains an up-to-date database of curated sequences for fungal mating-type genes. This portal allows for BLAST comparison of user query sequences against the curated MATch Maker dataset. The latter currently contains of 142 representative mating-type protein sequences, which includes 51 MAT1-1 and 91 MAT1-2 sequences from across the Pezizomycotina. The MATch Maker output is metadata-rich with descriptions of the available information for mating-type proteins, their known taxonomic distribution, protein functional domains, and references to verified and relevant literature. Also, the information generated by MATch Maker can be downloaded in a variety of formats to assist in accurate functional and structural annotation of *MAT1* genes and their inferred protein products. Users can further submit new sequences to the platform, and following curation, their sequences would become part of the MATch Maker dataset. In other words, our web-based platform presents an easy-to-use tool for supporting real-time updates on developments made in studying fungal mating-type genes. Its implementation will go a long way towards ensuring standardized application of the nomenclature guidelines for mating-type genes, and ultimately serves to support an improved understanding of fungal reproductive biology in both model and non-model fungal species.

372T Identifying novel sexual reproduction defects by TN-seq in *Schizosaccharomyces pombe* *Caroline Craig*[1], Blake Billmyre[1], Michael Eickbush[1], Jeffrey Lange[1], Christopher Wood[1], Rachel Helston[1], Sarah Zanders[1,2] 1) Stowers Institute for Medical Research, Kansas City, MO; 2) University of Kansas Medical Center, Kansas City, KS.

Traditional genetic analyses examine single isolated mutants. However, pooled analysis can reveal types of phenotypes not observed in single mutants. We have developed a genome-wide transposon insertion (TN-seq) screen to identify genes important for sexual reproduction. Using this approach, we found over 500 genes involved in sexual reproduction. Some of the mutants, such as those in *ifs1* (Important for Sex), are unable to produce viable spores. Two other mutants, *plb1*Δ and *alg9*Δ have a previously undescribed

phenotype, where mutant cells are capable of undergoing meiosis and sex but the spores produced are sick and delay germination. To analyze this phenotype, we used a competitive growth assay and found that *plb1*Δ and *alg9*Δ spores but not vegetative cells were outcompeted by wildtype. Furthermore, we live imaged mutant spores using a microfluidic device and used deep learning to analyze the size, rate of growth, and aspect ratio of the spores from these timelapse videos. These data revealed that *plb1*Δ and *alg9*Δ spores are smaller and have a delay in germination. Pooled analyses using TN-seq are an invaluable tool for studying sex and can reveal phenotypes that are difficult to detect using traditional approaches.

373F Epistatic genetic interactions govern morphogenesis during sexual reproduction and infection in a global human fungal pathogen *Sheng Sun*[1], Cullen Roth[2], Anna F. Averette[1], Paul Magwene[2], Joseph Heitman[1] 1) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC; 2) Department of Biology, Duke University, Durham, NC.

Cellular development is orchestrated by evolutionarily conserved signaling pathways, which are often pleiotropic and involve intra- and inter-pathway epistatic interactions that form intricate, complex regulatory networks. *Cryptococcus*species are a group of closely-related human fungal pathogens that grow as yeasts yet transition to hyphae during sexual reproduction. Additionally, during infection they can form large, polyploid titan cells that evade immunity and develop drug resistance. Multiple known signaling pathways regulate cellular development, yet how these are coordinated and interact with genetic variation is less well understood. Here, we conducted quantitative trait locus (QTL) analyses of a mapping population generated by sexual reproduction of two parents, only one of which is unisexually fertile. We observed transgressive segregation of the unisexual phenotype among progeny, as well as a novel large-cell phenotype under mating-inducing conditions. These large-cell progeny were found to produce titan cells both *in vitro* and in infected animals. Two major QTLs and corresponding quantitative trait genes (QTGs) were identified: *RIC8* (encoding a guanine-exchange factor) and *CNC06490* (encoding a putative Rho-GTPase activator), both involved in G-protein signaling. The two QTGs interact epistatically with each other and with the mating-type locus in phenotypic determination. These findings provide insights into the complex genetics of morphogenesis during unisexual reproduction and pathogenic titan cell formation and illustrate how QTL analysis can be applied to identify epistasis between genes. This study shows that phenotypic outcomes are influenced by the genetic background upon which mutations arise, implicating dynamic, complex genotype-to-phenotype landscapes in fungal pathogens and beyond.

374W Widespread tissue-specific gene expression during fruiting body development of Coprinopsis cinerea revealed by laser-capture microscopy coupled single-cell RNA sequencing *Torda Varga*[1], Balázs Bálint[1], Viktória Bense[1], László G. Nagy[1] 1) Biological Research Center, Eötvös Loránd Research Network (ELKH).

The fate of cells in a developing organism has always been a central question for biologists. While elaborate cell fate maps exist in animal and plant model systems, we have little knowledge about how the main tissue types are formed during multicellular development in fungi. Here we explore tissue differentiation of Coprinopsis cinerea using laser-capture microdissection (LCM) coupled with single-cell RNA sequencing (scRNA-Seq) and compare temporal and spatial mRNA expression changes in different cell populations to understand fine-scale patterns of early fruiting body development. We first adapted LCM and scRNA-seq to basidiomycetes, then performed ultra-low input RNA extraction in four biological replicates of six developmental stages and 26 tissue types.
We focused on two main events during early development to reveal major spatial and temporal gene expression patterns of the cell populations. The first is the transition from vegetative mycelium to primary and secondary hyphal knot stages, where growth mode switches from simple to complex multicellularity and a cell population becomes dedicated to fruiting body development. Second, we focused on the primordium stages, where major mushroom tissues are outlined: homogenous hyphal mass diversify into main tissue types such as cap, universal veil, gill, or stem.
Gene expression showed a high level of tissue-specificity within the mushroom primordium, implying significant spatial variation in the transcriptome. For example, defense-related and cell surface-protein encoding genes showed increased expression in tissues close to the environment (e.g., universal veil or cap) relative to inner fruiting body structures (e.g., nodulus, stipe). During the transition from vegetative mycelium to hyphal knot stages, a substantial temporal switch was exhibited. More specifically, cell division (cyclins, septins) and cell wall remodeling genes (e.g., glycoside hydrolases) were enriched and could have a pivotal role in the very beginning of the fruiting body development.
We showed that cell populations during the early development of the fruiting body follow a strict genetic program and gene expression exhibits a high tissue-specificity even at the early phases of cell differentiation. Our work could further extend our knowledge on the genetics of fruiting development and could generate new hypotheses for gene functions.

375T Role of a septin duplication in fruiting body development of a complex multicellular model fungus *Máté Virágh*[1], László G. Nagy[1] 1) Biological Research Center, Szeged, Hungary.

Septins are GTP-binding cytoskeletal proteins polymerizing into hetero-oligomeric complexes that can form higher-order structures. These proteins are conserved in most eukaryotes and play important roles in diverse biological processes such as cytokinesis and cell differentiation. In *S. cerevisiae*, core septins *cdc3*, *cdc10*, *cdc11,* and *cdc12* form hetero-octamers with *cdc11* occupying the terminal positions of the octamer. Orthologs of *cdc11* (along with other core septins) in filamentous fungi were reported to be important players of hyphal morphogenesis.
During their sexual cycle, fungi can form complex multicellular structures, called fruiting bodies. The most complex fruiting bodies can be found within Agaricomycetes. In a complex multicellular model fungus, *Coprinopsis cinerea,* the ortholog of *cdc3* was reported to play a role in stipe elongation, but nothing is known about the function of other septins during fruiting body morphogenesis.
Here we have found that all the core septins are present in Agaricomycetes and that *cdc11* went through a duplication in the order Agaricales, likely after the divergence of Pleurotaceae. Phylogenetic analysis also revealed selective loss of the newly gained paralog in a selected Agaricales species. We have found that both copies of the *cdc11* homologs are developmentally regulated during fruiting body morphogenesis in several fruiting body forming fungi, and in the case of *C. cinerea,* the two copies are expressed reciprocally at the beginning of the development. Multiple sequence alignment of Cdc11 proteins of 24 Agaricomycetes species showed a high sequence similarity between the Cdc11 paralogs. However, the broadly conserved Cdc11 (called Cdc11a) harbors a conserved short C-terminal motif, while the same C-terminal part of the new Agaricales-specific Cdc11 paralog (called Cdc11b) is highly variable.

This led us to the hypothesis that the altered C-terminal part of the newly gained Cdc11b plays a role in an Agaricales specific morphogenetic change during fruiting body development. As a first step to test this hypothesis we have generated deletion mutants of *cdc11a* and *cdc11b* in *C. cinerea* using a ribonucleoprotein-based CRISPR/Cas9 gene deletion strategy. We are phenotyping the deletion strains with special emphasis on characterizing the morphogenetic significance of this Agaricales-specific septin duplication.

376F Funtional characterization of regulatory genes in *Schizophyllum commune* identifies nine new genes related to mushroom development *Peter Jan Vonk*[1], Natalia Escobar[1], Julie Grosse-Sommer[1], Yuanyuan Wang[1,2], Éva Almási[1], Robin A. Ohm[1] 1) Microbiology, Utrecht University, Utrecht, The Netherlands; 2) College of Food Science and Technology, Huazhong Agricultural University, Wuhan, China.

The mushrooms in the order Agaricales are among the most complex developmental structures in fungi. These sexual structures contain a wide variety of morphologies and contain the basidia where sexual spores are formed for sexual propagation. Recent -omics approaches have identified a large number of potential regulators of mushroom development, but their function remained unclear. The deletion of multiple transcription factors and kinases that are developmentally regulated in *Schizophyllum commune* and/or conserved in mushroom-forming fungi, reveals a role in mushroom-development for many previously uncharacterized genes. Four transcription factors were identified to play a role in mushroom development, expanding the regulatory network of multiple stages of mushroom development. Furthermore, five kinases were identified that have an important role in spore formation. Deletion of these kinases resulted in a strong reduction of spore production, but did not affect morphology and viability of spores. The observed reduction of spore formation can be valuable in the industrial production of mushrooms due to their potential to cause respiratory diseases. Together these results further our understanding of the complex regulatory program of mushroom development.

377W Characterisation of sexual reproduction mechanisms of *Pyricularia oryzae* to determine genetic bases of male and female fertility. *Alexandre Lassagne*[1,2,3], Sylvain Brun[4], Henri Adreit[1], Fabienne Malagnac[5], Didier Tharreau[1], Elisabeth Fournier[2,3] 1) PHIM, Cirad, Montpellier; 2) PHIM, Inrae, Montpellier; 3) PHIM, Muse, Montpellier; 4) LIED, Université de Paris, Paris; 5) I2BC, Université Paris-Saclay, Orsay.

The reproductive system of an organism conditions the emergence and evolution of adaptive variants in response to selective constraints. Comprehension of the mode of reproduction in pathogens helps to understand their life history. Mechanisms and genes involved in sexual reproduction in Ascomycetes, are relatively well described in several model organisms such as *Neurospora crassa* or *Podospora anserina* (Brun *et al*., 2021). However, in *Pyricularia oryzae*, the fungal pathogen responsible for blast diseases on several species of Poaceae, the biology of sexual reproduction remains poorly documented and phenotyping of fertility is based on the result of sexual reproduction, ie the formation of perithecia (Saleh *et al*., 2012). Previous studies have reported microconidia (Chuma *et al*., 2009) and have hypothesized that they could be the male gametes. However, their role in sexual reproduction was not demonstrated.
By spraying microconidia, macroconidia or mycelial fragments on growing mycelium, we demonstrate, that only microconidia can fertilize female fertile strains of opposite mating type. Thus, we demonstrate that microconida are the male gametes in *P. oryzae*. Live-cell imaging experiments are in progress to observe the fertilization process.
The identification of microconidia as male gametes in *P. oryzae* allows a precise phenotyping of male fertility by counting the microconidia production. Following this new method, we measured the male fertility of strains in a recombinant field population. We also sequenced the genome of the same strains. A Genome Wild Association Study (GWAS) based on these phenotypic and genotypic datasets is in progress to identify genes involved in male fertility.

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378T The *ndrC* gene, which is regulated by *nsdD*, controls sexual development in *Aspergillus nidulans* *Yu Kyung Kim*[1], Kap-Hoon Han[1] 1) Woosuk University.

Aspergillus nidulans is a filamentous model fungus that has both of asexual and sexual life cycles, which depend on environmental factors such as nutritional conditions and stresses. The *nsdD* gene is a well-known GATA type transcription factor responsible for the regulation of sexual and asexual development. In this study, we identified a gene, named *ndrC* (*nsdD*-dependent regulation) by using RNA-seq experiment followed by DEG analysis. The NdrC protein encoded by the *ndrC* gene is conserved in some Aspergillus species but not in other organisms. It has no known domain except DUF4267 and reported as hypothetical protein. To characterize the function of the gene, deletion mutants were generated, and the phenotypes under the various differentiation induction conditions were observed. The colony size of the mutant was similar to the host strains and the control strains, but more conidia were produced compared to the control strains, suggesting that the gene is negatively regulate asexual development or conidia production. Microscopic observations showed that there was no cleistothecium or hüll cell formed after incubation of sexual induction condition. In wilt-type strain the *ndrC* gene expression was not detected in cleistothecia whereas the gene was expressed in conidia. Taken together, the *ndrC* gene is responsible for the sexual development and repression of asexual development in *A. nidulans*.

379F Dark stipe mutants in fruiting body development of *Coprinopsis cinerea* *Shanta Subba*[1], Botond Hegedüs[2], Laszlo G. Nagy[2], Chee Seng Man[1], Ursula Kües[1] 1) Molecular Wood Biotechnology and Technical Myycology, University of Goettingen, Goettingen, Germany; 2) Institute of Biochemistry, Biological Research Center, HAS, Szeged, Hungary.

Fruiting body formation in *Coprinopsis cinerea* is a complex morphological process employing successively > 30 different cell types in cap and stipe development. It follows a conserved developmental pathway defined by day and night phases, with well predictable distinct stages over the time. The differentiation process starts with loose aggregate formation primary hyphal knots (Pks) in the mycelium in the dark. Upon a light signal, these primary hyphal knots (Pks) turn into compact secondary hyphal knots (Sks) in which stipe and cap tissues start to differentiate. Primordial tissue development takes 4 days (stepwise recognized as primordia stages P1 to P4) until all basic tissue formation and differentiation is completed, with probasidia in the hymenia on the gills of the P4 primordia ready to receive a light signal to induce karyogamy. Nuclear fusion is finished on day 6 of development P5 stage primordia and culminates in meiosis and basidiospore production. Basidiospore production parallels stipe elongation and cap expansion for fruiting body maturation. Mature fruiting bodies autolyze on day 7 of the fruiting pathway to release the spores in liquid droplets. Development is strictly regulated by environmental conditions including nutrients, alternating light and dark phases, temperature and aeration (CO_2). Failure in daily illumination at the Sk to the P4 stages leads to the formation of so-called 'dark stipes', under proliferation of stipe tissues and blocks in cap development. Failure in dark signaling at night phases at the Sk to the P4 stages leads to the formation of 'dwarf primordia' with short stipes and bulky caps with retarded gill differentiation. 'Dark stipe' phenotypes are further formed from P3 and P4 stages under non-aerated conditions with normal light-dark changes. Scavenger experiments of CO_2 with KOH recovered the normal phenotypes in fruiting body development. 'Dark stipe' mutants with different genetic defects are available. *dst1* and *dst2* mutants form 'dark-stipes' from secondary hyphal knots and are defective in light regulation by mutations in the WC1 light receptor and in a FAD/FMN-binding enzyme of the GlcD superfamily. *dst3* and *dst4* mutants turn into dark-stipe phenotypes at stages P3 and P4, respectively, and appear to be defective in CO_2 signaling. The *dst3* and *dst4* mutants are defective in regulation at these later stages of primordial development while they are not blind with respect of light-induced oidiation. Genome sequencing identified defective candidate genes involved in CO_2 metabolism and the Cop9 signalosome. Functions in metabolic pathways producing CO_2 and CO_2 signaling pathways will be discussed.

380W DFG-5 plays a key role in the extracellular targeting of *Neurospora crassa* glycoproteins for incorporation into the cell wall and secretion into the growth medium Pavan Patel[1], *Stephen Free*[1] 1) University at Buffalo.

The GH76 family of alpha-1,6-mannanases are important cell wall biosynthetic enzymes. The *N. crassa dfg-5* gene encodes an alpha-1,6-mannanase needed for crosslinking glycoproteins into the cell wall. We investigated the role of DFG-5 in processing the N-linked galactomannans on cell wall glycoproteins and targeting the glycoproteins for cell wall incorporation. Site-directed mutagenesis of DFG-5 identifies four amino acids required for DFG-5 activity. Aspartate residues at positions 116 and 117 represent the active site. An aspartate at position 76 and a glutamate at position 130 are also required for DFG-5 to function in cell wall biogenesis. Co-purification experiments with HIS6-tagged DFG-5 demonstrate that DFG-5 forms stable interactions with a large number of cell wall glycoproteins. The presence of an N-linked galactomannan is required for these interactions. Using Western blot assays, we show that DFG-5 specifically associates with cell wall glycoproteins, and it does not form associations with secreted glycoproteins. DFG-5 binds to the N-linked galactomannans on cell wall glycoproteins and processes the galactomannans. The processed galactomannans are recognized by lichenin transferases, which attach lichenin to the processed galactomannan and thereby incorporate the glycoproteins into the cell wall. An important aspect of our report is that DFG-5 plays a central role in the extracellular trafficking of cell wall and secreted glycoproteins. By processing the galactomannans on cell wall glycoproteins, DFG-5 targets them for cell wall incorporation. Secreted glycoproteins are not recognized by DFG-5 and retain a full-length galactomannan, which is not a substrate for lichenin transferase-mediated incorporation into the cell wall. Secreted glycoproteins are therefore released into the growth medium. The ability of DFG-5 to discriminate between cell wall and secreted glycoproteins is the key event in targeting the two different groups of extracellular glycoproteins to their final destinations.

381T Defects on endocytosis cause alterations in actin and produce aberrant hyphal morphology of *Neurospora crassa* *Marisela Garduño-Rosales*[1], Diego L. Delgado-Alvarez[1], Rosa R. Mouriño-Pérez[1] 1) CICESE.

Endocytosis is highly concentrated in the subapex of filamentous fungi, which supports the removal of excess membrane generated by exocytosis and recycling of membrane-embedded proteins. The collar is composed of actin and many other proteins. We report the effect of the deletion of actin-binding proteins associated with endocytosis. We performed live-cell confocal microscopy, total internal reflection fluorescence microscopy and nanoscopy of the F-actin reporter LIFEACT tagged with the green fluorescent protein in the mutant backgrounds Δ*sla1* and Δ*fim1*. Both strains showed strongly affected phenotypes, including thinner hyphae, polarity loss and Spitzenkörper disorganization events. FM4-64 internalization was delayed in both strains. The localization and dynamics of the endocytic collar were different in the mutants compared with the wild-type (WT) strain. The Δ*sla1* mutant did not have an endocytic collar, only a few patches were visible throughout the hypha. However, actin cables were more abundant than in the WT strain. In the Δ*fim1* mutant, the collar was larger than in the WT strain and in many cases, actin patches were continuously present toward distal regions of the hypha. Actin cables were more abundant in the Δ*fim1* mutant compared with the WT strain as well. Nanoscopic analysis resolved individual cortical patches of the endocytic collar in all strains. We conclude that the actin-binding proteins SLA-1 and Fimbrin are necessary for the proper polymerization and cross-linking of actin filaments. The lack of these proteins results in defective endocytic collars and affects the integrity of the Spitzenkörper, which produces aberrant hyphal morphology.

382F Functional characterization of DENN domain proteins in *Aspergillus nidulans* *Steven Harris*[1,2], Samantha Reese[2,3] 1) Iowa State University; 2) University of Nebraska; 3) Meati Inc..

In *Aspergillus nidulans*, the *mesA* mutant was originally identified through a synthetic lethal interaction with mutations affecting the formin SepA. Functional analyses showed that MesA controls actin organization at hyphal tips. Subsequent genome annotation efforts revealed that MesA shares features with a larger family of proteins that include Avl9 and the DENN domain proteins. These proteins share conserved regions known as AH or DENN domains, and have been implicated in protein trafficking as regulators of Rab/Arf GTPases. The *A. nidulans* genome encodes three other proteins that possess canonical DENN domains; the Avl9 homologue AN1018, as well as the classical DENN homologues AN4349 and AN0575. Notably, the latter two are not conserved in the yeast *S. cerevisiae*. To better understand the function of the DENN domain proteins in filamentous fungi and their potential role in morphogenesis, we

characterized deletion mutants of *an1018*, *an4349*, and *an0575*, and also localized their cognate proteins. The loss of any single or double mutant combination of the three genes caused no obvious defect in viability or growth other than a modest reduction in colony diameter of mutants missing *an4349*. More significantly, *an4349* mutants displayed significant defects in conidiation and conidiophore morphology. Given their predicted function in protein trafficking, analysis of protein secretion was performed on each mutant. Results from these experiments suggest that the absence of AN4349 and AN0575 in particular leads to hyper-secretion of cellulases and amylases. The use GFP fusions to localize AN1018, AN4349, and AN0575 further emphasized their possible role in secretion. AN1018 and AN4349 both localize to the hyphal tip, with AN4349 also exhibiting potential localization to the Spitzenkorper and internal membrane structures. AN0575 displayed prominent localization to septa and the plasma membrane, with no obvious bias toward the hyphal tip. However, in the absence of AN1018, AN0575 markedly re-localizes to hyphal tips. These observations collectively suggest that the DENN domain proteins function in a spatially distinct manner to modulate protein trafficking in *A. nidulans*. In this context, because AN1018, AN4349, and AN0575 have been identified as substrates of the protein kinases PKA and MpkA, they might serve as key regulators of secretion in response to changing growth conditions and/or cell wall stress.

383W Trade-off between Plasticity and Velocity in Mycelial Growth *Norio Takeshita*[1] 1) University of Tsukuba.

Tip-growing fungal cells maintain cell polarity at the apical regions and elongate by *de novo*synthesis of the cell wall. Cell polarity and tip growth rate affect mycelial morphology. However, it remains unclear how both features act cooperatively to determine cell shape. Here, we investigated this relationship by analyzing hyphal tip growth of filamentous fungi growing inside extremely narrow 1 µm-width channels of microfluidic devices. Since the channels are much narrower than the diameter of hyphae, any hypha growing through the channel must adapt its morphology. Live-cell imaging analyses revealed that hyphae of some species continued growing through the channels, whereas hyphae of other species often ceased growing when passing through the channels, or had lost apical polarity after emerging from the other end of the channel. Fluorescence live-cell imaging analyses of the Spitzenkörper, a collection of secretory vesicles and polarity-related proteins at the hyphal tip, in *Neurospora crassa* indicates that hyphal tip growth requires a very delicate balance of ordered exocytosis to maintain polarity in spatially confined environments. We analyzed the mycelial growth of seven fungal species from different lineages, including phytopathogenic fungi. This comparative approach revealed that the growth defects induced by the channels were not correlated with their taxonomic classification or with the width of hyphae, but, rather, correlated with the hyphal elongation rate. This report indicates a trade-off between morphological plasticity and velocity in mycelial growth and serves to help understand fungal invasive growth into substrates or plant/animal cells, with direct impact on fungal biotechnology, ecology, and pathogenicity.

384T Evidence that the Vesicle Supply Center function of the Spitzenkörper resides in the Actin Cytoskeleton *Salomon Bartnicki-Garcia*[1], Diego Delgado-Alvarez[2] 1) CICESE Center for Scientific Research and Higher Education, Ensenada, B.C. Mexico; 2) National Laboratory of Advanced Microscopy at CICESE, Ensenada, B.C. Mexico.

The vesicle supply center (VSC) is a mathematically derived solution that explains how the Spitzenkörper (SPK) can create and shape the growing tip of a hypha. The VSC assumes that wall-building exocytic vesicles emanate in all directions from a linearly moving center. The net effect is the generation of a sharp gradient of cell wall growth from the hyphal apex to the subapex. It is well known that actin is a component of the SPK and it is widely accepted that exocytic vesicles move on actin filaments. A strain of *Neurospora crassa* expressing Lifeact-GFP was examined with the improved image resolution of a Laser Scanning Confocal Microscope Olympus FV1000 to reveal its actin cytoskeleton. We visualized numerous strands of actin emanating radially from the SPK of growing hyphae. The radial filaments were more prominent in the subapical region. We found a good similarity between the observed radial arrangement of these actin cables/filaments and the radial distribution of the vesicle paths predicted mathematically by the hyphoid equation y = x cot (xV/N). The actin filaments projecting backwardly from the SPK support the delivery of exocytic vesicles to the subapex and thus generate the typical shape of a fungal hypha. We propose that the SPK constitutes an efficient exocytic machine made of actin filaments radiating from the SPK center to the cell periphery. The continuous forward motion of the SPK while sending vesicles radially creates a gradient of exocytosis that constructs a cell wall with the characteristic hyphoid shape. The known presence of actin polymerizing formins (e.g. BNI-1 or SEPA) in the center of the SPK region supports the concept that a dynamic actin cytoskeleton drives the vesicle supply center.

385F Nanoscale imaging of dynamic cell wall formation in fission yeast Pascal Odermatt[1, 2, 3], Amilcar Perez[1,4], Kerwyn Huang[2], *Fred Chang*[1] 1) UCSF, San Francisco, CA, USA; 2) Stanford U., Stanford, CA, USA; 3) EPFL, Lausanne, Switzerland; 4) Johns Hopkins, Baltimore, MD, USA.

The cell wall is a critical structural element responsible for cell shape and mechanical properties. Many walled cells-- including fungi, bacteria, and plants– derive their shape by tip growth, in which new cell wall is assembled at the cell tip. Similarities in the shapes of tip growing cells across kingdoms and over several magnitudes in size suggest that these organisms utilize common physical mechanisms. Here, we used atomic force microscopy to visualize for the first time the dynamic behaviors of individual glucan fibers of the cell wall during tip growth in the fission yeast *Schizosaccharomyces pombe*. We embedded cells in a porous matrix to stand the cell on end, which allowed us to probe the growing tip through an entire cell cycle (hours) at minute time resolution with < 10-nm spatial resolution. Analyses reveal a mesh-like structure composed of filaments (likely to be glucan bundles) that reorganize continuously at the growing cell tip. Our findings provide evidence for insertion at discrete sites, followed by directed movement, with apparent stretching and remodeling of these fibers as they progress outwards towards the lateral walls. We further used single molecule imaging to track the behavior of the major cell wall synthase Bgs4. Single molecules of halo-tagged Bgs4 exhibited processive movements at sites of cell wall synthesis. These movements were not dependent on actin but were inhibited by caspofungin, an inhibitor of Bgs4 activity, suggesting that movement of the synthase is powered by glucan assembly. Together, these findings represent promising new avenues to probe dynamic mechanisms of cell wall assembly at the nanoscale.

386W *Aspergillus niger* conidial germination: 3D live cell exploration *Susanne Fritsche*[1,2], Matthias Steiger[1,2] 1) Austrian Centre of Industrial Biotechnology (ACIB GmbH), Vienna, Austria; 2) Technical University of Vienna, Vienna, Austria.

Conidial germination describes the transition of dormant spores to hyphal structures. Breaking dormancy is followed by isotropic and polarized growth with a cell wall constantly being remodeled.
To explore and understand the rearrangement and timing of key events in *A. niger*, we use the Nanolive stain-free live cell imaging system - a combination of holography and tomography. A temperature-controlled growth chamber is used for incubating the fungus on-stage of the microscope. With the 3D Nanoscopy technique, videos of the germination process can be recorded and morphological structures distinguished based on different refractive indices (RI). Images of germinating conidia and digital cell reconstruction in 3D based upon the cell's inherent physical properties revealed a ring formation that might direct germ tube formation.
This knowledge is critical to the development of future approaches to manipulate fungal growth for medical, agricultural or industrial purposes.

387T Investigating molecular mechanisms that underlie thermal dimorphism and pathogenesis of *Histoplasma capsulatum* *Sarah Heater*[1], Anita Sil[1] 1) University of California, San Francisco.

Histoplasma capsulatum (*Hc*) causes approximately 500,000 infections per year in the US and is a significant cause of morbidity and mortality. Similar to other thermally dimorphic fungi, *Hc* grows as hyphae in soil, producing infectious spores that readily aerosolize. Once inhaled by mammals, spores respond to body temperature and germinate to grow as pathogenic yeast. In vitro, this transition can be induced by temperature alone: ambient temperature induces hyphal growth while body temperature produces virulent yeast. However, the mechanism(s) by which *Hc* and other thermally dimorphic fungi sense temperature is unknown. We have thus far identified several candidate thermosensors, including the signaling mucin Msb2, based on their necessity for temperature-responsive change in morphology and transcription, as well as on sequence analysis, and the function of known orthologues. Msb2 is the first known *Hc* factor in the pathway inducing hyphal growth in response to ambient temperature and orthologues of Msb2 in other fungi are involved in sensing various stimuli. Here I will present my explorations of temperature-dependent alterations of Msb2, including identification of temperature dependent cleavage. To further our understanding of the mechanism which enables temperature response, I am performing a genetic screen to identify additional genes required for thermal dimorphism, and have isolated a mutant in the gene *MDH2* which fails to grow as hyphae at ambient temperature. This work contributes to our understanding of the mechanisms that underlie temperature-sensing and regulate *Hc* thermal dimorphism, a crucial feature of its pathogenesis.

388F Study of the physiological role of amyloid structures in the pathogenic yeast *Candida albicans*. *Thierry Mourer*[1], Sophie Bachellier-Bassi[1], Christophe d'Enfert[1] 1) Institut Pasteur, Université de Paris, INRA USC2019, Fungal Biology and Pathogenicity Unit, F-75015 Paris, France..

The human commensal fungus *Candida albicans* can, under some conditions, cross the digestive mucosa, disseminate into the bloodstream, and cause invasive candidiasis. *C. albicans* can also form structured communities, namely biofilms, attached on epithelia or indwelling medical devices. This yeast is a huge burden for healthcare systems worsened by the fact that *C. albicans* biofilms are strongly resistant to classical antifungal drugs and can also evade the immune system. Because of these issues, curing patients with invasive candidiasis remains challenging. A better understanding of biofilm formation at the molecular level could lead to new therapeutic strategies. Glycosylphosphatidylinositol anchored proteins (GAPs) are heavily glycosylated proteins associated to the cell wall. Over the past decade, many GAPs of *C. albicans* have been shown to contribute to biofilm establishment, although their precise molecular functions need to be elucidated. Overexpression of some genes encoding GAPs have been shown to affect biofilm formation. Among them, overexpression of *PGA59* increases adhesion forces to the substrate and between cells, and results in an increase of biofilm dry weight. Recently, our laboratory has uncovered that Pga59 displays amyloid properties. We have produced recombinant His-tagged Pga59 using *Escherichia coli* as a host, and using electron microscopy and Thioflavin T staining (a specific dye of amyloid fibers), we demonstrated that *in vitro* recombinant Pga59 was able to adopt an architecture reminiscent of beta amyloid fibers. We then investigated if Pga59 is a part of cell surface amyloid fibers in *C. albicans* and whether parietal amyloids impact cellular adhesion and biofilm formation. *In vivo* Thioflavin T staining allowed us to visualize amyloid structures in the cell wall of *C. albicans*. Interestingly, we showed that a mutant impairing Pga59 amyloid assembly impacts biofilm formation in a continuous-flow microfermentor system. Moreover, our results strongly suggest that amyloid properties of Pga59 are required to mediated cell-cell interactions. Altogether, information gathered on amyloid fibers formation in response to adhesion and on their functions could lead to the discovery of an unsuspected mechanism to regulate biofilm formation in *C. albicans*.

389W Putative oxidoreductase Cip1 is critical for pheromone-independent unisexual development in *Cryptococcus neoformans* *Nathan Glueck*[1], Xiaorong Lin[1] 1) University of Georgia.

Cryptococcus neoformans is a ubiquitous environmental basidiomycete capable of establishing a replicative niche within a human host. For immunocompromised individuals, pulmonary cryptococcal infections can disseminate to other organs, including the brain, causing cryptococcal meningoencephalitis (CME). Similar to other fungal pathogens, Cryptococcus is dimorphic, meaning it can grow in both yeast and hyphal forms. In the human host, Cryptococcus grows primarily as yeast with the hyphal morphology being immunogenic and attenuated in virulence. Sexual reproduction is requisite for hyphal morphogenesis. While sexual reproduction is primarily dependent on pheromone signaling, our lab has previously identified copper-induced, pheromone-independent unisexual reproduction. Here we found that the *CIP1* gene is drastically upregulated in this process. We show that the putative oxidoreductase Cip1 is required for robust hyphal morphogenesis. *CIP1* gene knockouts reduce expression of transcription factors *MAT2* and *ZNF2*, associated with sexual reproduction and hyphal morphogenesis, respectively. Noting that cadmium has been shown to increase *CIP1* expression, we supplemented media with cadmium to discover that (i) cadmium also induces pheromone-independent hyphal morphogenesis, and (ii) deletion of *CIP1* hinders cadmium-induced, pheromone-independent filamentation. Importantly, the *cip1*Δ mutant shows increased sensitivity specifically to copper and cadmium, two metals shown to induce pheromone-independent unisexual reproduction, indicating that Cip1's role in this pheromone-independent filamentation may have the effect of mitigating stress caused by these metals. We also profile the transcriptome of the *cip1*Δ mutant in filamentation inducing conditions to provide a more holistic view of the role this oxidoreductase plays in the regulatory networks governing the yeast-to-hyphae transition. We are also examining whether the function of Cip1 in mitigating metal stress contributes to cryptococcal virulence. This work provides a foundation for the further study of Cip1's function

relating to hyphal morphogenesis and fungal pathogenesis.

390T Temperature adaptation of biological phase separation *Amy Gladfelter*[1], Benjamin Stormo[1], Ian Seim[1], Ammon Posey[3], Fred Dietrich[2], Rohit Pappu[3] 1) UNC Chapel Hill; 2) Duke University; 3) Washington University at St. Louis.

Free-living microbes, plants and cold-blooded organisms survive in the face of temperature fluctuations that arise across many time scales. Climate change is increasing the amplitude and frequency of temperature variations in the natural world and biological phase separation may be a key mechanism of adaptation of the biosphere to climate change. The focus of this presentation will be on our recent work to identify how protein and RNA sequence encodes temperature sensitivity and how material properties of biomolecular condensates are maintained across temperatures fluctuations. For these studies, we focus on a model phase separation protein, Whi3, in the syncytial ascomycete fungus, *Ashbya gossypii.* This protein binds to and regulates specific RNAs important for cell cycle control and cell polarity. We have found natural sequence variation within the core protein/RNA components are sufficient to induce highly tunable temperature sensitivity for condensation. Sequence elements controlling protein-protein, protein-RNA and RNA-RNA interactions all contribute to modulating higher-order assembly and function in different temperature regimes. These studies indicate that small changes in protein and RNA sequences can promote organism adaptation to different climates providing potential mechanisms for adaptation of the biosphere to climate change.

391F Investigating the role of Hsp90 in the regulation of *Histoplasma capsulatum* morphology and transcriptional response *Jillian Freese*[1], Anita Sil[3], Sinem Beyhan[1,2] 1) J. Craig Venter Institute, La Jolla, CA; 2) University of California, San Diego, La Jolla, CA; 3) University of California, San Francisco, San Francisco, CA.

Thermally dimorphic fungal pathogens, *Histoplasma, Blastomyces, Paracoccidioides, Coccidioides, Sporothrix,* and *Talaromyces,* alter their vegetative morphology primarily in response to changes in temperature. In these fungal pathogens, human body temperature serves as the signal for conversion from an infectious to parasitic form. *Histoplasma*, a primary pathogen of humans, exists in a multicellular hyphal form in the soil and as a parasitic yeast form within host cells. Its transition from environmental to host-associated forms is critical for the expression of virulence factors and establishment of the disease histoplasmosis. Our long-term goal is to understand the mechanisms for temperature-sensing and its effect on the morphology and virulence traits of *Histoplasma*. The heat shock response plays a significant role in the adaptation and continued functioning of *Histoplasma* during the prolonged heat shock conditions experienced within its host. Making up 1-2% of all cellular protein, Hsp90 is central in the heat shock response. Hsp90 interacts mainly with transcription factors, kinases, and signaling receptors, making it a key player in signal transduction and transcriptional regulation. Hsp90 has been shown to regulate filamentation, development, and virulence in *Aspergillus* and *Candida* species. In *Histoplasma*, Hsp90 levels have been shown to be critical for virulence. In this study, we investigated the role of Hsp90 in regulating morphology and transcription in *Histoplasma*. Inhibition of Hsp90 with chemical inhibitors, including geldanamycin (GdA), Hsp990, and 17-AAG, resulted in filamentous growth at 37˚C in *Histoplasma*, while DMSO (carrier) had no effect on morphology. In GdA-treated cells, known hyphal-associated genes and kinases were upregulated along with heat shock response genes like Hsf1, Gac1, and Ssb1. Downregulation in GdA-treated cells aligned with yeast-associated cellular metabolism and membrane transporters. Continued analysis and characterization of hypothetical and unknown proteins that are differentially expressed in response to Hsp90 inhibition will identify additional genes involved in the transition between *Histoplasma* yeast and hyphae. Results of these analyses are critical as they may serve as a target for therapeutics that can block the transition of *Histoplasma* from an environmental form to a host-associated form.

392W Multi-omics Profiling Reveals New Pathways Regulating Hyphal Morphogenesis in *Candida albicans* *Kyung-hun Min*[1], Thomas Jannace[1], Haoyu Si[1], Krishna Veeramah[2], John Haley[3,4], James Konopka[1] 1) Department of Microbiology and Immunology, Renaissance School of Medicine, Stony Brook University (SUNY), Stony Brook, NY; 2) Department of Ecology and Evolution, Stony Brook University (SUNY), Stony Brook, NY; 3) Department of Pathology, Renaissance School of Medicine, Stony Brook University (SUNY), Stony Brook, NY; 4) Biological Mass Spectrometry Shared Resource, Renaissance School of Medicine, Stony Brook University (SUNY), Stony Brook, NY.

Fungi grow in a wide range of different morphologies that provide distinct advantages for survival and virulence. The human fungal pathogen *Candida albicans* switches between budding and filamentous hyphal morphologies in the host. Long hyphal filaments promote invasion into tissues, biofilm formation, and escape from macrophages. Adenylyl cyclase (Cyr1) has long been thought to be the master regulator of hyphal growth, through activation of cAMP signaling. However, it is difficult to define the roles of the cAMP pathway because *cyr1*Δ/Δ mutants grow very poorly and expresses abnormally low levels of genes needed for hyphal growth. Surprisingly, we discovered that faster growing *cyr1*Δ/Δ pseudorevertant (PR) mutants form hyphae in the absence of Cyr1 and cAMP. Genome analysis of multiple PR mutants revealed that their improved growth was due to loss of one copy of *BCY1*, the negative regulatory subunit of protein kinase A. Furthermore, hyphal morphogenesis was improved in some of PR mutants by multigenic haploinsufficiency resulting from loss of large regions of the left arm of chromosome 2, including global transcriptional regulators. Interestingly, the mutant cells were also able to induce hyphal associated genes in the absence of cAMP that are needed for virulence. This indicates that basal protein kinase A activity is required for hyphal induction, but further stimulation of PKA is not needed. Integrating information from different omics approaches identified cAMP-independent mechanisms that promote hyphal growth. Phosphoproteomic analysis indicated that the Cdc28 cyclin-dependent kinase and the casein kinase Yck2 play key roles in promoting polarized growth. In addition, integrating transcriptomic and proteomic data reveals that hyphal induction increased protein translation rate of the key transcription factors that are important for hyphal growth.

393T Investigating How the Septin Cytoskeleton Controls Morphogenesis in Marine Fungi *Ellysa Vogt*[1], Amy Gladfelter[1] 1) University of North Carolina at Chapel Hill, Chapel Hill, NC.

Complex cell morphologies are attributed to the dynamics and plasticity of the cytoskeleton. An understudied component of this framework is the septin cytoskeleton. Septins are filament-forming, GTP-binding proteins important for coordination of cell cycle progression, actin and microtubule organization, polarized cell growth, and membrane remodeling. Septins were discovered and have been most intensively studied in the budding yeast, *Saccharomyces cerevisiae*. In late G1 of budding yeast, septins form a cortical ring structure

near the bud site, and at the time of bud emergence, the ring expands into a rigid septin collar, spanning the whole bud neck and scaffolds other cytokinetic factors. Despite their conserved role in the eukaryotic cell cycle, many aspects of septin assembly and function in cell morphology remain mysterious. To study the biophysical properties of septins in the context of variable cell morphogenesis, we are using a set of species of black yeasts isolated from marine environments surrounding Woods Hole, MA. The marine environment presents a variety of stresses for fungi including high osmotic and oligotrophic conditions, UV exposure, temperature fluctuations, and limited substrates that necessitate particular morphological adaptations. To assess morphogenesis and division patterns at the single cell level, we filmed growth using high-magnification, differential interference contrast (DIC) time-lapse microscopy. Our single-cell analyses revealed remarkably distinct morphologies from the conventional model yeasts including multiple simultaneous budding events, division through a combination of budding and fission, and consecutive orthogonal septations. We hypothesize that some of these unique patterns of morphogenesis are linked to intrinsic features of septin polymerization and interactions of septins with downstream effectors. Bioinformatic comparisons of septin sequences show regions of divergence from the conventional yeast models *S. cerevisiae* and *S. pombe* that are conserved in the black yeast species. Many of these residue changes are located within key structural elements, such as the GTPase domain and C-terminal coiled-coil tail. This is interesting as septin oligomeric structures assemble via interactions between these domains; changes in these interactions could influence the biophysical properties of these proteins such as flexibility, annealing, fragmentation, and bundling, which could, in turn, alter their cellular function. Using comparative analysis of the genome, cell biology and biophysics of septins from these fungi, we can learn how the plasticity of the septin cytoskeleton can generate different cell morphologies.

394F Probing the unconvetional lifestyle of the multi-budding yeast, *Aureobasidium pullulans* *Claudia Petrucco*[1], Olivia Gorman[1], Daniel Lew[1] 1) Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC.

Aureobasidium pullulans is a ubiquitous black yeast with an unconventional lifestyle. Unlike *Saccharomyces cerevisiae*, *Candida albicans*, *Cryptococcus neoformans*, *Ustilago maydis*, and other well-studied yeasts, *A. pullulans* mother cells can be multinucleate and produce multiple buds within a single division cycle. Initial observations suggest that almost all buds inherit exactly one nucleus regardless of the number of nuclei or buds in the mother. These findings suggest that *A. pullulans* may exhibit novel cell biology with regard to polarity establishment, nuclear segregation, and possibly other phenomena. To investigate this interesting cell biology, we are developing a molecular genetics toolbox, including efficient transformation and fluorescent probes for intracellular structures of interest in order to transform *A. pullulans* into a tractable system for basic cell biology research. Initial findings from this effort suggest that *A. pullulans* undergoes a form of “semi-open” mitosis where the nuclear envelope remains intact but the nuclear pore complexes disassemble.

395W The Nma1 protein promotes long distance transport mediated by early endosomes in *Ustilago maydis* Karina Schneider[1], Theresa Farr[1,3], Niko Pinter[1,4], Kerstin Schmitt[2], Oliver Valerius[2], Gerhard Braus[2], *Jörg Kämper*[1] 1) Karlsruhe Institute of Technology, Institute of Applied Biosciences, Department of Genetics, Karlsruhe, Germany; 2) Department of Molecular Microbiology and Genetics and Göttingen Center for Molecular Biosciences, University of Göttingen, Germany; 3) State Academy and Research Institute for Viticulture and Fruit Cultivation, Weinsberg, Germany; 4) Institute for Surgical Pathology, Medical Center-University of Freiburg, Freiburg, Germany..

Early endosomes (EEs) are part of the endocytic transport pathway and resemble the earliest class of transport vesicles between the internalization of extracellular material, their cellular distribution or vacuolar degradation. In filamentous fungi, EEs fulfill important functions in long distance transport of cargoes as mRNAs, ribosomes and peroxisomes. Motility of early endosomes requires microtubule filaments and different motor proteins as kinesin and dynein. Formation and maturation of early endosomes is controlled by the specific membrane-bound Rab-GTPase Rab5 and tethering complexes as the hexameric CORVET (class C core vacuole/endosome tethering). In the basidiomycete *Ustilago maydis*, Rab5a is the prominent GTPase to recruit CORVET via its Vps8 subunit to EEs; in *rab5a* deletion strains, this function is maintained by the second EE-associated GTPase Rab5b. In contrast to *A. nidulans* and *S. cerevisiae*, the tethering- and core- subunits of CORVET are essential, buttressing a central role for EE transport and function in *U. maydis*.
While determining the CORVET composition by CoIP, we identified the previously uncharacterized Nma1-protein as an interactor for CORVET. Nma1 interacts with the CORVET subunit Vps3, by that stabilizing the binding of Vps3 to CORVET. CORVET is recruited to EEs by interaction of its Vps8 subunit to Rab5a, while Vps3 contacts Rab5a on independent endosomes as a first step for membrane fusion. The interaction of Nma1 with Vps3 could now diminish the Vsp3-dependent fusion and maturation processes. Indeed, deletion of *nma1* leads to a significantly reduced number of EEs, and an increased conversion rate of EEs to late endosomes. Thus, Nma1 modulates the lifespan of EEs to ensure their availability for the various long distance transport processes. As Nma1 also is associated with microtubles, this regulatory effect is directed mostly to the EE population moving on microtuble tracks.

396T Live cell imaging and changes to effector composition elucidate adaptations of *Magnaporthe oryzae* pathotypes to different plant genera *Tyler Suelter*[1], Giovana Cruppe[1], Ely Oliveira-Garcia[1,2], Chantal Solorzano[1,3], Monica Navia-Urrutia[1,4], Melinda Dalby[1], Sanzhen Liu[1], Barbara Valent[1] 1) Department of Plant Pathology, Kansas State University, Manhattan, KS; 2) Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA; 3) Dodge City, KS; 4) Department of Plant Pathology, University of Florida, Tropical Research and Education Center, Homestead, FL.

The blast pathogen *Magnaporthe oryzae* (synonym of *Pyricularia oryzae*) causes disease on a diverse array of graminaceous hosts worldwide, with specialized pathotypes (lineages) that infect economically important crops including rice, wheat, ryegrass, finger millet, and others. Live cell imaging of the fungus invading optically clear leaf sheath tissue has provided insight on how this hemibiotrophic fungus colonizes living rice cells to cause disease. Further imaging of biotrophic invasive hyphae expressing and secreting fluorescently-labeled effector proteins led to discovery of the specialized biotrophic interfacial complex (BIC), a highly localized structure involved in the translocation of secreted effectors inside rice cells. This assay has also provided a coupled view of the biotrophic interaction transcriptome during colonization of first-invaded host cells, leading to understanding of expression timing and movement of effector proteins in rice tissue. Comparing effector gene composition and dynamics among the rice-, wheat-, and ryegrass-adapted *M. oryzae* lineages will identify key differences and similarities in blast diseases on different host plants. Here we adapt the sheath assay

to wheat and ryegrass hosts in order to characterize, compare, and contrast the hyphal invasion strategies, effector gene expression and BIC formation across pathotypes and hosts. We report live cell imaging of wheat and ryegrass pathogens in wheat and ryegrass sheath cells, respectively, and we report initial studies on the wheat blast biotrophic interaction transcriptome. We continue to analyze cytoplasmic effector protein movement into and between plants cells. Characterization and understanding of the biology of blast effectors and their role in host infection will lead to enhanced blast disease control strategies.

397F Molecular characterization of Pex5b-dependent import of soluble cargo into peroxisomes of *Ustilago maydis* Julia Ast[1, 3], Nils Bäcker[1], Elena Bittner[1], Domenica Martorana[1,4], Humda Ahmad[1], Johannes Freitag[1], *Michael Bölker*[1,2] 1) University of Marburg, Marburg, Germany; 2) SYNMIKRO Center for Synthetic Microbiology, University of Marburg, Marburg, Germany; 3) Institute of Metabolism and Systems Research (IMSR), and Centre of Membrane Proteins and Receptors (COMPARE), University of Birmingham, Birmingham, UK; 4) University of Göttingen, Göttingen, Germany.

Peroxisomes are intracellular organelles that serve to degrade fatty acids and hydrogen peroxide. Peroxisomal matrix proteins are imported from the cytosol with the help of soluble receptor proteins. The majority of imported proteins contains a short C-terminal peroxisomal targeting signal type 1 (PTS1), which is recognized by tetratricopeptide repeat (TPR) containing proteins of the Pex5 family that mediate translocation into the lumen of a peroxisome.
The phytopathogenic basidiomycete *Ustilago maydis* contains two Pex5-like proteins, Pex5a and Pex5b. While Pex5a is not critical for import of PTS1 proteins, absence of Pex5b completely abolished peroxisomal localization of all matrix proteins . Both Pex5 proteins display a modular structure consisting of an N-terminal domain (NTD) and the C-terminal TPR-region. To determine the contributions of these domains, we have analyzed chimeric and truncated variants of Pex5a and Pex5b *in vivo.* The TPR domains of Pex5a and Pex5b are highly similar but differ in their affinity to variations of the PTS1 motif. Thus they contribute to peroxisomal targeting of distinct peroxisomal matrix proteins with a variable degree.
Strikingly, only the N-terminal domain (NTD) of Pex5b turned out to be able to interact with the peroxisomal import machinery to mediate translocation into peroxisomes. Therefore, peroxisomal import of PTS1-containing proteins bound to the TPR domain of Pex5a, also depends on interaction of this complex with the NTD of Pex5b. Proteins containing N-terminal peroxisomal targeting signals (PTS2) are recognized by the Pex7 receptor, which also depends on interaction with Pex5b for peroxisomal import. The NTD of Pex5b can recognize peroxisomal proteins that lack a canonical peroxisomal targeting signal such as the multifunctional protein Mfe2 involved in beta-oxidation of fatty acids. Interestingly, the NTD of Pex5a is also able to interact with specific PTS1-less cargo proteins. Together our data reveal that *U. maydis* employs a complex network of targeting factors that control import into peroxisomes. The NTD of Pex5b appears to play a pivotal role for targeting soluble proteins into peroxisomes.

398W Biofilm formation in the filamentous fungus *Fusarium graminearum* *Rebecca Shay*[1], Frances Trail[1] 1) Michigan State University, East Lansing, MI.

Biofilms are known to play important roles in bacterial pathogens of plants and animals where the formations help protect cells from defense responses and antimicrobial treatments. Although biofilms in bacterial plant pathogens are well studied, the role in filamentous fungal plant pathogens is virtually unexplored. Our studies focus on the economically important plant pathogen *Fusarium graminearum*, a filamentous fungus, which is the primary causal agent of the disease Fusarium head blight (FHB). We hypothesized that biofilms impact many steps in the disease cycle of FHB, from initial plant infection to colonization, to overwintering on crop residues in the field**.** Biofilm formation is initiated *in vitro* with the adhesion of propagules to a surface, followed by growth of the structures and development of an extracellular matrix, then dispersal of propagules and senescence of biofilms. We have documented this formation, along with the serial addition of components of the complex extracellular polymeric matrix around the biofilm formation. From there, we are now moving into work on the underlying genetic components of this process. Through the use of RNA-sequencing, the stages of biofilm development were compared sequentially for differentially expressed transcripts. We have profiled the transcriptome of biofilm formation in *F. graminearum* over time, providing candidate genes for functional analyses. We have characterized the formation of biofilms in *F. graminearum in vitro*, which, together with ongoing characterization of their association with host plants, is providing a basis for understanding the functionality of biofilms in the pathogen disease cycle.

399T Plant ATG8 as a Possible Extracellular Vesicle Marker *Huaitong Wu*[1], Grujic Nenad[2], Dagdas Yasin[2], Hailing Jin[1] 1) University of California at Riverside; 2) Gregor Mendel Institute of Molecular Plant Biology.

Extracellular vesicle (EV) has various important functions in plants including defense against pathogens, protein secretion and RNA transportation. Currently only three types of EV are known in plants yet none has clear relationship with program cell death (PCD). Since PCD in plants is an important response to pathogen infection which also induces EV release in plant cells, we hypothesize that in plants there should be a subclass of EV more directly associated with PCD, just like apoptotic bodies in mammalian cells. Since plants seem to lack apoptosis, we instead focus on autophagy, a major type of PCD in plants and many other species. Autophagy related gene 8 (ATG8) is a marker gene of autophagosomes and its homolog LC3 has been reported associating with EV in mammalian cells. We infected GFP-Atg8a and mcherry-ATG8e Arabidopsis with *Botrytis Cinerea* and found that both tagged ATGs seemed to be secreted to extracellular space, a process induced by Botrytis infection. Sucrose gradient showed the secreted ATG8s are associated with particles that have similar but not identical sedimentation coefficient with that of tetraspanin labeled vesicles. Under confocal, we've seen GFP-ATG8a signals forming vesicle like foci moving across plant cells under Botrytis infection. Together, it's possible that a subclass of EV related to autophagy and labeled by autophagy genes exist. Further research such as direct visualization, antibody pulldown, colocalization test with known tetraspanin markers, *atg* mutant analysis, RNA seq and proteomic analysis is needed for more validation and characterization of this potentially subclass of EV.

400F Role of the plasma membrane H^+-ATPase Pma1 in pH homeostasis, development and virulence of the fungal pathogen *Fusarium oxysporum* *Melani Mariscal*[1], Tânia R. Fernandes[2], Antonio Serrano[1,3], Robert Arkowitz[3], Antonio Di Pietro[1] 1) Departamento de Genética, Universidad de Córdoba, Campus de Rabanales, 14071 Córdoba, Spain; 2) GreenUPorto – Sustainable Agrifood Production Research Centre / Inov4Agro & DGAOT, Faculty of Sciences, University of Porto, Campus de Vairão, 4485-646

Vairão, Portugal; 3) Universite Cote d›Azur, CNRS, INSERM, Institute of Biology Valrose (iBV), Parc Valrose, Nice, France .

Ambient pH regulates fundamental processes in fungi, including cell growth, development and virulence. Expression of the ratiometric GFP-based pH sensor pHluorin in the fungal pathogen *Fusarium oxysporum* revealed that changes in external pH induce and transitory fluctuations in cytosolic pH (pH_{cyt}), resulting in rapid regulation of mitogen-activated protein kinase (MAPK) signaling cascades. How pH_{cyt} regulates MAPK-mediated functions such as hyphal growth, development and virulence is currently unknown. Here we used a combination of reverse genetics and live-cell imaging to investigate the role of the essential plasma membrane H^+-ATPase Pma1 in pH_{cyt} homeostasis. A gene knockout of *F. oxysporum* casein kinase 1, which functions a negative regulator of Pma1 in yeast, resulted in a marked increase of Pma1 activity and acidification of pH_{cyt}, but did not affect pH_{cyt}-mediated MAPK responses. Interestingly, the *ck1*Δ mutants exhibited reduced growth, were impaired in invasive hyphal growth and pathogenicity on tomato plants. Live-cell imaging of fluorescent Pma1-GFP revealed that this plasma membrane-localized H^+-ATPase is excluded from the hyphal tip, suggesting a possible role in regulating polarized growth. Overall, our results shed light on the regulation of pH_{cyt} and its role in development and virulence of fungal pathogens.

401W The ribonucleoprotein complex components JSN-1 and GUL-1 are involved in asexual development in *Neurospora crassa* *Anne Yenewodage*[1], Inbal Herold[1], Oded Yarden[1] 1) The Hebrew University of Jerusalem .

RNA-binding proteins (RBPs) are critical for the spatial localization, translational regulation and overall fate of mRNAs. In *N. crassa*, the RBP GUL-1 has been shown to bind over 2000 mRNA species, many of which encode genes involved in cell wall integrity. *gul-1* is an extragenic suppressor of the colonial, hyperbranched, *cot-1*(ts) mutant. Despite the role played by GUL-1, only minor morphological changes are observed when *gul-1* is inactivated, suggesting that additional proteins may have overlapping functions with GUL-1. To identify other potential components of the GUL-1 ribonucleoprotein complex (RNP), we used a protein co-immunoprecipitation approach. Four of the over 100 proteins identified harbored hallmarks of RBPs. Inactivation of a gene encoding one of them, JSN-1, resulted in partial suppression of *cot-1* (ts). The epistatic nature of *cot-1* suppression by *gul-1* and *jsn-1* support the possibility of a functional overlap between the two RBPs. Furthermore, both RBPs were found to affect MAK-1 phosphorylation under stress conditions. The increased JSN-1::GFP association with nuclei under stress conditions, as previously observed with GUL-1::GFP, also supports their common presence and possible roles within an RNP. GUL-1 and JSN-1 have additional roles in fungal development. While strains in which either of the two genes had been deleted exhibited normal vegetative reproduction, the Δ*jsn-1*;Δ*gul-1* strain was impaired in aerial hyphae formation and subsequent conidiation. We concluded that GUL-1 and JSN-1 are components of the same RNP, have partial overlapping functions within the COT-1 pathway and are jointly involved in asexual development.

402T Transcriptome investigation of *mpkB-mkkB* mutants related to secondary metabolism and sexual development of *Aspergillus nidulans* Sang-Cheol Jun[1], Jong-Hwa Kim[1], *Kap-Hoon Han*[1] 1) Woosuk University.

The MpkB MAP kinase plays a prominent role in the sexual differentiation signaling and secondary metabolism pathway, acting as the terminal MAPK activating expression of SteA and VeA. This MAP kinase is expected to balance the expression of genes encoding cell fusion, anastomosis, and secondary metabolism. Deletion of the *mpkB* gene causes conidiophore morphological abnormalities, a decrease in asexual spore production, and inhibition of homo-/heterothallic mating. This mutation has been reported to inhibit the production of sterigmatocystin (ST), a secondary metabolite, but our experiments did not find any evidence that ST production was inhibited in the *mpkB* mutant. In an effort to find other connections not yet known for this pathway, we constructed aspartic acid or alanine residues that could mimic the phosphorylation effect in amino acids 218 and 222 of MkkB MAPK kinase (upstream MAPK kinase of MpkB) to produce mutations that either constitutively phosphorylate or not phosphorylate MpkB MAPK. These mutants were analyzed for transcripts using the RNA-seq method under 16 hours of asexual stage. A total of 4 strains (wild type, *mkkB* knock-out mutant, MkkB constitutively activation mutant and MkkB constitutively inactivation mutant) generated more than 6 Gbp data by pair-end sequencing. When mutants were compared to the control strain, markedly altered expression levels of the relevant genes were identified, suggesting that MpkB signaling is involved with asexual sporulation, sexual differentiation and ST biosynthesis pathway, among others.

403F Local Translation and Nuclear Autonomy in a Multinucleate Fungus *Ashbya gossypii* *Ameya Jalihal*[1], Amy Gladfelter[1] 1) UNC Chapel Hill.

Multinucleate “syncytial” cells are found widely across all eukaryotic kingdoms, notably in filamentous fungi, embryos, and human tissues like placenta and muscle. It is unknown how nuclei in syncytia share and interpret shared cytosolic signals and to what extent they act autonomously. The filamentous ascomycete fungus *Ashbya gossypii* is a genetically tractable syncytial model system. Neighboring nuclei in *Ashbya* occupy the same cytoplasm yet show asynchronous nuclear division. Previous work in the Gladfelter lab has established that nuclear division asynchrony requires sequestration of cell-cycle transcripts into RNA-protein condensates which cluster around nuclei. This suggests that the cytosol in these cells is highly spatially heterogeneous, and nuclei don›t share all components equally. It is unclear how such spatial heterogeneity is maintained and exploited at the level of individual nuclei. One possibility is the existence of functional differentiated nuclei within the shared cytoplasm, whereby nuclear “territories” may behave as cells-within-cells, sensing and responding to the local environment semi-autonomously.
The goal of this work is to establish biophysical mechanisms that establish functional autonomy in nuclear territories in Ashbya, specifically, the role of local transcription and translation heterogeneity. We implemented a fluorescence in situ hybridization (FISH) based approach to visualize translation at single transcript resolution in Ashbya. By comparing localized translation of clustered cell-cycle transcripts with that of non-cell-cycle transcripts we expect to uncover how spatial regulation of translation proximal to nuclei can regulate nuclear territory functions. Ultimately, we hope to establish how the interplay of molecular localization and local translation control can give rise to nuclear autonomy in syncytial cells.

404W Evolutionary Morphogenesis, Chytrids and the rise of The Fungi *Edgar Medina*[1], Lillian Fritz-Laylin[1] 1) Department of Biology, University of Massachusetts Amherst.

Morphogenesis comprises the cellular processes that generate organismal shape. Despite being sister lineages, the morphogenetic programs of multicellular Fungi and Animals are remarkably divergent in their *materia prima* (e.g. cell wall vs. epithelia) and its mechanisms. How morphogenetic programs evolved and diversified in these lineages remains a key question in evolutionary cell biology. Chytrids are members of the deepest lineages of Fungi, and their life cycle alternates between an animal-like ciliated zoospore cell that swims and crawls and a sporangial cell with fungal traits like a cell wall and hyphae. The growth of the sporangial cell results in a multinucleated compartment that is more than a hundred times larger than the initial uninucleated zoospore. During late development, the sporangia switches developmental programs and undergoes cellularization to produce numerous zoospores that are discharged through a pore in the cell wall. During zoospore formation, chytrid sporangia are transiently multicellular and undergo morphogenetic transformations akin to those of animal embryonic development. These transformations include a cytokinetic event that produces a multicellular sphere of tightly packed uninucleated cells. This morula-like sphere undergoes ciliogenesis and synchronous abscission to form motile zoospores. This combination of fungal traits, such as cell wall and hyphae, and an animal-like developmental program makes chytrids uniquely suited to trace the evolution of animal and fungal morphogenesis. We recently developed the chytrid *Spizellomyces punctatus* as a genetically tractable model system for evolutionary cell biology. We combine live-cell imaging of F-actin, microtubules, myosin-II and membrane dynamics with pharmacological perturbations to characterize the developmental program and morphogenetic transformations underlying chytrid cellularization. Further, we show that Chytrids retain key potential regulators of cellularization that are shared with animals but absent from Dikaryotic fungi. These results provide new insights into the ancestral morphogenetic toolkit present in the common ancestor of animals and fungi as well as innovations unique to the Fungal morphogenetic program.

405T Distribution of non-canonical septins in fungi *Brent Shuman*[1], Michelle Momany[1] 1) University of Georgia, Athens, GA, USA.

Septins are the fourth component of the cytoskeleton. Three or four different septin proteins assemble to form nonpolar hetero-hexamers and hetero-octamers, respectively, which perform a variety of functions in fungi, most notably forming the septin ring necessary for cytokinesis. While four septin clades are conserved from yeast to humans, some fungi posses a fifth group of septins that is not evolutionarily similar any other septin group, and does not perform canonical functions. Present even in basal clades of the holomycota, likely this group was present in the opisthokont ancestor and lost in the holozoan ancestor. Deletion of a Group 5 septin in filamentous fungi causes mislocalization of hexamer septins and vice versa, but no distinct morphological phenotypes are observed. We are working to understand the evolutionary distribution of Group 5 septins in fungi and how that has selected for their non-canonical function.

406F Phosphorylation / dephosphorylation of the *Cochliobolus heterostrophus* stress-activated MAPK Hog1 in response to plant phenolic acids *Rina Zuchman*[1], Roni Koren[1], Tamar Ziv[1], Benjamin A. Horwitz[1] 1) Faculty of Biology, Technion–Israel Institute of Technology, Haifa, Israel.

Protein phosphorylation cascades are universal in cell signaling. While kinome diversity allows specific phosphorylation events, relatively few phosphatases dephosphorylate key signaling proteins. Fungal MAP kinases, in contrast to their mammalian counterparts, often show detectable basal phosphorylation levels. Dephosphorylation, therefore, could act as a signal. In *Cochliobolus heterostrophus*, the Dothideomycete causing Southern corn leaf blight, ferulic acid (FA), an abundant phenolic found in plant host cell walls, acts as a signal to rapidly dephosphorylate the stress-activated MAP kinase Hog1. To identify the protein phosphatases responsible, we constructed mutants in Hog1 phosphatases predicted from the genome by homology to yeast and other species. We found that *Cochliobolus heterostrophus* mutants lacking PtcB, a member of the PP2C family, exhibited altered growth, sporulation and attenuated dephosphorylation in response to FA. Loss of the dual-specificity phosphatase CDC14 led to slow growth, decreased virulence, and attenuated dephosphorylation. Mutants in two predicted tyrosine phosphatase genes PTP1 and PTP2 showed normal development and virulence. A functional Hog1:Gfp fusion protein partitioned to the nucleus in response to osmotic stress, but upon exposure to FA, accumulated in cytoplasmic granules. Hog1 is well-studied in the context of high osmolarity stress, but the FA-induced pathway studied here led to new insights regarding the roles of Hog1 in virulence and development. The results indicate a complex relationship between Hog1 dephosphorylation and the response of the fungal cell to chemical stress by plant phenolics. Our results reveal multi-phosphatase regulation of Hog1 mediated by serine/threonine phosphatases rather than tyrosine phosphatases. The cytoplasmic granules where Hog1:Gfp accumulates following exposure to FA resemble stress granules. However, the phosphorylation state of this MAPK is decreased rather than increased, as currently known in stress conditions. These observations reinforce the view of dephosphorylation as a stress signal, together suggesting a novel MAPK stress response. By biochemical and proteomic methods, we are addressing (1) the sequence of phosphorylation and dephosphorylation events on Hog1 in response to FA and osmotic stress; (2) the subcellular localization of Hog1 upon exposure to FA.

407W Assessing the impact of regulator of G protein signaling proteins in *Neurospora crassa* using conventional phenotypic assays and a quantitative image analysis algorithm *Katherine Borkovich*[1], Ilva Cabrera[2], Yagna Oza[1], Alexander Carrillo[1], Logan Collier[1] 1) University of California, Riverside; 2) University of California, San Diego.

Heterotrimeric (αβγ) G protein signaling pathways are critical membrane-associated environmental sensing systems found in eukaryotic cells. Exchange of GDP for GTP on the Gα subunit is stimulated by guanine nucleotide exchange factors, leading to its activation. In contrast, GTP hydrolysis on the Gα subunit is accelerated by Regulator of G protein Signaling (RGS) proteins, resulting in a return to the GDP-bound, inactive state. Here, we implement standard phenotypic assays and an image analysis algorithm for determining cell size in strains with knockout mutations in the seven identified RGS genes (*rgs-1* to *rgs-7*) in *Neurospora crassa.* We compared phenotypes to those of strains with either knockout mutations or GTPase-deficient, constitutively activated alleles for one of the three Gα subunit genes (*gna-1, gna-2* or *gna-3*). Our data showed that six RGS mutants have taller aerial hyphae than wild type and all seven mutants exhibit reduced asexual sporulation, phenotypes shared with strains expressing activated alleles for *gna-1* or *gna-3*. In contrast, Δ*rgs-1* and Δ*rgs-3* were the only RGS mutants with growth rate phenotypes, while Δ*rgs-1* and Δ*rgs-2* possessed sexual development defects. Measurements of cell compartment dimensions revealed several RGS mutants with phenotypes. Epistasis analysis uncovered multiple genetic interactions between *rgs-2* or *rgs-3* and the *gna-1* and *gna-3* Gα genes, and Δ*rgs-3* was observed to

suppress the sexual cycle phenotypes of both Δ*gna-1* and Δ*gna-3* mutants. Expression levels of candidate regulated genes were also determined using qRT-PCR in the group of strains. Taken together, the data support multiple RGS proteins acting on each Gα subunit in *N. crassa*.

408T ***Aspergillus nidulans*** **septa appear to be indispensable for surviving cell-wall stress** *Mark Marten*[1], Cynthia Chelius[1], Walker Huso[1], Ryland Spence[1], Alex Doan[1], Harley Edwards[1], Garrett Hill[1], Ryan Jordan[1], Jyothi Kumar[2], Samantha Reese[2], Nicole Beauregard[3], Stephen Lincoln[3], Ranjan Srivastava[3], Steven D. Harris[4] 1) Univ Maryland, Baltimore County (UMBC); 2) University of Nebraska-Lincoln; 3) University of Connecticut; 4) Iowa State University.

To assess system-wide, dynamic response to cell-wall stress, we perturbed *A. nidulans* cultures with the echinocandin micafungin, known to activate the Cell Wall Integrity (CWI) signaling pathway and result in expression of numerous wall-repair genes. Multiple samples were collected from perturbed cultures, over 10 and 120 min, for phosphoproteomic and transcriptomic analysis respectively, with over 1800 genes and 430 phosphorylation sites showing significant change with time. Our collective data indicate previously unknown connections with septum formation, leading to the hypothesis that septa are critical for survival during cell wall stress. To test this hypothesis, we treated wild type *A. nidulans* with micafungin and find the number of septa per hyphal volume increases significantly compared to a control. In addition, we subjected four different, known, septation-deficient, *A. nidulans* mutants (Δ*sepH*, Δ*bud3*, Δ*bud4*, and Δ*rho4*) to six antifungal compounds – three of which activate the CWI pathway and three of which (nominally) do not. Our results show that deficiencies in septation lead to fungi which are more susceptible to cell-wall perturbing compounds, but are no more susceptible to other antifungal compounds than a control. This implies septa play a critical role in surviving cell wall stress but may be dispensable regarding survival in other types of stress.

409F **Investigating germination initiation in the pathogenic fungus** ***Aspergillus fumigatus*** *Justina Stanislaw*[1], Michelle Momany[1] 1) University of Georgia, Athens, GA 30602 USA.

Aspergillus fumigatus is a ubiquitous fungus that can cause human infection, especially in immunocompromised individuals. Examining the development of A. fumigatus may uncover important mechanisms that could be essential for organismal growth, and may potentially be used in uncovering new drug targets for treatment of human hosts. In Aspergillus species, germination is well-characterized. The fungus produces small dormant spores that can be dispersed by wind, and when they contact a carbon source they break dormancy, swell, and extend germ tubes. If a susceptible host inhales the spores, they can break dormancy and grow in the lungs causing invasive disease. Although the germination process is essential for disease, the genetic factors imposing and maintaining dormancy are not known.

In the present study, we investigate the germination process by analyzing A. fumigatus RNA-seq transcriptome data to identify the most highly differentially expressed genes in dormant conidia versus hyphae. We hypothesize that potential factors inhibiting germination will be fungal specific genes with unknown function that are highly differentially expressed in conidia over hyphae. Future work with this group of candidate genes will include phenotypic characterization of their roles in germination and development, drug resistance, and pathogenicity.

410W **The conidial coin toss: asymmetric spore adhesion in** ***Colletotrichum graminicola*** *Brian Shaw*[1], Joseph Vasselli[1], Hope Hancock[1], Ellen Kainer[1], Thomas Chappell[1] 1) Texas A&M University.

Colletotrichum graminicola is an economically significant fungal pathogen of maize. The primary infective conidia of the fungus, the macroconidia (also called falcate conidia), are splash dispersed during rain events. The adhesion of the macroconidia is required for the development of infection structures. Macroconidia are capable of immediate adhesion due to hydrophobic interactions with the substrate. We report that rapid adhesion in *C. graminicola* is asymmetric, with a strip of adhesive material running the length of a single side of the conidium. This strip of adhesive is co-localized with dynamic transverse actin cables, and both the adhesive strip and actin cables are formed prior to adhesion to the infection court. These polarized adhesives determine early adhesion, and increases in adhesion rates can be induced by applying force to flip conidia onto their adhesive faces. We hypothesize that this selective adhesion helps to increase the dispersal of the spores beyond their initially site of deposition.

411T ***Aspergillus fumigatus*** **hexameric septin complex is involved in spore cell wall organization and immune evasion** Alban Sinani[1], Wyatt Boyer[1], *José Vargas-Muñiz*[1] 1) Microbiology Program, School of Biological Sciences, Southern Illinois University at Carbondale.

Aspergillus fumigatus is the major etiology of invasive aspergillosis, a leading cause of death in immunocompromised patients. Septins are conserved GTPases involved in septation, conidiation, and cell wall organization. The requirement of septins for tissue invasion and virulence has been demonstrated in the human pathogenic yeasts *Candidaalbicans* and *Cryptococcus neoformans. Aspergillus* spp. contain five genes encoding for septins (AspA-E), Aspergillus septins can interact to form a hexameric complex (AspA, AspB, and AspC) and octameric complex (AspA, AspB, AspC, and AspD). Previous studies showed that the octameric complex is required for full conidiation. Furthermore, the electron micrograph of Δ*aspB* strain spores shows that the spores lack the characteristic electron-dense outer layer. Based on their role in conidiation, we hypothesized that septins are also required for conidia cell wall organization. To test this hypothesis, we are using a combination of microscopy techniques and cell culture-based methods. We utilized Atomic Force Microscopy to determine the organization of the spore surface. Using this technique, we found that spores from the Δ*aspB* strain have a disorganized rodlet layer compared to the parent strain. Concomitant with a disorganized rodlet layer, spores from the Δ*aspA*, Δ*aspB*, Δ*aspC* strains have more exposed chitin. However, Δ*aspD* chitin exposure is similar to those of the wild-type, indicating that the octameric complex is dispensable for conidia surface organization. Previously, it was reported that *Galleria* infected with these strains showed rapid melanization, which is a response of the immune system to pathogens. This points out a possible role of the septin hexamer in regulating spore-host interaction via the assembly of the conidia cell wall. To test this, we first exposed macrophage-like cells (J744.1) to septin deletion strain's spores and measured TNF-α production to determine spore immunogenicity. As expected, only the deletion of any gene involved in the hexameric complex leads to a significant increase in TNF-α. Taken together, these results

suggest the septin hexameric complex is at least sufficient for *A. fumigatus* spore cell wall organization and immune evasion.

412F Developmental genetics of host invasion initiated by fungal conidia *Soumya Moonjely*[1], Wang Zheng[3], Jeffrey P Townsend[3, 4, 5], Frances Trail[1, 2] 1) Department of Plant Biology, Michigan State University, East Lansing, MI, USA; 2) Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI, USA; 3) Department of Biostatistics, Yale School of Public Health, New Haven, CT, USA; 4) Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT, USA; 5) Program in Computational Biology and Bioinformatics, Yale University, New Haven, CT, USA.

Conidial germination is a key step for initiating the colonization of fungi on any host or substrate. We are using an evolutionary approach to identify genes that have shifted in expression to acquire new roles in evolving lineages of fungi. We are examining seven ecologically and morphologically diverse lineages in the Sordariomycetes with a common ancestor. Comparative transcriptomics provides a powerful tool to identify the genes related to developmental differences between closely related organisms and as part of this project we are examining, *Fusarium graminearum* and *Metarhizium anisopliae*. *F. graminearum* is a plant pathogenic fungus and is a causal agent of Fusarium head blight in cereal crops, whereas *M. anisopliae* is an endophytic fungus that can form a mutualistic association with plant hosts. *M. anisopliae* is also well described as an insect pathogenic fungus, hence widely used as a biocontrol agent against agricultural pests. Four designated conidial germination stages were selected for transcriptome analysis: fresh conidia, polar growth, doubling of the long axis, and first hyphal branching. We examined the transcriptional differences between orthologous genes in these species across the four stages on a common growth medium and on the natural host, barley. Orthologs that have greatly increased in expression in one lineage when compared to another are hypothesized to have taken on new roles in that species. These orthologs, identified via ancestral character estimation, are then targeted for functional assays through gene knock-outs. Combining the transcriptome data with functional gene knock-out assays allows us to identify the network of genes necessary for conidial germination, pathogenesis/mutualism and the evolutionary path to new symbiotic relationships.

413W Developmental genetics of spore germination in *Epichloë festucae* *Esteban Valverde Bogantes*[1], Frances Trail[1,2], Carolyn Young[3] 1) Department of Plant Biology, Michigan State University, East Lansing, MI; 2) Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI; 3) Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK.

Species of *Epichloë* form endophytic relationships that range from mutualistic to pathogenic with grasses from the subfamily Pooideae. Plant colonization by *Epichloë* is persistent and systemic, with the fungus being present in most host tissues except for the roots. These endophytic fungi are known to provide certain benefits to the host plant, which include growth promotion, improved nutrient acquisition, as well as protection to the plant host from pests and pathogens due to the production of bioactive alkaloids that are toxic or deterrent to herbivores. Conidia production in *Epichloë* tends to be scarce and little is known about the genes relevant for spore germination. This project aims to use comparative genomics and evolutionary biology to reveal genes that are critical for spore germination, subsequent growth, and host colonization in *Epichloë*.
To stimulate spore production, the *E. festucae* E2368 isolate was inoculated on 2% water agar plates and incubated for two weeks at 22 °C. The spores were collected by flooding the medium with 4 mL of sterile water and a cell spreader was used to dislodge the spores. The suspension was filtered through two layers of miracloth to remove mycelial fragments and the conidia concentration was adjusted to $1x10^6$ spores/mL. The spores were inoculated onto cellophane membranes placed on top of PDA and incubated at 22 °C. Samples were collected at four different stages of conidia germination, namely, (i) fresh spores – 0 h, (ii) polar growth – 12 h, (iii) doubling of long axis – 24 h, and (iv) first hyphal branching – 42 h. RNA extraction and RNA sequencing from the samples are ongoing. Samples are also being collected from germinating conidia on plant host tissue. Gene expression will be compared with data from seven species in the Sordariomycetes class, which include phytopathogenic, entomopathogenic, endophytic, mycopathogenic, and a nonpathogenic model species. To increase the discovery rate of genes relevant to spore biology, candidate genes will be identified by estimating ancestral gene expression using Continuous Ancestral Character Estimation. Genes with the greatest shifts in expression from ancestor to descendant will be targeted for knockout, and phenotypes will be evaluated. Understanding the genetics underlying spore germination in *Epichloë* and related species will provide insight into this process and how it has evolved in related fungi with differing lifestyles.

414V The 14-3-3 homologs (Bmh1 and Bmh2) individually contribute to the proper integrity of the budding yeast kinetochore *Guhan kaliyaperumal Anbalagan*[1], Santanu Kumar Ghosh[1] 1) Indian Institute of Technology Bombay, Mumbai, India.

The 14-3-3 family of proteins are conserved across eukaryotes and serve myriad important regulatory functions of the cell. Homo/heterodimers of these protein homologs, majorly recognize their ligands via conserved motifs from a plethora of cellular proteins to modulate the localization and functions of those effector ligands. In most of the genetic backgrounds of Saccharomyces cerevisiae, disruption of both 14-3-3 homologs (Bmh1 and Bmh2) are either lethal or survives with severe growth defects with a gross chromosomal missegregation. To elucidate their roles in chromosome segregation, in this work we investigated their roles in the centromere-kinetochore related functions. Analysis of appropriate deletion mutants shows that both Bmh1 and Bmh2 proteins individually contribute to the proper integrity of the kinetochore ensemble. Despite very high sequence homology between Bmh1 and Bmh2 proteins, they may exhibit unshared functions at the kinetochore as revealed by the performance of the corresponding mutants in the assays including transcriptional read-through across centromere, di-centric plasmid stability, sensitivity to anti-microtubule drugs and genetic interaction with the kinetochore mutants. We conclude that Bmh1 and Bmh2 proteins facilitate proper kinetochore assembly perhaps by promoting physical interactions among the kinetochore proteins and their centromeric localization.

415V Cell wall dynamics in fast growing fungal hyphal cells *Louis Chevalier*[1], Mario Sala[2], Miguel Peñalva[2], Nicolas Minc[1] 1) Institut Jacques Monod, CNRS, Paris, France; 2) Departamento de Microbiología Molecular, Centro de Investigaciones Biolo´gicas C.S.I.C., Madrid , Spain.

Tip growth is a highly polarized cellular process used by walled cells of fungi, plants, or bacteria to colonize space, reproduce or infect. Tip growing cells are encased in a rigid cell wall that ensures surface integrity and limits cell growth, yet these cells can elongate at un-

usually high speeds of up to few mm/hrs. These considerations raise the fundamental question of how the cell wall may be dynamically assembled at cell tips to safeguard integrity while allowing rapid surface shape changes. We implemented a sub-resolution imaging approach to map cell wall thickness spatio-temporal dynamics, cell wall elasticity, and turgor pressure in very rapid growing hyphal cells of the filamentous fungus *Aspergillus nidulans.* We found that hyphal cells grow with a near homogenous cell wall thickness of about 80nm, and a marked gradient in cell wall bulk elastic modulus, with hyphal tips being twice softer than cell sides. By co-imaging cell wall thickness dynamics and secretory vesicle accumulations that deliver new cell wall material to cell tips, we found that both fluctuated with typical amplitudes of up to 150% and periods of 1-2 min during growth. Affecting secretory vesicle transport or fusion caused a rapid loss of polarity, growth arrest, and rapid thickening of the cell wall at cell tips. These data provide unprecedented details on cell wall dynamics, from synthesis to assembly and deformation, and suggest important dynamic coupling mechanisms between surface material synthesis and deformation rates, likely essential to support rapid growth and cell viability.

416V Trehalose metabolism is differentially modulated during cold stress response of postharvest pathogenic fungi *Carmit Ziv*[1], Lavanya Gunamalai[1], Kamal Tyagi[1], Ginat Raphael[1] 1) Agricultural Research Organisation.

Fungal pathogens are the leading cause of postharvest losses of fresh fruits and vegetables, estimated at approximately 30% of total crop yield worldwide. Low temperature (LT) storage is an efficient practice to prolong the postharvest performance of fresh produce with minimal negative implications on human health and the environment. LT decreases cellular respiration and metabolic activities, which inhibits the growth of pathogenic microorganisms. However, some phytopathogenic fungi are highly tolerant to LT (near 0□C) and can grow, infect and cause fruit deterioration during cold storage.
The necrotrophic fungal pathogen, *Botrytis cinerea,* causing gray mold disease of many plants, is highly tolerant of LT conditions and can develop during cold storage, causing rots of many economically valuable fresh produce. *B. cinerea* morphology was significantly affected by the temperature of growth. Growth at LT (2-5□C) was accompanied by hypo-branching, reduced conidiation, induction of sclerotia formation, and enhanced virulence. Interestingly, although infection at LT was significantly slower than at ambient temperatures, *Botrytis* virulence was not impaired, and conidiation was not suppressed by the LT during pathogenic interactions.
To further study the molecular basis of fungal tolerance to LT, we compared the physiological response of the cold-tolerant *B. cinerea* to another prevalent postharvest pathogen, the cold-sensitive *Colletotrichum gloeosporioides*. Transcriptome analysis indicated significant differences in the response of the two fungi to cold shift (22□C to 5□C for up to 48 h), and specifically different cold-stress respons (1-4 h after the shift). Specific differences were observed in several pathways, including ROS production and detoxification, and biosynthesis of osmoprotectants like trehalose, which was later validated by GCMS quantification. Further analysis indicated the induction of several genes known to be regulated by carbon catabolite repression, suggesting that *Botrytis* but not *Colletotrichum* was experiencing carbon starvation during the early stages of the cold shift. These molecular differences were correlated with altered morphological and pathogenicity characteristics of the two pathogens at LT. Uncovering the molecular basis for cold tolerance in phytopathogenic fungi may pave the way to reduce food loss by developing environmentally sustainable control treatments against these pathogens in cold storage.

417V Centromere Evolution during Fungal Pathogens *Pneumocystis* Adaption to Mammals *Ousmane H. Cisse*[1], Shelly Curran[1], Yueqin Liu[1], Lisa Bishop[1], Liang Ma[1], Jason Brenchley[2], Sergio Hassan[3], John P. Dekker[4], Pavel P. Khil[4], Honghui Wang[1], Sally Davis[5], Joseph Kovacs[1] 1) Critical Care Medicine Department, NIH Clinical Center, National Institutes of Health (NIH), Bethesda, MD, USA; 2) Laboratory of Viral Diseases, NIAID, NIH, Bethesda, MD, USA; 3) Bioinformatics and Computational Biosciences Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892; 4) Bacterial Pathogenesis and Antimicrobial Resistance Unit, National Institute of Allergy and Infectious Diseases (NIAID), NIH, Bethesda, MD, USA; 5) Diagnostic Medicine/Pathobiology, Kansas State University College of Veterinary Medicine, Manhattan.

Centromeres (CEN) are specific loci that directs the formation of kinetochore multiprotein complex for chromosomal segregation during mitosis. Yet despite their essential function across eukaryotes, centromeres display a great evolutionary diversity. *Pneumocystis*, a genus of mammal specific fungal pathogens, is a sister clade of the fission yeasts. *Pneumocystis* cannot be continuously maintained *in vitro*. As a result, their cell cycle is poorly defined and their CENs are uncharacterized. Here, we characterized the centromeres in three species of *Pneumocystis* which infect macaques, rats, and mice (*Pneumocystis macacae, P. carinii* and *P. murina,* respectively) using short term culture and animal models. These species are important models to study the human *Pneumocystis* pneumonia caused by *P. jirovecii*. We delineated the centromeres by identifying the centromeric histone H3 variant (CENP-A), the scaffold protein CENP-C and the kinetochore protein mis12 bound regions using ChIP-seq. Each species has monocentric chromosomes with short regional centromeres (6 – 8 kilobases), that spawn active genes, are devoid of silent chromatin (H3K9m2/3), as well as 5mC DNA methylation marks. Using MNase-ChiP-seq, we found that centromeres are organized by a single histone H3 nucleosome in their core surrounded by CENP-A nucleosomes and flanked by pericentromeric heterochromatin similar to *S. pombe* centromeres. These results represent the first step to determine the structure of centromeres in *Pneumocystis* and will guide the development of molecular tools to study their sexual cycle.

418V Investigating Gene-dependent cell death in *Cryptococcus neoformans* *Madhura Kulkarni*[1], Yining Liu[1], Zachary Stolp[1], J Marie Hardwick[1] 1) Johns Hopkins University, Bloomberg School of Public Health.

Fungal pathogens pose an increasing threat to public health and limited clinical drug regimens challenge infection control. Limited antifungal therapies and the increased threat of multi-drug resistant infections should be addressed by identifying new? targets for the development of fungicidal drugs. A novel approach is to activate the intrinsic cell death pathways encoded by fungi, yet the molecular details of cell death mechanisms in pathogenic fungi are not yet delineated. Here we demonstrate methods to detect molecular kinetics? identify death-promoting genes in Cryptococcus neoformans in response to transient heat stress. Deletion strains from the C. neoformans knockout collection were treated with a heat-ramp to uncover cell death-resistant knockouts. The C. neoformans Δapl5/CNAG_02468 mutant and C. neoformans Δchs5/CNAG_04321 knockout strains were found to be death resistant. Apl5p is a component of the AP-3 adaptor complex that traffics cargo from the Golgi compartment to the yeast vacuole, and Chs5p is a component of the yeast exomer trafficking complex that selectively transports cargo from the Golgi compartment to the plasma membrane. The identi-

fication of the conserved role of vesicle trafficking pathways in promoting cell death suggests the possibility that killer cargo proteins trafficked by AP-3 and exomer complexes function to promote cellular suicide upon stress. Δchs5 knockout strain was further tested for virulence phenotypes in mice and preliminary evidence suggests Δchs5 is more virulent than wildtype in 129s6 and CD-1 mice. Application of this novel assay has revealed death-resistant phenotypes in clinical isolates of Cryptococcus neoformans which further supports the investigation of novel connections between cell death resistance and virulence.

419V Novel putative seven-transmembrane receptors may underlie complex multicellularity in mushroom-forming fungi *Csenge Földi*[1], Zsolt Merényi[1], László Galgóczy[1], László Nagy[1] 1) Synthetic and Systems Biology Unit, Institute of Biochemistry, Biological Research Centre, Eötvös Loránd Research Network.

The evolution of multicellularity is almost universally associated with the sophistication of cell-to-cell communications systems, which in the metazoa is manifested in the expansion of G-protein coupled receptors (GPCRs) encoding genes. The complexity level of multicellular fungi is comparable with that of simple animals, however, we apparently can not see evidence for similar receptor expansion in their case. GPCRs are seven-transmembrane domain (7TM) cell-surface receptors that mediate cellular response to various stimuli by transmitting the signal through heterotrimeric G-proteins. GPCRs play a role in environmental signal sensing and cell-cell communication. 7TM proteins with known GPCR motifs are called classical GPCRs. In comparative genomic assays, we screened for predicted 7TM proteins in 443 fungal species and observed that the diversity of classical GPCR genes in fungi is surprisingly low (2-70, mean 14.7). In contrast, the predicted 7TM proteins without classical GPCR motifs are abundant (2-539, mean 85) in complex multicellular Agaricomycotina and Pezizomycotina, while they are strongly depleted in genomes of yeasts and dimorphic fungi. Several predicted 7TM proteins are particularly interesting because they show high expression dynamics during fruiting body development. We hypothesize that these 7TM proteins are hitherto uncharacterized, non-classical GPCRs, and that they are associated with diverse fungal functionalities, including multicellularity. To test this hypothesis, we use the inky cap mushroom (*Coprinopsis cinerea*) as a model system. By using functional assays, we set out to prove that selected predicted 7TM proteins of *C. cinerea* are bona fide GPCRs. This includes reverse genetic experiments to disrupt predicted 7TM protein genes, which show the highest expression dynamics during primordium initiation, using the CRISPR-Cas9 system. We expect our results will contribute to understanding the receptor repertoire of fungi and the function of novel receptors in development.

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420V The annexin ANXC4 plays a substituting role for ANX14 during Ca^{2+}-mediated membrane damage responses in *Neurospora crassa* *Linda Matz*[1], Marcel Schumann[1], André Fleißner[1] 1) Technische Universität Braunschweig.

During colony establishment, germinating spores of *Neurospora crassa* fuse with each other to form a mycelial network. The fusion process involves cell wall degradation and plasma membrane merger, which bears the risk of lysis and cell death.
We identified three proteins, ANX14, ANXC4 and PEF1, as part of different Ca^{2+}-dependent membrane repair mechanisms with overlapping functions. Subcellular localization and live-cell imaging revealed that PEF1 and ANX14 accumulate at the fusion point upon fusion-induced lysis. Since membrane damage can also be induced by membrane-targeting chemicals, we tested the subcellular dynamics of all three proteins in response to such compounds, including the anti-fungal drug nystatin and the plant defense compound α-tomatine. PEF1 and ANX14 are recruited to the plasma membrane in response to α-tomatine, while only PEF1 is responding to nystatin. ANXC4 is not responding to any of these compounds. Interestingly, however, ANXC4 shows robust membrane recruitment in a Δ*anx14* mutant background, suggesting a kind of backup function, when the regular repair machinery is defective.
Deletion of either *anx14* or *anxc4* resulted in increased lysis rates of fusing germling pairs compared to the wild type or the Δ*pef1* mutant. Lysis further increased by 2-fold in a Δ*anx14* Δ*pef1* double mutant, suggesting that PEF1 and ANX14 function independently. There is, however, no increase in the Δ*anx14* Δ*anxc4* mutant, indicating that ANX14 and ANXC4 function in the same pathway. When grown on α-tomatine, Δ*pef1* is more sensitive to the toxin than Δ*anx14* or Δ*anxc4*. Taken together, these observations suggest that the annexins are more important for the repair of mechanically-induced damage, while PEF1 is mainly involved in the response to membrane-targeting drugs. Based on these observations, we hypothesize that membrane repair constitutes the first step of resistance against membrane damaging compounds. Future studies aim at revealing and fully characterizing the different membrane repair mechanisms in *N. crassa*.

421V Characterization of Bud3 domains sufficient for bud neck targeting in *S. cerevisiae* Madison Schrock[1,2], *Yao Yan*[1], Megan Goeckel[1,3], Erianna Basgall[1,4], Isabel Lewis[1,5], Katherine Leonard[1,6], Megan Halloran[1,7], Gregory Finnigan[1] 1) Kansas State University, Manhattan, KS; 2) University of Utah, Salt Lake City, UT; 3) Washington University in St. Louis, School of Medicine, St. Louis, MO; 4) University of Utah, Salt Lake City, UT; 5) University of Texas Medical Branch, Galveston, TX; 6) Memorial Sloan Kettering Cancer Center, New York, NY; 7) University of Kentucky, Lexington, KY.

The cytoskeleton serves a diverse set of functions in both multi- and unicellular organisms including movement, transport, morphology, cell division, and cell signaling. The septin family of cytoskeletal proteins are found within all fungi and metazoans and can generate three-dimensional scaffolds *in vivo* that promote membrane curvature, serve as physical barriers, and coordinate cell cycle checkpoints. In budding yeast, the septins organize into polymerized filaments that decorate the division site between mother and daughter cells during mitosis; assembly of this structure at the “bud neck” is critical for completion of cytokinesis and execution of numerous other cellular events. One such pathway includes bud site selection and the recruitment of proteins such as Bud4 and Bud3 that are responsible for promoting an axial budding pattern in haploid yeast. While Bud4 appears to be recruited to the septins independent of the presence of Bud3, it is likely that Bud3 can localize to the bud neck using both Bud4-dependent and Bud4-independent mechanisms. Furthermore, it remains unclear the precise domain(s) within Bud3 that are both necessary and sufficient for optimal association at the septin structure. In this study, we examined the localization of GFP-Bud3 constructs in otherwise WT haploid yeast cells expressing Cdc10-mCherry using fluorescence microscopy; we tested a collection of N- and C-terminal truncations and fusions of separate Bud3 protein elements to identify the smallest domain(s) responsible for bud neck localization. We found that

the coordinate action of the central amphipathic helix (residues 847-865) and a partially conserved C-terminal motif (residues 1172-1273) was sufficient to promote bud neck recruitment in the presence of endogenous Bud3. This domain is considerably smaller than the previously characterized C-terminal portion required to physically interact with Bud4 (1221-1636) and utilizes a similar mechanism of pairing membrane association with a separate localization domain similar to other non-septin proteins targeted to the division site during cell division.

422V Tools for *Knufia petricola*: new techniques for CRISPR/Cas9-based genome editing *Eileen Erdmann* [1,2], Sarah Nitsche[1,2], Anna Gorbushina[1,2], Julia Schumacher[1,2] 1) Bundesanstalt für Materialforschung und -prüfung (BAM), Berlin, Germany; 2) Freie Universität Berlin, Germany.

Black microcolonial fungi represent a group of ascomycetes with similar adaptations for existing in natural and anthropogenically created extreme habitats. They exhibit slow yeast-like or meristematic growth, do not form specialized reproduction structures and accumulate the black pigment 1,8-dihydroxynaphthalene (DHN) in the multilayered cell walls. We chose the rock inhabitant *Knufia petricola* of the Chaetothyriales as a representative for developing methods for genetic manipulation, simulation of mineral weathering and study of symbiotic interactions. Here, we report on the expansion of the genetic toolkit by more efficient multiplex CRISPR/Cas9 using a plasmid-based system for expression of Cas9 and multiple sgRNAs and three additional resistance selection markers. The targeted integration of expression constructs by replacement of essential genes for pigment synthesis allows for an additional color screening of the transformants. The black-pink screening due to the elimination of *pks1* (melanin) was applied for promoter studies using GFP fluorescence as reporter, while the black-white screening due to the concurrent elimination of *pks1* (melanin) and *phs1* (carotenoids) was used to identify transformants that contain the two expression constructs for co-localization or bimolecular fluorescence complementation (BiFC) studies. In addition, two intergenic regions (*igr1*, *igr2*) were identified in which expression constructs can be inserted without causing obvious phenotypes. Plasmids of the pNXR-XXX series (Schumacher, 2012) and new compatible entry plasmids were used for fast and easy generation of expression constructs and are suitable for use in other fungal systems as well.

423V *Schizophyllum commune* in radioactive/metal contaminated environments *Erika Kothe*[1], Lea Traxler[1], Katrin Krause[1] 1) FSU.

Radioactive contamination resulting from major nuclear accidents presents harsh environmental conditions. Inside the Chernobyl exclusion zone, the model basidiomycete *Schizophyllum commune* was inoculated and survived for over a year in soil as well as grew at least 1m in the soil at a spread rate of approx. 8 mm per day in the contaminated soil. Metal tolerance and hyphal transport of metals could be shown, and adaptation towards Sr - without changes in cell or colony morphology – allowed for gene expression profiles under metal stress growing on soil versus artificial medium. A higher impact of a structured surface for growth on soil than with different metal concentrations was observed. The adapted strain revealed reduced stress response and metal transport along hyphae was shown. For intracellular signaling, inositol monophosphatase *imp1* overexpression verified its involvement in intracellular trafficking and metal tolerance, associated with induced expression of kinases and repression of phosphatases within the inositol cycle. The presence of the heavy metals Sr, Cs, Cd, and Zn lowered intracellular calcium levels. We could develop a model integrating inositol signaling in the known signal transduction pathways governed by Ras, G-protein coupled receptors, and cAMP, and elucidate their different roles in development.

424V BRO1 localizes to a specific subpopulation of vesicular structures which mediate cell-cell fusion in *N. crassa.* *Hamzeh Haj Hammadeh*[1], Ulrike Brandt [1], André Fleißner[1] 1) Technische Universität Braunschweig.

Colony initiation of filamentous fungi commonly involves fusion of germinating vegetative spores. Studies in *Neurospora crassa* revealed an unusual cell-cell communication mechanism mediating this process, in which the fusion partners coordinately alternate between two physiological stages, probably related to signal sending and receiving. This “cell dialog” involves the alternating, oscillatory recruitment of the SO protein and the MAK-2 MAP kinase module to the apical plasma membrane of growing fusion tips.
We recently identified BRO1, the homolog of the mammalian *ALG-2-interacting protein X* (ALIX), as a new factor essential for germling interaction and fusion. In *N.crassa,* BRO1 is essential. Subcellular localization and live cell imaging revealed that BRO1-GFP localizes to the cytoplasm and to vesicular structures in non-interacting germlings and in mature hyphae. BRO1-GFP accumulates at the tips of interacting germlings in a dynamic, oscillating manner, such that high signal intensity of BRO1-GFP in one tip correlates with low signal intensity at the tip of the fusion partner. The co-localization of BRO1-GFP and different vesicles markers indicated that BRO1 marks a sub-population of vesicles, which specifically accumulate at cell fusion sites. Downregulation of *bro1* partially relieves the phenotype of the plasma membrane *Prm1* fusion mutant, in which the membranes frequently fail to merge, and aberrant engagement of the fusion machinery results in cell lysis. When *bro1* is partially repressed, membrane fusion is still blocked but cell lysis is absent. We therefore hypothesize, that BRO1 might be involved in transporting the fusion machinery to the plasma membrane of fusing cell tips. While studying BRO1 dynamics, we identified a new mode of hyphal fusion, which takes place at randomly occurring hyphal contact sites. We hypothesize that the BRO1 carrying vesicles represent the earlier reported Spitzenkörper-like structures, that accumulate at hyphal fusion sites.
Together these data indicate that BRO1 plays distinct roles in cell-cell communication and plasma membrane fusion. Future analysis of its molecular function will greatly contribute to our understanding of the unique “cell dialog” mechanism, the molecular bases of fungal cellular communication, and membrane fusion.

425V COT-1 kinase activity is required for proper conidial germination and directed hyphal growth in *Neurospora crassa* *Lucas Well*[1], Oded Yarden[2], Ulrike Brandt[1], André Fleissner[1] 1) Technische Universitaet Braunschweig; 2) The Hebrew University of Jerusalem.

Cot-1 (Colonial Temperature sensitive 1) was one of the first temperature sensitive mutants found in an early forward genetics mutant screen of *Neurospora crassa*. When grown at restrictive temperatures, the mutant loses the activity of the serine/threonine protein kinase COT-1, which results in a hyperbranching dendritic-spine like phenotype. Besides this severe polarity defect, it also generates more septa and thicker cell walls compared to the wildtype. To investigate the kinase COT-1 via live cell imaging we employed a

chemical genetics approach. By exchanging a single amino acid residue of the ATP-binding pocket, the kinase is rendered sensitive to an ATP-analog. Addition of this inhibitor inactivates the kinase within minutes, thereby allowing its functional characterization at specific growth stages. Inhibition of the analog-sensitive COT-1 variant in germinating conidiospores results in a cell polarity defect, characterized by the formation of numerous germtubes and a swollen spore body. In the wild type, germinating conidia usually undergo cell-cell interactions, in order to form a supracellular network. When COT-1 is inhibited, these germling interactions are highly reduced and occur only when the cells are in close proximity.
In interacting wild-type cells, the proteins MAK-2 and SO accumulate at the growing tips in an oscillating antiphase manner, suggesting a dialog-like cellular communication. When COT-1 is inhibited, both proteins permanently accumulate at the growing tips at the same time, which indicates that the cell-cell communication is corrupted. The unusual tip accumulation of the two proteins also occurs in all branches of the *Cot-1* specific hyperbranching hyphae, indicating a relationship between the observed polarity defect and the altered subcellular protein dynamics.

426V The dual roles of Pef1, a penta-EF-hand protein, in plasma membrane integrity and polarized growth in *Candida albicans* *Martin Weichert*[1], Marcel René Schumann[1], Ulrike Brandt[1], Alexandra C. Brand[2], André Fleißner[1] 1) Institute of Genetics, University of Braunschweig, Braunschweig, Germany; 2) Medical Research Council Centre for Medical Mycology (MRC CMM), University of Exeter, Exeter, United Kingdom.

Many naturally occurring or clinically applied antifungal compounds, such as polyenes and plant metabolites, directly disrupt the plasma membrane of fungi by binding to ergosterol and forming membrane pores. We have previously shown that in the saprophytic mold and model organism, *Neurospora crassa*, maintaining membrane integrity in the presence of pore-forming compounds involves the rapid recruitment of the cytosolic Ca^{2+}-binding penta-EF-hand protein, PEF1, to sites of plasma membrane damage. While the mammalian ortholog, ALG2, initiates the Ca^{2+} influx-triggered assembly of a membrane repair machinery, our understanding of the instant response of fungal cells to acute types of membrane disruption remains limited.

Membrane integrity is also crucial for pathogenic fungi during attack by immune cells and antifungal agents. Here, we are using the polymorphic fungus, *Candida albicans*, which is a leading cause of mycosis in humans, to test the hypothesis that the instant cellular response to pore-forming substances is conserved in distantly related fungal species. Consistent with our results in *N. crassa*, loss of the Pef1 protein in *C. albicans* caused strong susceptibility to the saponin, tomatine, but not to the polyenes, nystatin and amphotericin B. In cells treated with these pore-forming compounds, functional GFP-tagging of Pef1 in the *pef1*Δ/Δ mutant revealed that the protein quickly accumulated at the plasma membrane and also in puncta at intracellular locations. However, in the absence of membrane stress, GFP-Pef1 primarily localized at sites of cell polarity during yeast and hyphal growth. The *pef1*Δ/Δ mutant showed incomplete separation of daughter yeast cells and a delay in hypha formation, further indicating a role of Pef1 during polarized growth in *C. albicans*. To decipher the molecular mechanisms associated with Pef1, we are currently exploring the contribution of cytosolic Ca^{2+} influx as well as factors involved in Ca^{2+} homeostasis and Ca^{2+}/calcineurin signaling to the function of Pef1 in polarized growth and membrane integrity.

Taken together, these studies indicate that a conserved PEF hand protein is involved in the rapid response of fungi to acute plasma membrane damage. In *C. albicans*, Pef1 exerts dual roles during membrane repair and polarized growth, suggesting that its underlying function in maintaining membrane integrity is common to both processes in this pathogenic fungus.

427V The role of the *A. niger* antimicrobial peptide AnAFP in the cell's homeostasis of life and death *Stephan Starke*[1], Sascha Jung[1], Vera Meyer[1] 1) TU Berlin, Applied Molecular Microbiology.

Antimicrobial peptides, such as AnAFP from *Aspergillus niger*, are used by various organisms to fight off predators, invaders, or microbial competitors and can be found virtually in all domains of life. Yet, recent insights into the expression profile of AnAFP point towards a second, endogenous function.
For example, *anafp* is expressed in axenic cultures and only for a short period of time that coincides with carbon depletion, where it parallels the expression of genes predicted to function in autophagy, nutrient recycling, and development. We therefore proposed earlier that AnAFP could act as a cannibal toxin that drives development and programmed cell death. It kills a subpopulation of *A. niger* cells to support the survival of another subpopulation, to ultimately secure the survival of the species.
To verify this hypothesis, we put *anafp* expression under the control of the doxycycline-dependent Tet-On gene switch. Indeed, reduced growth of *A. niger* was measured during cultivation in presence of doxycycline, in either shake flask or on solid agar media, when *anafp* expression was induced prematurely, i.e. before carbon depletion.
To determine whether the reduced growth is the result of an enhanced regulated cell death, *A. niger* mycelia expressing *anafp*, were monitored regarding autophagy and apoptosis, using confocal fluorescence microscopy, qRT-PCR, and co-immunoprecipitation. Corresponding data will be presented.

428V Swe1 homologs in *Cryptococcus neoformans*: Roles in stress response, virulence, and the G_2/M Checkpoint *Rodney Colón-Reyes*[1], Jin-Tae Choi[2], Seung-Heon Lee[2], Nicole Bodner[3], Srikripa Chandrasekaran[3], Yong-Sun Bahn[2], Lukasz Kozubowski[1] 1) 1: Department of Genetics and Biochemistry, Clemson University, SC USA; 2) Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, Seoul, Republic of Korea; 3) Department of Biology, Furman University, Greenville, SC USA.

Wee1 kinase is a conserved cell cycle regulator acting in cell cycle checkpoints in fungi and animals. In *Saccharomyces cerevisiae*, Wee1 homologue, Swe1 kinase phosphorylates conserved Y-19 residue on cyclin dependent kinase (CDK) Cdc28 and enforces a pre-mitotic delay in response to defects in cellular morphogenesis. In contrast, in vertebrates, Wee1 homologues phosphorylate CDK tyrosine and negatively regulate the S and the M phase and act in cell-cycle-checkpoints in response to genotoxic stress. Here we characterize the two Wee1 homologs, CnSwe1 and CnSwe102, present in the genome of the human fungal pathogen *Cryptococcus neoformans*. Single deletion strains *swe1*Δ and *swe102*Δ were generated and a strain that harbors *swe102*Δ deletion and expresses *SWE1* from a promoter suppressed by copper was also constructed. These mutant strains were subjected to various DNA

damaging agents and stressors such as osmotic and ER stress. Strikingly, cells lacking CnSwe102 and expressing CnSwe1 at reduced levels were significantly more sensitive to hydroxyurea and MMS, agents that interfere with replication. CnSwe1 and CnSwe102 were tagged with a fluorescent tag, mCherry and used to complement the single deletions and to elucidate their subcellular localization. Both proteins exhibited an overall diffuse localization throughout the cell. Both deletion strains exhibited slower growth at 30 and 39˚C as compared to the WT. Consequently, the *swe102*Δ strain was less virulent than the wild type in animal infection models. Heterologous expression of CnSwe1 or CnSwe102 in the *Saccharomyces cerevisiae swe1*Δ mutant has indicated that both *C. neoformans* homologues phosphorylate the conserved Y-19 residue on Cdc28. Overexpression of CnSwe1 or CnSwe102 in *S. cerevisiae* resulted in a distinct elongated shape of the yeast cells, indicative of the morphogenesis checkpoint. Our data suggest that the two *C. neoformans* Wee1 homologues play partially redundant roles in regulation of cell cycle progression in response to replication stress and are together essential for growth.

429V Unraveling the Essential Transcription Factors in *Cryptococcus neoformans* *Seung-Heon Lee*[1], Kyung-Tae Lee[1], Yu-Byeong Jang[1], Jin-Tae Choi[1], Jae-Won La[1], Alexander Idnurm[2], Yong-Sun Bahn[1] 1) Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, Seoul, Republic of Korea; 2) School of BioSciences, The University of Melbourne, Australia.

Cryptococcus neoformans is the fungal pathogen that causes cryptococcosis in both immunocompromised and healthy individuals, and is one of the leading causes of death among HIV patients. However, antifungal drugs for cryptococcosis are facing limitations because of limited in vivo efficacy, the emergence of resistant strains, and toxic side effects. Therefore, developing antifungal drugs satisfying those unmet clinical needs is crucial. To this end, essential fungal proteins can be notable as they are directly required for growth and therefore considered as fungicidal targets. Essential transcription factors (TFs) are particularly valuable because TFs are evolutionarily divergent among eukaryotes and therefore we can avoid toxicity issues. In this study, we aim to systematically identify essential TFs of *C. neoformans* and characterize their pathobiological roles. Our previous research revealed that 23 genes could be putative essential TFs in *C. neoformans* because they cannot be knocked out even after repeated attempts. Besides Hsf1, which we reported to be essential, here we constructed conditional expression strains for the remaining 22 TFs by replacing their native promoters with the copper-regulated CTR4 promoter. Under repressive condition, conditional expression strains of 12 TFs showed growth defect, implying that these TFs are at least required for the growth of *C. neoformans.* To further verify their essentiality for the viability of the fungus, we constructed heterozygous mutants of the 22 TFs with engineered diploid strain AI187 using the nourseothricin resistance marker (NAT), and are currently performing spore analysis. After harvesting basidiospores from heterozygous mutant, we analyze the genotypes of haploid progenies. If we could get spores with nourseothricin resistance, we would classify the target genes as non-essential and uncover their roles with null mutants. On the other hand, if we were unable to acquire spores with the drug resistance, we would regard the target genes as essential and study their functions with constitutive overexpression strains. So far, 4 TFs are found to be essential, and 3 TFs are turned out to be non-essential for the viability of the fungus. By discovering essential TFs and their traits through this study, we are expecting not only to broaden our understanding of transcription factor networks in *C. neoformans*, but also to suggest potential targets for developing next-generation antifungal agents.

430V GAG, a polysaccharide cytotoxin? *Caitlin Zacharias*[1], Fabrice Gravelat[1], Francois Le Mauff[1], Hong Liu[2], Scott Filler[2,3], Donald Sheppard[1,4] 1) Infectious Diseases in Global Health Program, Research Institute of the McGill University Health Centre, Montreal, QC, Canada; 2) Division of Infectious Diseases, Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, CA, United States of America; 3) David Geffen School of Medicine at UCLA, Los Angeles, CA, United States of America; 4) Departments of Medicine, Microbiology, and Immunology, McGill University, Montreal, QC, Canada.

Aspergillus fumigatus is a ubiquitous filamentous mold that causes necrotizing pneumonia in immunosuppressed individuals. One of the virulence factors of *A. fumigatus* is the synthesis of the adhesive, cationic exopolysaccharide GAG, which is mediated by the products of a five gene cluster. One of these genes, *ega3* encodes a glycosyl hydrolase that is specific for deacetylated GAG that is anchored to the cell membrane of *A. fumigatus*. We hypothesized that Ega3 is necessary for GAG synthesis, and to test this hypothesis, we sought to disrupt *ega3* in *A. fumigatus.*
Multiple attempts to disrupt *ega3* by conventional methods were unsuccessful. Switching to CRISPR/Cas9 generated a single Δ*ega3* clone. As with other mutations in the GAG cluster, this mutant did not produce deacetylated GAG. Complementation with an *ega3* allele failed to restore deacetylated GAG production, suggesting the presence of a secondary mutation in this strain. Analysis of the expression of the rest of the GAG cluster genes revealed that *agd3*, encoding the GAG deacetylase, was not expressed in the Δ*ega3* mutant. We therefore hypothesized that *ega3* is conditionally essential in the presence of deacetylated GAG production. To test this, *agd3* was expressed in the *ega3* null mutant under the control of a tetracycline-inducible promotor (Δ*ega3*-Tet ON-*agd3*). Under *agd3*-expressing conditions, GAG production was restored, however fungal growth was inhibited. We hypothesized that GAG may be toxic to the cell membrane of *A. fumigatus* and that Ega3 is necessary for degrading GAG near the membrane. To test this, ATP was measured in fungal culture supernatants as a proxy for cell leakage. Induction of *agd3* expression in the Δ*ega3*-Tet ON-*agd3* mutant causes increased ATP release, suggesting that deacetylated GAG disrupts the fungal cell membrane.
Since GAG causes membrane permeability in Δ*ega3*, we hypothesized that it may cause host cell injury. This was tested in a cell damage assay where epithelial cells were loaded with radioactive chromium and exposed to culture supernatants of wild-type *A. fumigatus* with and without recombinant Ega3. *A. f umigatus*-induced epithelial cell damage was nearly abolished in the presence of Ega3. Similar findings were observed using propidium iodide staining of bone marrow-derived macrophages exposed to GAG with or without Ega3. This data suggests that cationic GAG is cytotoxic to both fungal and host cells, and that Ega3 can serve as an antitoxin to mitigate these effects.

431V Understanding the impact of the combination of *hapE* and *hmg1* mutations in *A. fumigatus* clinical triazole drug resistance *Ana C O Souza*[1], Jeffrey M Rybak[1], Wenbo Ge[2], Jarrod R Fortwendel[2], P. David Rogers[1] 1) St Jude Children›s Research Hospital; 2) University of Tennessee Health Science Center.

Aspergillus fumigatus is a ubiquitous environmental mold than can cause severe disease in immunocompromised patients, as well as

chronic disease in individuals with underlying lung conditions. Triazole antifungals are the first-line therapy for *A. fumigatus* infections, but unfortunately resistant isolates have been increasingly reported worldwide, threatening the clinical use of these antifungals and reinforcing the need for a better understanding of resistance mechanisms. In this study, we investigated a pan-azole resistant clinical isolate (DI15-105) with mutations in *hapE* and *hmg1*. Both *hapE*^{P88L} and *hmg1*^{F262del} have been previously shown to individually contribute to triazole resistance, although this is the first report of both mutations simultaneously present in a clinical isolate. Using *in vitro* assembled CRISPR/Cas9-mediated gene editing, we performed allele swaps and either corrected or inserted *hapE* and *hmg1* mutations in the clinical isolate DI15-105 or Cea10, respectively. Antifungal susceptibility testing according to CLSI guidelines was performed to determine the Minimum Inhibitory Concentration (MIC) to Voriconazole (VRC), Isavuconazole (ISA), Itraconazole (ITR) and Posaconazole (POS). Correction of the *hapE* mutation in DI15-105 decreased MIC for VRC, ISA and ITRA, while insertion of the *hapE*^{P88L} mutation increased MIC of all triazoles. On the other hand, correction of the *hmg1* mutation in DI15-105 decreased MIC for all triazoles, whereas insertion of the *hmg1*^{F262del} mutation in Cea10 increased MIC. Correction of both genes intensified recovery of triazole susceptibility in DI15-105, reducing the MIC for all azole drugs, while double insertion of *hapE*^{P88L} and *hmg1*^{F262del} in Cea10 increased MIC in comparison to single mutants. Radial growth analysis revealed that *hapE* gene correction increased growth of DI15-105, whereas insertion of the *hmg1*^{F262del} mutation in Cea10 generated a significant growth impairment that was enhanced by the presence of the *hapE*^{P88L} mutation in the double-mutant strain. Besides, presence of the *hapE*^{P88L} mutation in the Cea10 background led to increased sensitivity of either the single or double mutants to the cell wall disrupting agent Calcofluor White. Our results suggest that the combination of both *hapE*^{P88L} and *hmg1*^{F262del} mutations have a synergistic impact on triazole resistance through a mechanism that remains to be defined.

432V Establishing minimal conditions sufficient for titanization in *Cryptococcus neoformans*/*gattii* species complex *Mariusz Dylag*[1,2], Rodney Colon-Reyes[1], Lukasz Kozubowski[1] 1) Clemson University, Clemson, SC, USA; 2) University of Wroclaw, Wroclaw, Poland.

Opportunistic pathogens of the *Cryptococcus neoformans*/*gattii* species complex are characterized by unique virulence factors that enabled evolutionary adaptation to pathogenesis. Among the factors contributing to cryptococcosis is a morphological transformation into giant (Titan) cells. Recently established *in vitro* protocols demonstrate that 5 or 10% fetal bovine serum combined with 5% CO_2, 37°C and sufficiently low cell density triggers titanization in cells that are obtained from stationary-phase culture (Serum protocols). However, the exact contribution of serum components in Serum protocols remains incompletely characterized. In search for minimal conditions necessary for triggering titanization, we performed a study where we eliminated serum from the protocol (Serum-free protocol) and instead systematically adjusted amount of glucose, source of nitrogen (ammonium sulfate) and the pH. We found that exposing stationary-phase cells to neutral pH and 0.05% of glucose, 0.025% ammonium sulfate in the presence of 5% CO_2 and 30 or 37°C triggers Titan cell formation to the same degree as previously established protocol that utilized 10% serum as the sole nutrient source. Titans obtained from the Serum-free protocol were characterized by cell body size over 10 microns, a single enlarged vacuole, thick cell wall, extensive polysaccharide capsule, and increased ploidy, all classic hallmarks of titanization. Strikingly, in both, Serum and Serum-free protocols, relatively acidic pH (5.5) prevented titanization and promoted robust proliferation, whereas alkaline pH (~8.0) had a profound growth inhibitory effect and resulted in cell death when cells were incubated in the absence of 5% CO_2. Our study suggests that the re-entry to the cell cycle under conditions that allow only restricted proliferation in the presence of 5% CO_2 is sufficient for Titan cell formation in *Cryptococcus neoformans*/*gattii* species complex.

433V Unraveling the biology of Nematophagy During a Fungal-Nematode Predator-Prey Interaction Using Time-Course Transcriptomic analysis *Hung-Che Lin*[1], Guillermo Vidal-Diez de Ulzurrun[1], Sheng-An Chen [1], Ching-Ting Yang[1], Pedro Gonçalves [1], Chih-Yen Kuo [1], Tsung-Yu Huang[1], Erich Schwarz [2], Yen-Ping Hsueh [1] 1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; 2) Cornell University, Ithaca, NY.

Nutritional deprivation triggers a saprotrophic to predatory lifestyle switch in soil dwelling nematode-trapping fungi (NTF). In particular, *Arthrobotrys oligospora* has evolved to secrete food and sex cues to lure their prey – Nematoda animals – into an adhesive network of traps, specialized structures that originate from the vegetative mycelium. Upon capture, the nematodes are invaded and digested by the fungus, thus serving as a food source. We employed RNA-sequencing to examine the response of *A. oligospora* upon exposure to the model nematode *Caenorhabditis elegans*. A dynamic transcriptomic reaction that indicated a strong reliance on protein secretion was observed. A comprehensive prediction of the secretome of *A. oligospora* resulted in 1084 transcripts, 64% of which are upregulated in the presence of *C. elegans* at all tested time points. We found a large number of genes related to ribosome biogenesis induced at early time point, 2hr-post *C. elegans* exposure, suggesting that the TOR signaling pathway might be critical for sensing the presence of nematodes. Rapamycin treatment inhibited both trap development and function. Moreover, a plasma membrane t-SNARE protein, SSO2, involved in membrane fusion of secretory vesicles, plays a major role in nematode-adhesion. We subsequently predicted the putative effectors of *A. oligospora* and found that they represent approximately 19% of the secretome and that their expression peaked after 10 hours of introduction of nematodes. Specifically, we found that genes of the Egh16 family were highly upregulated upon nematode exposure. *In situ* hybridization reveals the accumulation of the top three highly expressed *Egh16* transcripts in the traps cell. Thus, we named these gene family as Trap-enriched Secreted Protein (TSP). Gene deletion of the highest expressed gene *TSP1* impairs the function of trap. Lastly, Egh16 gene family is highly expanded in the genomes of several nematode-trapping fungi, suggesting that this gene family may have a critical role for the evolution of the predatory life style in Ascomycetes.

434V A model of the bud emergence 46 (BEM46) protein mode of action based on transcriptomics data in *Neurospora crassa* *Krisztina Kollath-Leiss*[1], Hossein Emami[2] 1) Christian-Albrechts-Universität zu Kiel; 2) UKSH Lübeck.

Despite the ubiquitous presence of BEM 46 homologs in eukaryotic organisms [1], the function of BEM46 is still not completely understood. To gain an improved understanding of the role of BEM46 in *N. crassa*, a transcriptomic study of different developmental stages in different bem46 backgrounds was performed. Our data approve the distinct developmental mode of action of BEM46 in *N. crassa*. Most differentially expressed genes were detected in germinating conidiospores in bem46-overexpressing background, which corresponds with the significant phenotype of these transformants showing delayed germination and reduced growth [2,3]. GO analysis on the differen-

tially expressed genes showed an enrichment of membrane components, signaling- and phosphorylation-associated, auxin biosynthetic and transporter genes. Our results suggest defective eisosome formation in bem46 overexpressing conidiospores, which could affect the positioning of integral membrane proteins. Additionally, based on the relationship of BEM46 to auxin biosynthesis, a model can be established, in which both BEM46 and auxin can influence plasma membrane subdomain structuring, providing a platform for signaling proteins.

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435V Uniparental nuclear inheritance following bisexual mating in fungi *Vikas Yadav*[1], Sheng Sun[1], Joseph Heitman[1] 1) Duke University Medical Center, Durham, NC.

Some remarkable animal species require an opposite-sex partner for their sexual development but discard the partner's genome before gamete formation, generating hemi-clonal progeny in a process called hybridogenesis. Here, we discovered a similar phenomenon, termed pseudosexual reproduction, in a basidiomycete human fungal pathogen, *Cryptococcus neoformans*, where exclusive uniparental inheritance of nuclear genetic material was observed during bisexual reproduction. Analysis of strains expressing fluorescent reporter proteins revealed instances where only one of the parental nuclei was present in the terminal sporulating basidium. Whole-genome sequencing revealed the nuclear genome of the progeny was identical with one or the other parental genome. Pseudosexual reproduction was also detected in natural isolate crosses where it resulted in mainly *MAT*a progeny, a bias observed in *Cryptococcus* ecological distribution as well. The mitochondria in these progeny were inherited from the *MAT***a** parent, resulting in nuclear-mitochondrial genome exchange. The meiotic recombinase Dmc1 was found to be critical for pseudosexual reproduction. These findings reveal a novel, and potentially ecologically significant, mode of eukaryotic microbial reproduction that shares features with hybridogenesis in animals.

436V Role of microtubules and actin in the intracellular organization in the entomopathogenic fungus *Metarhizium brunneum* *Olga A. Callejas-Negrete*[1], Alejandra I. Hernández-Saiz[1], Abraham A. Gasca-Venegas[1], Gloria A. González-Hernández[2], Juan C. Torres-Guzmán[2], Rosa R. Mouriño-Pérez[1] 1) CICESE; 2) Universidad de Guanajuato.

Metarhizium genus is an important group of filamentous fungi employed for pest control due to its virulent and parasitic ability in more than 200 different insect species. Recently, *M. robertsii* has been reported to be rhizosphere-competent stimulating plant root development. While interacting with either insects or plants, the fungus must defy different barriers and toxic compounds, which trigger events of cell differentiation and organelle-transport to achieve homeostasis. In fungi, cell polarity is an essential process for proper growth and morphogenesis. Microtubules and the actin cytoskeleton are essential to maintain cell shape and intracellular transport and organelle organization (vesicles, mitochondria, peroxisomes, lipid droplets, etc.). To describe the role of the actin and microtubular cytoskeleton in the intracellular organization of *M. brunneum*, we used the anti-microtubules drug benomyl (BML) and the anti-actin drug latrunculin B (Lat B). Peroxisomes labeled with the protein KAT-GFP, were observed as bright fluorescent spots evenly distributed along the hypha. Peroxisomes moved in anterograde and retrograde fashion at different speeds. Exposure to Lat B did not affect peroxisomes movement but their distribution was abnormal. In the presence of BML, peroxisomes stop moving and their dynamics was different to the WT. Lipid droplets stained with BODYPI were observed as fluorescent particles localized throughout the hypha with slow anterograde and retrograde motion. In cells treated with BML, lipid droplets remained static and started to accumulate throughout the hypha. The Spitzenkörper (Spk) labeled with FM4-64 was localized in the apical dome moving in the growth direction; with BML treatment SPK was smaller. Mitochondria accumulated in the subapex, and their dynamics and positioning were independent of microtubules. Cell wall stained with calcofluor white was thicker in the hyphal tip when compared to the subapical and basal regions. Nevertheless, during exposure to BML, the cell wall was thinner in the apical dome. We concluded that microtubules are involved in chitosomes, peroxisomes and lipid droplets transport but not in mitochondria position, and actin is necessary for peroxisomes positioning.

437V From a Cap to a Collar, ontogeny of the subapical endocytic collar in filamentous fungi *Rosa R. Mouriño-Pérez*[1], Marisela Garduño-Rosales[1], Salomón Bartnicki-García[1] 1) CICESE.

Hyphal morphogenesis depends mainly on the establishment and maintenance of polarized growth. This is accomplished by the orderly migration and discharge of exocytic vesicles carrying cell membrane and wall components. We have been searching for evidence that endocytosis, an opposite process, could also play a role in hyphal growth, polarity maintenance, and conidial germination. We analyzed proteins involved in the different stages of endocytic vesicles formation (AP180, SLA1, LAS17, MYO-1, ARP2/3, FIM-1, CRN-1) and their respective deletion mutants during the various stages of development in the filamentous fungus *Neurospora crassa*. We found that patches labeled with endocytic reporters accumulate in the apex of the germinating tube of conidia forming a cap. This position is maintained until the germ tube reaches about 150 microns thereafter patches begin to form a collar in the subapex, a conspicuous localization maintained in mature hyphae. The presence of the endocytic collar coincides with the changes in growth speed from a germling to a mature hypha and the establishment of the polarize growth apparatus.

438V Investigating the cell biology of plant infection by the rice blast fungus *Magnaporthe oryzae* *Berlaine Quime*[1], Xia Yan[1], Vincent Were[1], Lauren Ryder[1], Nicholas Talbot[1] 1) The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, Norwich, United Kingdom.

Rice blast disease is one of the most important limiting factors in rice production. The disease is caused by the filamentous ascomycete rice blast fungus, *Magnaporthe oryzae.* Blast infection starts when a three-celled conidium lands on the leaf surface, carried by wind or splash dispersal. To enter the rice leaf, the fungus forms a dome-shaped infection structure called an appressorium. The melanized appressorium develops a penetration peg, which differentiates into a narrow primary invasive hypha (IH) and subsequently into bulbous invasive hyphae that colonize the first host cell. Previous live-cell imaging studies have demonstrated that IH are surrounded by the

extra-invasive hyphal membrane (EIHM), an invagination of the host plasma membrane, which bounds IH as they proliferate in epidermal rice cells. We have generated a series of rice (cv Kitaake) and barley (cv Golden Promise) transgenic lines expressing green fluorescent protein (GFP)-labelled subcellular components, including the plasma membrane, endoplasmic reticulum (ER), Golgi, nuclei, chloroplast, F-actin, microtubules, early and late endosomes. Using these transgenic lines, we aim to characterize the temporal development of blast disease and create a spatial atlas of the major cellular changes associated with blast infection, in both compatible and incompatible interactions. We have shown that blast infection is associated with depolymerization of the F-actin cytoskeleton in host cells. We have also classified major changes in membrane organisation and integrity during invasive growth and formation of transpressoria, which are used to move between host cells. We will report the major plant cellular changes that accompany host tissue colonization by *M. oryzae.*

439V Chemical inhibition of nuclear division and migration during appressorium development in the rice blast fungus, *Magnaporthe oryzae* *Brandon Mangum*[1], Mariel Pfeifer[1], Chang Hyun Khang[1] 1) Department of Plant Biology, University of Georgia, Athens, GA.

To successfully nucleate appressoria in *Magnaporthe oryzae*, duplicated spindle pole bodies (SPB) must migrate to opposite poles of the nucleus to form a bipolar spindle, allowing for the separation of sister chromatids and the migration of the daughter nucleus to the appressorium. Kinesin-5 motor proteins have a canonical function of walking towards the plus-ends of microtubules and exerting an outward force on SPBs during mitosis. We used live-cell imaging of an *M. oryzae* strain with tdTomato-tagged histone H1 and GFP-tagged beta-tubulin together with an aminobenzothiazole (ABT) compound to investigate the role of kinesin-5 in bipolar spindle formation. ABT has been shown to inhibit kinesin-5 motor activity in *Candida albicans*. We found that ABT inhibited *M. oryzae* appressorium development in a dosage dependent manner. In a block and release experiment, we treated cells with ABT for ten minutes. We observed a failure in establishing a bipolar spindle. After rinsing, the cells recovered, and a bipolar spindle was able to form. Prolonged ABT treatment resulted in intriguing mitotic phenotypes. The nucleus could migrate into the germ tube with a monopolar spindle. Cells that escaped inhibition displayed any combination of variable nuclear movement, aberrant wave-like spindle structures, and a severe lagging chromosome phenotype that could leave chromosomes stranded in the germ tube. Our data suggests kinesin-5 plays a critical role in SPB separation and coordinated migration of chromosomes.

440V Identification and functional characterization of the putative cyclin FlpA as a regulator of the metula-to-phialide transition during conidiophore development of *Aspergillus nidulans* Ziortza Agirrezabala[1], Ainara Otamendi [1], Eduardo A. Espeso[2], *Oier Etxebeste*[1] 1) Lab. Biology, Dept. of Applied Chemistry, Faculty of Chemistry, University of the Basque Country; 2) Aspergillus Cell Biology group. Lab 247, Dept. of Cellular and Molecular Biology, Centro de Investigaciones Biológicas Margarita Salas CSIC.

The genus Aspergillus includes industrially, medically and agriculturally important species. All of them, as do fungi in general, disperse to new niches and hosts principally by means of asexual spores. When it comes to the study of the genetic/molecular mechanisms controlling asexual development, *Aspergillus nidulans* is the main reference. Two main pathways control in *A. nidulans* the production of conidiophores, asexual structures containing thousands of asexual spores known as conidia. The UDA pathway transduces environmental signals, determining whether the CDP pathway and thus the required morphological changes are induced. The transcriptional regulator BrlA links both pathways, while loss-of-function mutations in *flb* (UDA) genes block *brlA* transcription and, consequently, conidiation. However, the aconidial phenotype of some *flb* mutants can be reverted under salt-stress conditions. A library of Δ*flbB* mutants unable to conidiate on medium supplemented with NaH_2PO_4 (0.65M) (FLIP, *fluffy in phosphate* mutants) was generated by us. Mutants were grouped according to their phenotypical features. Here, we have identified a Gly347Stop mutation within *flpA* as responsible for the mutant phenotype FLIP57. Deletion of the gene causes a significant reduction in germination, radial extension, quantity of cleistothecia and conidia production. Functional characterization of the putative cyclin FlpA suggests that it is necessary in the transition from metulae to phialides during conidiophore development. The ortholog of FlpA in *Schizosaccharomyces pombe* has been described to form a complex regulating RNA pol II activity together with orthologs of *A. nidulans* Stk47 and FlpB. Here, it is shown that the corresponding single-null mutants show the same phenotype, the three proteins are localized into nuclei and this localization is based on specific functional dependencies. Overall, this work adds new elements to the complex networks of proteins coordinating growth with sexual and asexual developmental programs.

441V A Tor1 N-terminal region required for *Candida albicans* anabolic- and stress regulation *Wanjun Qi*[1], Maikel Acosta-Zaldívar[1], Peter Flanagan[2,3], Ning-Ning Liu[1,4], Niketa Jani[1,5], José Fierro[6], María Andrés[6], Gary Moran[2], Julia Köhler[1] 1) Division of Infectious Diseases, Boston Children's Hospital/Harvard Medical School, Boston, MA; 2) Division of Oral Biosciences, School of Dental Science, Trinity College Dublin, Ireland; 3) Department of Clinical Microbiology, St. James's Hospital, Dublin, Ireland; 4) School of Public Health, Shanghai Jiao Tong University School of Medicine, Shanghai, China; 5) BioAgilytix, Boston, MA; 6) Laboratory of Oral Microbiology, University Clinic of Dentistry (CLUO), and Department of Functional Biology (Microbiology), Faculty of Medicine, University of Oviedo, Oviedo, Asturias, Spain.

For eukaryotic cells, Target of Rapamycin Complex 1 (TORC1) makes an essential decision to either direct cellular resources toward growth and proliferation in favorable conditions, or toward stress responses in adverse environments. Loss of TORC1 function is lethal. A TORC1 inhibitor like rapamycin could be a potent antifungal against *Candida albicans*, but this agent that targets the highly conserved Tor kinase domain, is also severely toxic to human cells.
The least conserved region of fungal and human Tor kinases are the N-terminal HEAT domains. We here examined the role of the 8 most N-terminal HEAT repeats of *C. albicans* Tor1 during nutritional- and stress responses. Using cells expressing N-terminally truncated Tor1 from repressible *tetO* (*tetO-TOR1ΔHEAT*), full-length Tor1 from *tetO (tetO-TOR1*) or wild type Tor1 from the native promoter, we found specific stress responses to be significantly impaired by loss of Tor1 N-terminal HEAT repeats, including those to oxidative-, cell wall-, and heat stress. Specifically, during oxidative stress, translation was inappropriately upregulated in *tetO-TOR1ΔHEAT* cells, while activation of the oxidative stress response MAP kinase Hog1 was weak. In contrast, plasma membrane stress and antifungal agents that disrupt plasma membrane function were tolerated by *tetO-TOR1ΔHEAT* cells.

Cells lacking N-terminal HEAT repeats were unable to take advantage of favorable nutritional conditions by accelerating their growth. Genome-wide expression analysis showed simultaneous activation of both anabolic- and starvation responses in *teTO-TOR1ΔHEAT* cells in the absence of stress, accompanied with mis-regulation of carbon metabolism and translational machinery biosynthesis. Current work comparing the responses of cells lacking this region of Tor1 to cells with constitutively active Tor1 kinase will distinguish phenotypes due to inappropriate Tor1 activation during stress, from lack-of-function phenotypes in these cells. Targeting fungal-specific Tor1 N-terminal HEAT repeats with small molecules might abrogate fungal viability, especially when during infection multiple stresses are imposed simultaneously by the host immune system.

442V Cell wall and turgor pressure affect the release of extracellular membrane vesicles in filamentous fungi Yuka Iwahashi[1], Riki Mikami[1], Shusaku Kanematsu[1], Norio Takeshita[1], Masanori Toyofuku[1], *Shyun-ichi Urayama*[1], Daisuke Hagiwara[1] 1) University of Tsukuba.

Extracellular membrane vesicles (eMV) are small, membrane-enclosed structures released from a cell into the surrounding environment. eMV contains cargo molecules such as nucleic acids, proteins and chemical compounds, and affects diverse biological processes, including virulence, horizontal gene transfer and cell-to-cell communication. Recent studies demonstrated that some filamentous fungi can produce biologically active eMV under lab culture conditions. However, a comprehensive understanding of eMV-producing ability and mechanism have not been established. In this study, we used several filamentous fungi such as Aspergillus species to identify eMV production during their cultivation. All fungi released eMV at the stationary phase, while few fungi released eMV at the log phase. In particular, *Aspergillus oryzae* released unstructured lipid fragments and eMV at the log and stationary phase, respectively. To identify the factors affecting lipid or eMV release in *A. oryzae*, we applied different culture conditions and used mutant strains. Under high osmotic pressure, the amount of released unstructured lipid fragments was reduced. The cell wall mutants released eMV instead of unstructured lipid fragments. These analyses suggested the significance of fungal cell wall and turgor pressure in eMV production. Microscopic observation also supported the release of eMV during cell growth and cell lysis (burst and autolysis). Our data suggested that most fungi release plasma membrane-derived eMV at cell lysis. This study provides a scientific base to understand the function and release mechanisms of fungal eMV.

443W Karyotype evolution via chromosome fusion-inversion in *Kwoniella,* the sister genus to *Cryptococcus* *Marcia David-Palma*[1], Marco A. Coelho[1], Minou Nowrousian[2], Terrance Shea[3], Sheng Sun[1], Joseph Heitman[1], Christina Cuomo[3] 1) Department of Molecular Genetics and Microbiology, Duke University, Durham NC, USA; 2) Lehrstuhl für Molekulare und Zelluläre Botanik, Ruhr-Universität, Bochum, Germany; 3) Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA.

Kwoniella is the sister genus to the *Cryptococcus* clade that includes human fungal pathogens such as *C. neoformans*. Chromosome-level genome assemblies were generated for 15 *Kwoniella* species, covering most of the known diversity in the genus. Genome analysis revealed that the chromosome number of *Kwoniella* species differs remarkably from species in the *Cryptococcus* genus. While most *Cryptococcus* species have 14 chromosomes, nearly all *Kwoniella* species have fewer, with some having as few as 3, with no substantial reduction in genome size (averaging 22.8 Mb). In the *Kwoniella* species with only 3 chromosomes, synteny analysis revealed their largest chromosome (~16-18 Mb) to be the product of fusion of 11 extant individual chromosomes present in two early branching *Kwoniella* species with 14 chromosomes. Each chromosome-chromosome fusion involved an inversion near a centromere, which may have inactivated one centromere at each fusion step, thus avoiding the generation of an unstable dicentric chromosome with two functional centromeres. While similar chromosome fusion-inversion patterns were observed in all *Kwoniella* species with less than 14 chromosomes, this was, however, absent in all 13 *Cryptococcus* species with chromosome-level genome assemblies.
To further corroborate these genome assemblies and explore this chromosome fusion-inversion hypothesis, we experimentally validated the centromeric regions of 3 representative *Kwoniella* species. This was done by expressing a mCherry-tagged allele of the kinetochore protein Cse4/CENP-A followed by chromatin immunoprecipitation sequencing (ChIP-seq). The results from ChIP-seq validated all the centromere regions that had been predicted in silico. Additionally, the genome-wide distribution patterns of the histone modification H3K9me2 assessed by ChIP-seq for 5 representative Kwoniella species showed to be largely coincident with the centromeric regions predicted in silico and validated by the Cse4/CENP-A occupancy. Chromosome conformation capture followed by high throughput sequencing was also performed for *Kwoniella* species with distinct karyotypes, further confirming the genome assemblies generated. Together, this robust genomic data set, combined with computational and experimental centromere validation, provides a foundation to elucidate mechanisms that drive centromere loss and chromosome-chromosome fusion, leading to remarkably distinct karyotypes in closely related fungal species.

444T Periodic DNA patterns associated with chromatin regulation in Fungi *Stephen Mondo*[1], Juna Lee[1], Guifen He[1], Hugh Salamon[1], Catherine Aime[2], Ronan O'Malley[1], Igor Grigoriev[1] 1) DOE Joint Genome Institute, Lawrence Berkeley National Lab, Berkeley, CA; 2) Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN.

Across Eukaryota, one of the most highly conserved and fundamental complexes is the nucleosome. Nucleosomes are the basic repeating units, each spanning ≈150bp, that structure DNA in the nucleus and precise positioning of these are critical for regulating gene activity. Consequently, a substantial number of epigenetic modifications in eukaryotes serve to move these complexes, making genes easier (or more difficult) to access by transcriptional machinery. However, previous studies in model eukaryotes have revealed the potential for DNA itself to play an important role in nucleosome organization, although its broader significance across taxa is currently poorly understood. Here, we discovered the presence of ≈150bp Periodic DNA Patterns (PDPs) across several diverse eukaryotes at key genomic positions, particularly surrounding transcriptional start sites and coding sequence start sites. PDPs were particularly abundant in the Basidiomycota and were enriched at highly conserved genes. Through *in vitro* and *in vivo* assays on multiple fungi we confirmed that the presence of these sequences is associated with increased nucleosome occupancy, particularly in the Basidiomycota, indicating an ancient contribution of DNA to organizing chromatin in this phylum. Occupied DNAs harbored AT-rich cores with peaks of GC ±37bp from nucleosome centers. This GC profile, coupled with local DNA structural features were the most important contributors to generation of nucleosome-favorable DNA landscapes. Using these features, we created a model for predicting *in vitro* nucleosome occupancy which showed agreement with both *in vitro* and *in vivo* occupied sites in the Basidiomycota phylum. Importantly, outside the

Basidiomycota, lineages without PDPs showed little agreement between predicted and actual nucleosome-bound sites, indicating that the contribution of DNA to nucleosome organization can vary widely across taxa. This analysis brings to light the potentially substantial role DNA sequence might play in nucleosome organization in some organisms and allows us to more profoundly explore its relationship with epigenomic modifications and chromatin remodelers for final organization of eukaryotic chromatin.

445F Long transposon-rich regional centromeres in the oomycete *Phytophthora sojae* reveal divergence of centromere features in the Stramenopila-Alveolata-Rhizaria (SAR) lineages *Yufeng "Francis" Fang*[1,5], Marco A. Coelho[1], Haidong Shu[2], Klaas Schotanus[1,6], Bhagya C. Thimmappa[3,7], Vikas Yadav[1], Han Chen[2], Kaustuv Sanyal[3], Suomeng Dong[2], Minou Nowrousian[4], Joseph Heitman[1] 1) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina; 2) College of Plant Protection, Nanjing Agricultural University, Nanjing, China; 3) Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India; 4) Lehrstuhl fuer Molekulare und Zellulaere Botanik, Ruhr-Universitaet Bochum, Bochum, Germany; 5) GreenLight Biosciences Inc, Research Triangle Park, North Carolina; 6) Bejo, Trambaan 1, 1749 CZ Warmenhuizen, the Netherlands; 7) Department of Biochemistry, Robert-Cedergren Centre for Bioinformatics and Genomics, University of Montreal, 2900 Edouard-Montpetit, Montreal, H3T1J4, QC, Canada.

Centromeres are chromosomal regions that serve as platforms for kinetochore assembly and spindle attachments, ensuring accurate chromosome segregation during cell division. Despite functional conservation, centromere DNA sequences are diverse and often repetitive, making them challenging to assemble and identify. Here, we describe centromeres in the oomycete *Phytophthora sojae* by combining long-read sequencing-based genome assembly and chromatin immunoprecipitation for the centromeric histone CENP-A followed by high-throughput sequencing (ChIP-seq). *P. sojae* centromeres cluster at a single focus at different life stages and during nuclear division. We report an improved genome assembly of the *P. sojae* reference strain, which enabled identification of 15 enriched CENP-A binding regions as putative centromeres. By focusing on a subset of these regions, we demonstrate that centromeres in *P. sojae* are regional, spanning 211 to 356 kb. Most of these regions are transposon-rich, poorly transcribed, and lack the histone modification H3K4me2 but are embedded within regions with the heterochromatin marks H3K9me3 and H3K27me3. Strikingly, we discovered a *Copia*-like transposon (CoLT) that is highly enriched in the CENP-A chromatin. Similar clustered elements are also found in oomycete relatives of *P. sojae*, and may be applied as a criterion for prediction of oomycete centromeres. This work reveals a divergence of centromere features in oomycetes as compared to other organisms in the Stramenopila-Alveolata-Rhizaria (SAR) supergroup including diatoms and *Plasmodium falciparum* that have relatively short and simple regional centromeres. Identification of *P. sojae* centromeres in turn also advances the genome assembly.

446W Stable genome transformation via zoospore electroporation in the Chytridomycota *Andrea Vu*[1], Nicolas Buchler[1] 1) North Carolina State University, Raleigh, NC.

Functional genomics relies on methods for stable genome transformation. Recently, Agrobacterium-mediated transformation (AMT) was developed for the soil chytrid *Spizellomyces punctatus*. This method was used to deliver and randomly integrate exogenous genes and fluorescent protein fusions into the genome through non-homologous end joining. However, unlike other methods of gene delivery, AMT does not allow for the introduction of biomolecular complexes into the cell, such as CRISPR ribonucleoprotein, for precision genome-editing. Chytrids make zoospores, which are motile, single-nuclear fungal spores that lack a cell wall. Alternative transformation methods, such as zoospore electroporation, have been successfully used to transform non-fungal zoosporic organisms, such as *Phytophthora* species. The goal of this study was to develop zoospore electroporation for genome transformation of *S. punctatus*.

Informed by the oomycete literature, we developed a protocol for zoospore electroporation and transformation of *S. punctatus*. For proof of concept, a histone 2B-tdTomato fusion and hygromycin resistance gene were transformed and randomly integrated into the genome through non-homologous end joining. Resultant transformants were validated by microscopy and whole genome sequencing. We are developing methods for gene replacement in *S. punctatus* by targeting and replacing *URA3* with the hygromycin resistance gene via homologous recombination. Preliminary results in other chytrids species will be discussed. The development of this transformation method will allow for broad evolutionary and comparative studies in this group of early diverging fungi.

447T Genes of unknown function conserved across fungi: a call for action Asaf Salamov[1], Igor Shabalov[1], *Igor Grigoriev*[1,2] 1) US DOE Joint Genome Institute, LBNL, Berkeley, CA; 2) Plant and Microbial Biology Department, University of California Berkeley, Berkeley, CA.

The ever-increasing number of sequenced genomes presents us with an exciting opportunity to discover highly conserved gene families of unknown function and characterize them experimentally. Over 18 million proteins encoded in ~1300 fungal genomes from JGI MycoCosm were clustered into families using cascaded MMseqs2 with default parameters (Steinegger et al, 2017). A subset of 142 clusters of proteins with the following properties has been selected: (i) proteins conserved across large phylogenetic distances, i.e. present in either >50% of all fungal genomes or >90% of genomes in the clade; (ii) proteins of unknown function, with neither known Pfam domains except for the domains without specific function, like DUF or UPF, nor Blastp hits against Swissprot; (iii) at least 20% of genes of each selected cluster are transcriptomics based models.
We have detected these gene families across the kingdom Fungi and invite the international research community to functionally characterize their individual members and propagate their annotations across the Fungal Tree of Life (https://mycocosm.jgi.doe.gov/conserved-clusters/run/run-2020;FRJbyJ). Investigators can register, login, click on any cluster, and add notes to any protein from the list along with the methods used for functional characterization. Once characterized and annotated, each of such proteins enables us to expand the annotation to the entire cluster composed of proteins from many fungal species. Besides the targeted single protein biochemical characterization this list could focus more high-throughput methods on predicting function of these proteins: structure-based functional predictions or gene knockouts or various multiomics capabilities for characterization for sequenced genomes through JGI Community Science Program. Examples of these approaches will be presented.

448F Diversity of genomic adaptations to post-fire environment in higher fungi points to a crosstalk between charcoal

tolerance and sexual development *Andrei Stecca Steindorff*[1], Kyungyong Seong[1,2], Akiko Carver[1,2], Sara Calhoun[1], Monika Fischer[2], Kyra Stillman[2], Haowen Liu[2], Elodie Drula[3,4], Bernard Henrissat[5,6], Hunter Simpson[7], Jonathan Schilling[8], Anna Lipzen[1], Guifen He[1], Mi Yan[1], Jasmyn Pangilinan[1], Kurt LaButti[1], Vivian Ng[1], Matthew Traxler[2], Thomas Bruns[2], Igor Grigoriev[1,2] 1) US DOE Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA, USA; 2) Plant and Microbial Biology, UC Berkeley, Berkeley, CA, USA; 3) Architecture et Fonction des Macromolécules Biologiques (AFMB), CNRS, Marseille, France; 4) INRAE, Architecture et Fonction des Macromolécules Biologiques (AFMB), Marseille, France; 5) Department of Biological Sciences, King Abdulaziz University, Jeddah, Saudi Arabia; 6) DTU Bioengineering, Technical University of Denmark, Lyngby, Denmark; 7) Department of Bioproducts and Biosystems Engineering, University of Minnesota, St. Paul, MN, USA; 8) Department of Plant and Microbial Biology, University of Minnesota, St. Paul, Minnesota, USA.

Understanding post-fire soil systems are essential because they have significant direct and indirect effects on global carbon storage. Soil microbes are likely involved in the degradation of pyrolyzed organic matter (PyOM), yet little is currently known about the organisms or metabolic processes involved. So far, we have sequenced and annotated four pyrophilous Basidiomycetes and seven Ascomycetes genomes. In our previous work on Basidiomycetes fungi, we found expansion of genes potentially involved in the degradation of the hydrophobic layer, pyrolyzed organic matter, and mushroom formation. In this work, we focused on the seven ascomycetes genomes and compared them with the other 12 non-pyrophilous in the same order and 124 genomes at a larger scale, including pyrophilous Basidiomycetes and other organisms with heterogenous lifestyles. Additionally, we explored enriched Pfam domains and CAZymes to identify patterns associated with these organisms' "fire-loving" lifestyle. Our analyses uncovered gene families related to the degradation of pyrolyzed organic matter, but these gene families were distinct from those expanded in the pyrophilous fungi in Basidiomycota. The enrichment analysis revealed families like peritrophin-A, arthropod defensin, β-glucosidases, heat shock proteins, and fungal fucose-specific lectin. These families might be involved with the pyrophilous fungi' capacity to survive in a toxic environment like post-fire soil. We found a CAZyme CBM14 expanded exclusively in the Pyronemataceae family. This family is mainly found in insects and some fungi. Since it is a chitin-binding domain, this suggests that secreted CBM14 domain proteins might protect the fungus from microbial attacks in its soil habitat. Another interesting finding is that pyrophilous fungi have significantly larger proteins and higher GC3 content than non-pyrophilous, being in an intermediate state to thermophiles. Pyrophilous fungi are commonly found fruiting after fire events, passing through their sexual stages in this process. To make an in-depth comparison of these conditions, we analyzed the available transcriptomic data of *Pyronema domesticum* grown in charcoal and during sexual development. We performed a co-expression network analysis and found two modules with the most differentially expressed genes in charcoal and sexual development. Gene Ontology categories like chitin/carbohydrate/lipid/superoxide metabolism and transport were found in both modules, showing that such processes are likely required to grow in the presence of charcoal and sexual development. Also, the transcription factors STE12, LreA, LreB, VosA, and EsdC involved in mating response and environmental cues in yeasts and filamentous ascomycetes were up-regulated in charcoal, revealing a crosstalk between charcoal tolerance and sexual development.

449W A Pangenomic assessment of a Cercospora beticola global population *Nathan Wyatt*[1], Rebecca Spanner[1,3], Viviana Rivera-Varas[2], Gary Secor[2], Melvin Bolton[1] 1) Sugarbeet and Potato Research Unit, United States Department of Agriculture, Fargo, ND 58102, United States.; 2) Department of Plant Pathology, North Dakota State University, Fargo, ND 58102, United States.; 3) Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile; ANID—Millennium Science Initiative—Millennium Institute for Integrative Biology (iBIO), Santiago, Chile.

Cercospora beticola, causal agent of Cercospora leaf spot (CLS), is the most economically damaging pathogen of sugar beet (*Beta vulgaris* subsp. *vulgaris)*. Population genetic analysis have shown *C. beticola* populations to have high levels of genetic diversity and gene flow between local and geographically distant populations. Relationships among pathogen populations can reveal important information relevant to disease epidemiology including sources of origin, population dynamics, and patterns of dispersal. This information is crucial for predicting the capacity of particularly virulent strains to disperse to geographically distant locations. To gain an understanding of *C. beticola* population dynamics on a global scale, a large population of *C. beticola* isolates from geographically distinct regions was subject to whole genome sequencing. Using this global data set, we conducted a pangenomic analysis to provide novel insights into the pathogen's evolution, genome dynamics, and virulence genes relevant to crop disease outbreaks. Core and accessory genomic components were assessed as well as population-specific presence/absence variation to provide a foundation for characterizing the adaptive landscape of the genome of *C. beticola*. Further, a genome wide association study (GWAS) approach was employed with this population to identify genomic loci associated with adaptation to CLS management practices. Preliminary results of a GWAS identified four strong marker trait associations in the *C. beticola* genome corresponding to virulence on an economically important sugarbeet cultivar. These results set the foundation for larger comparative population genomic analysis assessing origin, patterns of dispersion, and shifts in the global population of *C. beticola*.

450T Analysing the Pangenome of Aspergillus Fumigatus to Uncover Accessory Genes Involved in Azole Resistance *Harry Chown*[1], Johanna Rhodes[2], Paul Bowyer[1], Michael J. Bromley[1], Matthew C. Fisher[2] 1) Manchester Fungal Infection Group, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester Academic Health Science Centre, Core Technology Facility, Manchester, UK; 2) MRC Centre for Global Disease Analysis, Imperial College London, London, UK.

The opportunistic fungal pathogen *Aspergillus fumigatus* shows an increasing trend of resistance towards the first-line antifungal azole drug group. Due to its clinical importance, understanding the acquisition of drug resistance genotypes from the environment is crucial to public health. Furthermore, novel resistance mechanisms can be overlooked in comparative genomics due to comparisons towards a reference strain.

Here, we present a pangenome analysis of 218 *A. fumigatus* isolates across the United Kingdom and Republic of Ireland to uncover accessory genes that are associated towards resistant isolates. First, we identify the total number of genes within the population as 13,206 and that the core-gene set makes up around 50% (6,705) of the total gene-pool; whilst the accessory gene content varies between isolates and the total number of strain-specific genes is equal to 12% (1,592) of the population gene-set. Next, we show that the pangenome is still considered "open" with this many isolates. Finally, we use association tests, between gene content and azole

minimum inhibitory concentrations to identify the importance of accessory genes in azole resistance, demonstrating the intricacies between clade structure and drug resistance. Results from this study help us to understand the extent of genomic variation in populations of *A. fumigatus* and how this influences pathogenicity.

451F ***Aspergillus fumigatus*** **pan-genome analysis identifies genetic variants associated with human infection** *Amelia Barber*[1], Tongta Sae-Ong[1], Kang Kang[1], Bastian Seelbinder[1], Jun Li[2], Grit Walther[1], Gianni Panagiotou[1,2], Oliver Kurzai[1,3] 1) Leibniz Institute for Natural Product Research and Infection Biology-Hans Knoell Institute, Jena, Germany.; 2) University of Hong Kong, Hong Kong, China; 3) Institute for Hygiene and Microbiology, University of Wuerzburg, Wuerzburg, Germany.

Aspergillus fumigatus is an environmental saprobe and opportunistic human fungal pathogen. Despite an estimated annual occurrence of more than 300,000 cases of invasive disease worldwide, a comprehensive survey of the genomic diversity present in *A. fumigatus*—including the relationship between clinical and environmental isolates and how this genetic diversity contributes to virulence and antifungal drug resistance—has been lacking. In this study, we define the pan-genome of *A. fumigatus* using a collection of 300 globally sampled genomes (83 clinical and 217 environmental isolates). We found that 7,563 of the 10,907 unique orthogroups (69%) are core and present in all isolates and the remaining 3,344 show presence/absence of variation, representing 16–22% of the genome of each isolate. Using this large genomic dataset of environmental and clinical samples, we found an enrichment for clinical isolates in a genetic cluster whose genomes also contain more accessory genes, including genes coding for transmembrane transporters and proteins with iron-binding activity, and genes involved in both carbohydrate and amino-acid metabolism. Finally, we leverage the power of genome-wide association studies to identify genomic variation associated with clinical isolates and triazole resistance as well as characterize genetic variation in known virulence factors. This characterization of the genomic diversity of *A. fumigatus* allows us to move away from a single reference genome that does not necessarily represent the species as a whole and better understand its pathogenic versatility, ultimately leading to better management of these infections.

452W **Giant** ***Starship*** **elements mobilize accessory genes in fungal genomes** *Emile Gluck-Thaler*[1,2,3], Timothy Ralston[3], Zachary Konkel[3], Cristhian Grabowski Ocampos[4], Veena Devi Ganeshan[3], Anne E. Dorrance[3], Terry L. Niblack[3], Corlett W. Wood[2], Jason C. Slot[3], Horacio D. Lopez-Nicora[3,5], Aaron A. Vogan[6] 1) University of Neuchâtel, Switzerland; 2) University of Pennsylvania, USA; 3) The Ohio State University, USA; 4) Universidad Nacional de Asunción, Paraguay; 5) Universidad San Carlos, Paraguay; 6) University of Uppsala, Sweden.

Accessory genes are variably present among members of a species and are a reservoir of adaptive functions. In bacteria, differences in gene distributions among individuals largely result from mobile elements that acquire and disperse accessory genes as cargo. In contrast, the impact of cargo-carrying mobile elements on eukaryotic evolution remains largely unknown. Here, we show that variation in genome content within multiple fungal species is facilitated by *Starships*, a novel group of massive mobile elements that are 110 kb long on average, share conserved components, and carry diverse arrays of accessory genes. We found hundreds of *Starship*-like regions across every extant class of filamentous Ascomycetes, including 32 unique Starships that range from 27-393 kb and last shared a common ancestor ca. 400 mya. Using new long-read assemblies of the plant pathogen *Macrophomina phaseolina*, we characterize 4 distinct *Starships* whose past and ongoing activities contribute to standing variation in genome structure and content. One of these elements, *Voyager*, inserts into 5S rDNA and contains a candidate virulence factor. Phenotypic assays revealed that *Voyager* copy number has contrasting associations with pathogenic and saprophytic growth, suggesting its activity underlies an ecological trade-off. We propose that *Starships* are eukaryotic analogs of bacterial integrative and conjugative elements based on parallels between their conserved components, and may therefore represent the first known agents of active gene transfer in a eukaryote. Together, our results suggest that *Starships* have shaped the content and structure of fungal genomes for millions of years, revealing a new concerted route for evolution across an entire eukaryotic kingdom.

453T **Within-species variability of the insect-pathogenic fungus** ***Metarhizium acridum*** **revealed by pangenomic analysis** *Dinah Parker*[1], Carolina Nogueira[1], Tue Kjærgaard Nielsen[1], Knud Nor Nielsen[1], Lars Hestbjerg Hansen[1], Henrik H. De Fine Licht[1] 1) Department of Plant and Environmental Sciences, University of Copenhagen, Denmark .

The genus *Metarhizium* comprises a diverse set of entomopathogenic fungi that exhibit a wide spectrum of host ranges. Within this genus, *M. acridum* is a specialist pathogen that primarily infects insects in the order Orthoptera, and is currently used as an environmentally friendly mycoinsecticide. Despite being a species with a cosmopolitan distribution across tropical and sub-tropical regions around the world, much of the current genotypic and phenotypic characterization is based on only two isolates (CQMa102, ARSEF324). However, our preliminary data provides evidence for high SNP diversity and phenotypic variation in virulence among globally sampled isolates. Here, we continue in our efforts to elucidate intra-specific genomic diversity by establishing a reference-quality pangenome of *M. acridum* based on six assembled genomes of isolates from four continents. While previous studies suggested that specialist species in this genus have fewer genes compared to generalists (~9,160), our current results suggest a wide range from 9,725 to 10,367 genes, a first indication of higher genetic diversity than previously described. We also identified the core and accessory orthogroups within this species, and examined regions that exhibit large-scale structural variation across the genome. Many pathogenic organisms are also known to carry genes associated with pathogenesis within highly variable regions or even on accessory chromosomes. Therefore, gene enrichment analysis was used to investigate differences in gene function between the core and accessory gene sets, to validate functional differences. Taken together, we characterize a novel pangenome of an entomopathogenic fungus to further our understanding of how genomic diversity affects host-pathogen interactions.

454F **Comparison of long-read sequencing platforms for de novo genome assemblies of the fungal cereal pathogen** ***Bipolaris sorokiniana*** *Shaobin Zhong*[1], Yueqiang Leng[2], Yang Du[3], Jason Fiedler[4] 1) North Dakota State University; 2) North Dakota State University; 3) Valley City State University; 4) USDA-ARS.

Pacific Biosciences (PacBio) and Oxford Nanopore Technologies (ONT) can generate longer sequence reads than the short-read sequencing technologies such as Illumina, and thus have become more and more used for sequencing and assembling whole genomes of many organisms, including bacteria, fungi, plants and animals. In this study, we sequenced one isolate (ND85F) of *Bipolaris*

sorokiniana (syn: *Cochliobolus sativus*), the fungal pathogen that cause spot blotch, common root rot, and kernel blight in barley and wheat, using two sequencing platforms (PacBio, and ONT). The objective of the research was to compare the continuity, base accuracy, and cost-effectiveness of genome assemblies resulted from these long-read sequence data sets. Two PacBio protocols (Sequel II CLR and Sequel II HiFi) with one SMRT cell for each were used to generate 126.9 Gb of CLR subreads (n = 9576935, ave = 13249.90 bp, largest = 221005 bp) and 16.4 Gb of HiFi reads (n = 990282, ave = 16567.79 bp, largest = 44703 bp) for ND85F. One ONT flow cell was used to produced 14.0 Gb of long reads (n = 400330, ave = 34960.12 bp, largest = 223477 bp) using the Ligation Sequencing Protocol. We also generated 12 Gb of 125 bp paired-end reads from the Illumina sequencing technology. Assemblies with Sequel II HiFi reads had the highest consensus accuracy, while those with ONT data had the lowest base accuracy. However, the assemblies with ONT reads had the highest genome contiguity with a total of 30 scaffolds generated, 17 of them containing at least one telomere. The assemblies from Sequel II CLR subreads were intermediate between those from the other two data sets (HiFi and ONT) in terms of base accuracy and contiguity. The quality of the assemblies from ONT reads could be enhanced by using the short reads from Illumina for base correction and polishing. The ONT-Illumina hybrid approach would be more cost-effective for achieving a high-quality assembly for fungal genomes.

455W Pangenome analyses of *Fusarium* isolates infecting banana reveals evolutionary dynamics of the adaptive genome *Anouk van Westerhoven*[1,2], Einar Martinez de la Parte[1], Harold Meijer[1,3], Edgar Chavarro Carrero[1], Gert Kema[1], Michael Seidl[2] 1) Laboratory of Phytopathology, Wageningen University &Research, Wageningen, the Netherlands ; 2) Theoretical Biology and Bioinformatics, Department of Biology, Utrecht University, the Netherlands ; 3) BU Biointeractions and Plant Health, Wageningen University & Research, Wageningen, the Netherlands.

Many plant pathogens evolved multispeed genomes with conserved core and adaptive genomic compartments. The adaptive genome often harbors genes encoding effectors that mediate host colonization. Since these regions are often hypervariable between species or strains, they are thought to facilitate the rapid adaptation of effector genes in the co-evolutionary arms races with the host's immune system. The fungus *Fusarium oxysporum* (*Fo*) is a major plant pathogen that causes wilting disease in hundreds of different hosts. The genome of *Fo* carries adaptive regions which can encompass entire chromosomes. Importantly, transfer of some of these adaptive chromosomes can modulate pathogenicity and host range, as these chromosomes carry essential effector genes. However, it is not yet known how these adaptive regions evolve and how they differ between *Fo* of the same host range. Here we will report our ongoing efforts to study the emergence and evolution of *Fo* isolates able to infect banana (*Foc*), the most popular fruit and an essential staple crop in various regions worldwide. We applied pan-genomics approaches to study the genome diversity and dynamics of a global panel of more than 70 isolates. As reported previously, these *Fusarium* isolates are genetically diverse and can be grouped into multiple races based on their capacity to infect a subset of banana cultivars. We observed that the adaptive genome differs considerably between races and can range from entire chromosomes to specific regions on the core chromosome. With future comparative work we aim to characterize host-specific as well as race-specific genomic regions, which is essential to uncover effectors associated with specificity towards individual banana cultivars. Ultimately, our study contributes to understanding the pathogenicity and supports breeding of disease resistant banana varieties.

456T Seeing red: investigations of spatial expression in hyphae indicate horizontally acquired bikaverin production in *Monosporascus* limits fungal-fungal interactions *Aaron Robinson*[1], Demosthenes Morales[1], Julia Kelliher[1], Karen Davenport[1], La Verne Gallegos-Graves[1], Amy Zheng[2], Patrick Chain[1] 1) Los Alamos National Laboratory, Los Alamos, NM; 2) Vanderbilt University, Nashville, TN.

The biological gene cluster (BGC) responsible for production of the fungal pigment bikaverin has been acquired by multiple fungal isolates through apparent horizontal gene transfer events with *Fusarium* species. Bikaverin has been implicated in a number of fungal processes, most notably the production of bikaverin to limit unfavorable interactions with certain bacteria. A complete bikaverin BGC has been recently identified in the genome of a *Monosporascus* isolate acquired from a desert grassland. The genomes of several other *Monosporascus* isolates from the same environment only contain fragments of the BGC, possibly indicating the cluster was acquired in a common ancestor but was not maintained in all lineages. Co-cultures of this *Monosporascus* isolate with diverse bacteria from the same environment did not result in the characteristic red phenotype associated with increased bikaverin production, but the red phenotype was observed in co-cultures of *Monosporascus* isolates. Chemical analysis of the red *Monosporascus* co-cultures confirmed substantial changes in bikaverin production. Spatial examinations of the expression of the genes in the bikaverin BGC using smFISH indicate a directed response in hyphae proximal to the other *Monosporascus* isolate. These observed interactions raise several questions about potential roles of horizontally acquired BGCs in fungal sensing and response to other organisms, while also indicating exciting potential for genetic engineering. Additionally, bikaverin shows promise for furthering the understanding of mating genetics and sexual interactions in the Xylariales, which remain largely unknown.

457F Comparative genomics in *Coccidioides* *Kelsey Aadland*[1], Marc Orbach[2], Lisa Shubitz[2], John Galgiani[2], Jason Stajich[1] 1) University of California, Riverside, CA; 2) University of Arizona, Tucson, AZ.

Coccidiomycosis, also known as Valley Fever, is a respiratory disease whose etiological agents consist of two species of dimorphic fungi, *Coccidioides immitis* and *Coccidioides posadasii*. The fungi are endemic to arid and semi-arid regions in both North and South America and can cause disease in humans and a wide range of other mammals. The disease typically initiates from inhaled spores often liberated by soil disruption where the saprotrophic form of the fungi resides outside of an animal host. Spores develop into infectious structures called spherules that grow and erupt in the lungs, releasing endospores and continuing the cycle. *C. immitis* and *C. posadasii* are unique fungal pathogens that are capable of infection of both immunocompromised and immunocompetent individuals, and the scale of coccidiomycosis severity ranges from no discernible symptoms to systemic fatal infections. Currently there are limited antifungal therapeutics and efforts have been made towards development of a vaccine due to retained immunity among those exposed and recovered from coccidiomycosis. Genetic variability within and between species occurs for isolates from varying endemic regions, and is believed to be a cause of differences in virulence of some *Coccidioides* strains. Previous work has produced multiple genome assemblies for *Coccidioides* species including closed assemblies for *C. immitis* strain RS and *C. posadasii* strain Silveira. We have

further used long-read PacBio HiFi sequencing to assemble genomes of two *C. posadasii* and three *C. immitis* strains. The two *C. posadasii* strains, RMSCC 1038 and RMSCC 3700 are of interest due to their hypovirulence. RMSCC 1038 has been used to develop a murine chronic disease model of coccidioidomycosis. Two of the *C. immitis* strains, B10637 and B10992, are clinical and soil isolates from Washington state, and the last strain is a clinical isolate from a Utah patient with disseminated disease. The quality of these genomes was assessed to be high based on high completeness with BUSCO and presence of telomere repeats on most scaffolds and compared full-length chromosomes among strains/species. We have also annotated the genes in assemblies using the funannotate pipeline and compared the protein coding genes and repetitive content. We evaluated orthologous gene clusters among the strains and compared duplications, deletions and losses, allowing us to look at gene and transposable element content in context of nearly complete full length genome assembly. Using these comparative methods we not only gained information on genetic variations in strains of *Coccidioides*, but were also able to look into the benefits of updating reference genomes.

458W Domestication history and its relation to clonal heterogeneity and microevolution of *Saccharomyces cerevisiae* in the human host *Walter Pfliegler*[1], Alexandra Imre[1], Hanna Rácz[1], Péter Oláh[2], Zsuzsa Antunovics[1], Ilona Dóczi[3], Renátó Kovács[1], László Majoros[1], Ksenija Lopandic[4], Devin Bendixsen[5], Rike Stelkens[5], István Pócsi[1] 1) University of Debrecen, Debrecen, Hungary; 2) University of Pécs, Pécs, Hungary; 3) University of Szeged, Szeged, Hungary; 4) University of Natural Resources and Life Sciences, Vienna, Austria; 5) Stockholm University, Stockholm, Sweden.

The yeast *Saccharomyces cerevisiae* is ubiquitous in the food industry and widely used as a probiotic. Many of the species' described clades are specifically adapted to traditional or modern industrial fermentation environments and are often characterized by high levels of admixture, extensive genome structure variations, the ability for rapid adaptation, and elevated stress tolerance. These phenomena are considered as hallmarks of millennia-long domestication of this microbe. In addition to its manifold uses, *S. cerevisiae* may also be regarded as a member of our extended microbiome and may also be capable of long-term commensal and even pathogenic colonization.
In this work, we utilized (1) a review of early to mid-20th century reports on industrial yeasts and strain improvement efforts, (2) short- and long-read whole genome sequencing from altogether 600 previously sequenced or newly isolated yeast samples of industrial and human origin, (3) benchmarking phylogenomic and comparative genomic pipelines, (4) and assessing geno- and phenotypic clonal microevolution of yeasts to understand how domestication and deliberate breeding affected the adaptability and pathogenic potential of *Saccharomyces*.
We were able to trace back many dozens of commensal and pathogenic human isolates to commercially available food industry (mainly baking) strains and probiotic yeasts. Our phylogenomic analysis furthermore uncovered human isolates of the wild subclade of the Wine/European yeasts, and a sample closely related to a 2800-years-old archeological isolate. We showed that the low intra-clade diversity and the highly admixed genomes of baking yeasts are products of artificial strain crossing strategies and manufacturing companies' policies, not of natural selection.
The genomic characteristics and breeding history had remarkable effects on the in-host microevolution of baking strains. These preferably colonize the female genital tract and display large-scale and rapidly arising genome structure variations independent of meiotic processes. This leads to high geno- and concomitant phenotypic clonal heterogeneity. Such an anatomic niche preference and such levels of variation are not characteristic to human isolates of other clades.
Our study shows that even if *S. cerevisiae* is a true member of the human microbiome, it is now mostly present in us in the form of non-natural, domesticated, and often artificially bred clades.

459T High-throughput genetics and essential gene discovery in *Cryptococcus neoformans* *Blake Billmyre*[1], Michael Eickbush[1], Caroline Craig[1], Sarah Zanders[1,2] 1) Stowers Institute for Medical Research, Kansas City, MO; 2) University of Kansas Medical Center, Kansas City, KS.

Advances in sequencing technologies have revealed dramatic variation in gene content and sequence between different species and even between different isolates within individual species. However, our ability to understand the functional consequences of these changes has lagged behind significantly. The *Cryptococcus* genus is an ideal model fungal system to explore these questions. There are at least seven species within this genus amenable to lab experimentation that diverged around the same time as humans and birds. In addition, *Cryptococcus* species are pathogens of humans, causing nearly 200,000 deaths annually. There are also additional tractable non-pathogenic sister species of *Cryptococcus* to serve as outgroups. We have developed a high-throughput quantitative genetics approach in the human fungal pathogen *Cryptococcus neoformans* by using massively parallel insertional mutagenesis coupled with targeted sequencing and machine learning to determine the set of genes important for growth under a given condition. Using this method, we have identified the set of essential genes in *C. neoformans*. This set includes dozens of genes that are nonessential in other fungi but required in *C. neoformans*. We also identified many genes that are essential in other species but dispensable in *C. neoformans*. We are now exploring genes important for drug resistance and quorum-regulated behaviors. Taken together, this work will produce a global understanding of drug resistance and a map of essential genome function to guide future drug development in *C. neoformans*. In addition, this project will establish an experimental tool and framework that can be used to analyze other non-model fungal pathogens and help bridge the gap between sequence and function.

460F Genetic interaction mapping via pooled CRISPR-Cas9 insertional mutagenesis in the human fungal meningitis pathogen *Cryptococcus neoformans* *Manning Huang*[1], Hiten Madhani[1] 1) University of California, San Francisco.

Cryptococcus neoformans is responsible for about 15% of global HIV/AIDS associated mortality. About 230,000 cases of cryptococcal meningitis occur every year with an associated 81% global case mortality. Multiple virulence factors permit *C. neoformans* to evade the host immune system and colonize the lungs from which the pathogen can disseminate to other organs, including the brain. However, our understanding of these processes remains incomplete. Large-scale strategies using unbiased forward genetic screens of a genome-wide deletion collection in our lab have begun to identify *C. neoformans* genes required for infection. However, many genes that may play roles during infection do not have well-annotated orthologs, making it critical to develop methods to decipher the molecular mechanisms of these unannotated virulence associated genes. A powerful strategy for approaching this challenge is genetic

interaction mapping.
To this end, we are developing a novel pooled CRISPR-Cas9 insertional mutagenesis strategy to rapidly generate genome wide pools of gene disruption mutants. This strategy inserts a DNA cassette containing a selectable marker, unique molecular identifier (UMI), and sgRNA expressing construct at the Cas9 cut site via nonhomologous end joining. The approach is designed such that sequencing of the UMI and sgRNA will permit quantification of the abundance of specific mutants within the pool. Initial experiments show that gene disruption with this strategy is highly efficient (>98%) with few off-target effects. Ongoing experiments seek to validate strategy by employing a junction-sequencing approach to directly identify loci where marker-sgRNA cassettes have been inserted. We have begun to use this system in genetic interaction mapping experiments aimed at systematically disrupting all nonessential genes in a range of mutant strain backgrounds. Results of these experiments will be presented which are anticipated illuminate relationships between virulence associated genes and to develop testable hypotheses for gene function.

461W A versatile selection free CRISPR-Cas9 transformation system for *A. fumigatus* *Norman van Rhijn*[1], Takanori Furukawa[1], Lauren Dineen[1], Tim Baltussen[2], Jochem Buil[2], Paul Verweij[2], Willem Melchers[2], Michael Bromley[1] 1) University of Manchester; 2) Radboud University Nijmegen Medical Centre.

Improvements in methods that facilitate genetic modifications in fungi are required to aid research in this area. *Aspergillus fumigatus* is a saphrophytic fungus that is the cause of more than 300,000 life-threatening infections annually. The development of rapid and versatile gene editing methodologies for *A. fumigatus* is essential. Unlike to model yeast *Saccharomyces cerevisiae*, targeted allele replacement in *Aspergillus fumigatus* is complicated by low rates of homologous recombination and the fact that replacement cassettes require long homology arms of c. 1kb. CRISPR-Cas9 mediated transformation has been widely using as a genome editing tool to overcome some of these issues. However, successful editing normally relies on time consuming multi-step cloning procedures paired with the use of selection markers, which can result in a metabolic burden for the host and unintended transcriptional modifications at the site of integration. Recently we published data showing that an in vitro CRISPR-Cas9 assembly methodology could be used to perform genome editing without the need for selectable markers. Here we show how the method can be used to perform epitope-tagging, site-directed mutagenesis and insertion of genetic constructs. We have introduced a functional GFP-epitope tag to the N- and C-terminus of the *pacC* and *srbA* protein. In addition, we generate targeted point mutations in the *pyrG* and *pyrE* genes. Lastly, we use a batch screening method to rapidly evaluate over 500 viable colonies from selection free CRISPR-mediated transformations. This enables rapid identification of transformed colonies. Overall, our selection free method decreases the time required for complex construct synthesis and can potentially be translated to other fungi.

462T The narrow footprint of ancient balancing selection surrounding nonself recognition genes in *Aspergillus fumigatus* *Ben Auxier*[1], Jianhua Zhang[1], Eveline Snelders[1], Alfons Debets[1] 1) Laboratory of Genetics, Wageningen University, NL.

Fusion within a fungal individual is necessary for many life processes, yet is restricted between individuals. The genetics of this have been worked out particularly in *Neurospora* and *Podospora*. The similarity of the genetics, if any, was unknown in any *Aspergillus*. To address this, we used heterokaryotic complementation of auxotrophic nitrate assimilation mutants in *Aspergillus fumigatus*. We assessed compatibility between ~150 progeny of a sexual cross between two environmental strains. We recovered ~3% of offspring were compatible, consistent with segregation of 5 genes. 4 of these loci had a strong effect, while the fifth only delayed, not prevented, heterokaryon formation. Using whole-genome sequences of each offspring, we identified 5 candidate het loci, 4 of which could be identified to a single gene. Heterologous expression of candidate alleles from autonomous plasmids confirmed the causal role of 3 of these loci, and validation of the remaining is ongoing. The 4 identified strong effect genes include het gene mechanisms known from other species, including NLR and patatin-like proteins. One gene, *het*B, appears to involve a protease containing a CARD domain known to interact in mammalian apoptosis which fungi do not use. Phylogenetic analysis of these genes shows strong trans-species polymorphisms, with alleles being shared between *A. fumigatus, A. lentulus, A. fisheri,* and *A. udagawae*, spanning >10 million years of evolution. Suprisingly, the diversity surrounding these identified *het* genes is restricted to only +/- 200bp of the coding sequence, potentially related to the high recombination rate in this species. Knowledge of the het gene complement in this species presents an opportunity to investigate the downstream cell death pathways, which may have clinically relevant opportunities.

463F Meiosis in the human pathogenic fungus *Aspergillus fumigatus* produces the highest known number of crossovers Ben Auxier[1], Alfons Debets[1], Joost van den Heuvel[1], *Eveline Snelders*[1] 1) Wageningen University.

The decade-old discovery of a sexual cycle, combined with population genetic data, indicates that sex is common in the fungus *Aspergillus fumigatus*. However, the impact of sex remained unclear. Here we show that meiosis in *A. fumigatus* produces the highest known number of crossovers between chromosomes. Using genome-wide markers, we observed 29 crossovers per chromosome on average. This remains the highest known crossover rate after correcting for genome size or chromosome number. We show that this calculated high recombination rate, combined with abundant sexual progeny, can explain the origin of highly antifungal resistant haplotypes from individual tightly linked antifungal resistance mutations. Understanding the consequences of this unparalleled crossover rate not only enriches our population-level understanding of this emergent human pathogen, but of meiosis in general.

464W Global analysis of circuitry governing *Candida albicans* morphogenesis within host immune cells *Nicola Case*[1], Kwamaa Duah[1], Teresa O'Meara[2], Brett Larsen[3], Cassandra Wong[1,3], Anne-Claude Gingras[1,3], Luke Whitesell[1], Amanda Veri[1], Nicole Robbins[1], Leah Cowen[1] 1) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan, United States of America; 3) Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, Ontario, Canada.

The evasion of killing by host immune cells is crucial for fungal survival in the host. For the human fungal pathogen *Candida albicans*, the morphogenetic transition from yeast to filament upon internalization by macrophages is a key intracellular survival strategy that occurs through mechanisms that remain largely enigmatic. To identify the *C. albicans* genes that orchestrate filamentation in macrophages, we performed a functional genomic screen of conditional expression mutants covering ~50% of the *C. albicans* genome and identified 127 genes important for filamentation upon phagocytosis. Notably, twenty-six of the genes were dispensable for filamentation

in host-relevant culture conditions (RPMI with 3% serum, 37°C, 5% CO_2), demonstrating specificity in the program governing morphogenesis within macrophages. Gene ontology enrichment of the genes specifically required for morphogenesis in macrophages revealed a key role for the mitochondrial ribosome, aerobic respiration, and gluconeogenesis, suggesting that *C. albicans* may rely on non-fermentative metabolism to enable intraphagosomal filamentation. Further, we explored filament-inducing stimuli within or produced by the macrophage and determined that macrophage lysate is sufficient to induce morphogenesis. Bioactivity-guided fractionation coupled to mass spectrometry identified the immune modulator, prothymosin alpha (PTMA), as a potential macrophage-derived trigger of filamentation. Immunoneutralization of PTMA within macrophage lysate abolished its ability to stimulate *C. albicans* filamentation, strongly supporting PTMA as a filament-inducing component of macrophage lysate. This work is the first to implicate a host protein as a trigger of filamentation and identifies key elements of the regulatory circuitry that specifically governs *C. albicans* morphogenesis in response to phagocytosis by host immune cells.

465T Systematic genetic analysis of *Candida albicans* filamentation in response to elevated temperature *Emma Lash*[1], Nicole Robbins[1], Leah Cowen[1] 1) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada.

Fungal diseases impose a significant burden on human health, killing at least as many people as malaria and tuberculosis each year. To establish infection in humans, fungi must be able to grow at mammalian body temperature, and this requirement is only met by a small subset of species within the fungal kingdom. One such species is *Candida albicans*, a leading human fungal pathogen that can cause life-threatening infections in immunocompromised individuals. The inextricable link between temperature and virulence becomes even more prominent in *C. albicans* through the impact of temperature on morphogenesis. The capacity to transition from a yeast to filamentous growth state is tightly coupled to its pathogenicity and is induced by a number of cues, including temperatures exceeding 39˚C, with most other cues that induce filamentation requiring a minimum temperature of 37˚C. However, the genetic mechanisms through which elevated temperature triggers this developmental switch in *C. albicans* remain largely enigmatic. To explore this, I screened a mutant collection covering ~40% of the *C. albicans* genome and identified 40 genes required for filamentation at 39˚C, conditions relevant to febrile episodes in the human host. Interestingly, the results obtained suggested a key role for mRNA splicing via the spliceosome and actin cortical patch organization in governing morphogenesis induced by this environmental cue. Further hypothesis-driven experiments will elucidate the mechanisms through which these processes regulate filamentation at high temperature. Overall, this work illuminates genes important for morphogenesis in response to high temperature, with implications for understanding *C. albicans* pathogenicity.

466F A New Genetic Toolset Reveals Regulators of *Candida auris* Morphogenesis *Darian Santana*[1], Teresa O'Meara[1] 1) Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI.

Candida auris is an emerging healthcare-associated pathogen of global concern. Recent reports have identified *C. auris* isolates that grow in cellular aggregates or filaments, often without a clear genetic explanation. To investigate the regulation of *C. auris* morphogenesis, we applied an *Agrobacterium*-mediated transformation system to all four *C. auris* clades. We identified aggregating mutants associated with disruption of chitin regulation, while disruption of *ELM1* produced a novel filamentous morphology. We developed a transiently-expressed Cas9 and sgRNA system for *C. auris* that significantly increased targeted transformation efficiency across the four *C. auris* clades. Using this system, we confirmed the roles of *C. auris* morphogenesis regulators. Morphogenic mutants showed dysregulated chitinase expression, attenuated virulence, and altered antifungal susceptibility. Our findings provide novel insights into the genetic regulation of aggregating and filamentous morphogenesis in *C. auris*. Furthermore, the genetic tools described here will allow for efficient manipulation of the *C. auris* genome.

467W tRNA-ome of the human fungal pathogen Aspergillus fumigatus: high-throughput functional analysis reveals a valine tRNA isodecoder involved in Azole sensitivity *Lauren Dineen*[1], Ressa Lebedinec[1], Marcin Fraczek[1], Can Zhao[1], Daniela Delneri[1], Paul Bowyer[1], Mike Bromley[1] 1) University of Manchester.

Aspergillus fumigatus is an opportunistic human fungal pathogen responsible for an alarming number of life-threatening infections worldwide. There are limited antifungal treatments currently available to clinicians, and rates of resistance of A. fumigatus to several key antifungals is increasing. A growing number of transcriptional and post translational factors have defined roles in antifungal drug resistance however little is known about the role of translational factors. Transfer RNAs (tRNAs) are ancient RNA molecules with an integral role in translation. Recently, tRNAs have been implicated in complex stress responses and adaptive translation (Torrent et al 2018, Thompson et al 2008, Begley et al 2007). To investigate the significance of tRNAs in drug resistance, a genome wide barcoded tRNA knock out library has been generated in A. fumigatus. Through library generation we have identified 5 tRNA genes that are essential for viability. We further show that under optimal growth conditions, A. fumigatus displays robustness to tRNA gene deletion. By employing a competitive fitness (bar-seq) screening approach we have identify a set of tRNA deletion mutants displaying both sensitivity and resistance to antifungals including a unique valine tRNA isodecoder that plays a role in Azole sensitivity.

468T Characterizing genomic and phenotypic traits of the human pathogen *Aspergillus flavus* and its non-pathogenic close relatives *E. Anne Hatmaker*[1], Manuel Rangel Grimaldo[2], Rafael W. Bastos[3], Huzefa Raja[2], Sonja L. Knowles[2], Hadi Pourhadi[2], Gustavo Goldman[3], Nicholas Oberlies[2], Antonis Rokas[1] 1) Vanderbilt University, Nashville, TN, USA; 2) University of North Carolina at Greensboro, Greensboro, NC, USA; 3) Universidade de São Paulo, Ribeirão Preto, Brazil.

Although fungal diseases affect millions of humans each year, fungal pathogens of humans remain understudied. The mold *Aspergillus flavus* is a causative agent of both aspergillosis and fungal keratitis infections. Although *A. flavus* is commonly isolated from patients with these infections, species closely related to *A. flavus* are rarely, if ever, isolated from patients and are not considered clinically relevant. To gain insights into why this is the case, we compared phenotypic and genomic traits between *A. flavus* and three closely related non-pathogenic species, namely *A. arachidicola* and *A. parasiticus*, and *A. nomius*. Using the invertebrate model of fungal disease *Galleria mellonella*, we found that strains of the same species varied widely in their virulence profiles, and that *A. flavus* strains were not more virulent than strains of the non-pathogenic species. Characterization of secondary metabolites for all four species for two clinically relevant conditions, the temperature of the human body and salt concentration of human tears, revealed that temperature

changes impacted metabolite production in all species. In contrast, we found a surprising lack of impact of salt on secondary metabolite production. Although our chemical analyses showed that each species produced few unique metabolites, our genomic analyses revealed that *A. flavus* strains shared seven biosynthetic gene clusters that were absent in strains from the three non-pathogenic species. Furthermore, we identified over 2,000 orthologous proteins unique to *A. flavus*, which were enriched in the gene ontology categories of transmembrane transport and oxidoreductase activity. Our work provides additional puzzle pieces in the study of *A. flavus* pathogenicity and its prevalence in human infections compared to its close, non-pathogenic relatives.

469F COFUN: Final report on the construction of the genome wide-knockout library in *A. fumigatus* *Michael Bromley*[1], Can Zhao[1], Lauren Dineen[1], Isabelle Storer[1], Thorsten Heinekamp[2], Axel Brakhage[2], Daniela Delneri[1], Paul Bowyer[1], Ressa Sinaia Lebedinec[1] 1) University of Manchester, Manchester Fungal Infection Group, Manchester, United Kingdom ; 2) HKI, Jena, Germany.

Genome-wide knockout (KO) libraries have been used to great effect to establish an in depth understanding of microbial functional genomics. Despite their obvious value, no KO collection is available in a pathogenic filamentous fungus. To address this, in 2017 we initiated the COFUN project to generate a genome-wide collection of KO mutants in the leading mould pathogen *Aspergillus fumigatus*. Here we will present our final report, updating on our progress and highlighting how the libraries can be accessed. We will also describe how the libraries can be used in competitive fitness studies to elucidate interconnected networks of genes that are critical for stress adaptation in *A. fumigatus*.

470W QTL Mapping and Bulk Segregant Analysis to determine natural polymorphisms associated with CO_2 tolerance in *Cryptococcus neoformans* *Benjamin Chadwick*[1], Xiaofeng Xie[1], Xiaorong Lin[1] 1) University of Georgia.

Cryptococcus neoformans is a ubiquitous free-living soil yeast. It is also an opportunistic pathogen that causes about 223,100 cases of cryptococcal meningitis per year, killing over 180,000 people. The pathogenicity of *C. neoformans* relies on its adaptation to the host conditions. An important difference between its natural environment and the mammalian host is concentration of CO_2. CO_2 levels of ambient air is about .04%, and the percent CO_2 in the host is over 100 fold higher (~5%). It was recently found that while clinical isolates are tolerant to high levels of CO_2, many environmental isolates are sensitive and therefore attenuated in animal models. The genetic basis responsible for the adaptation of clinical strains and some environmental isolates to this high level of CO_2 is unknown. To determine what genetic loci are associated with CO_2 tolerance in natural isolates, we used quantitative trait loci (QTL) mapping with close to 400 F1 progeny of a cross between the CO_2-tolerant clinical isolate H99 and a CO_2-sensitive environmental isolate A7-35-23. The progeny of this cross exhibited a wide range of growth ability in host levels of CO_2, with some more extreme than either parent, indicating CO_2 tolerance is a complex trait controlled by multiple regions throughout the genome. Our QTL mapping analysis reflected this observation, as we found multiple significant interacting genetic loci contributing to CO_2 tolerance. Bulk segregant analysis coupled with pooled genome sequencing of F5 progeny with distinct growth in CO_2 was applied for finer mapping of the specific polymorphisms which contribute to this complex trait. The role of a few genes identified in these QTL regions in CO_2 tolerance was verified by targeted gene deletion. Further characterization of these polymorphic alleles will help uncover mechanisms behind CO_2 tolerance and their contribution to Cryptococcosis.

471T Transcriptome Analysis of the Entomopathogenic Fungus *Culicinomyces clavisporus* *Dana Foresman*[1], Aurelien Tartar[1] 1) Nova Southeastern University.

Culicinomyces clavisporus is an entomopathogenic fungus that can infect mosquito larvae. It has been shown to be effective against vectors of public health importance, such as *Culex quinquefasciatus, Aedes aegypti, and Anopheles stephensi*. Whereas most fungal entomopathogens infect hosts through the cuticle, *C. clavisporus* initiates infection through ingestion (*per os*). This unique infection strategy suggests that the *C. clavisporus* genome may be mined for novel pathogenicity factors with the potential for vector control. To this end, a transcriptome analysis was initiated. The strain *C. clavisporus* ARSEF 582 was grown in modified PYG liquid cultures that was supplemented with whole, insect larvae (*Galleria mellonella*) in an effort to elicit the expression of genes involved in host-pathogen relationships. After a 14 day growth period, total RNA samples were extracted and processed for cDNA library construction and Single Molecule Real Time (SMRT) sequencing (PacBio platform). A total of 3,512,145 sequences have been produced, with an average sequence length of 1732 bp. Assembly of these reads was completed using CD-HIT-EST and revealed 8,266 unigenes. Transcript annotation will aid in understanding the molecular basis of *C. clavisporus* pathogenicity process as well as its potential as a bioinsecticide.

472F Conservation, expansion and functional adaptation of Transcriptional Factor Repertoire in the *Fusarium oxysporum* Species Complex *Houlin Yu*[1], Sajeet Haridas[2], Richard Hayes[2], Hunter Lynch[1], Sawyer Andersen[1], Alex Liu[1], Gengtan Li[1], Domingo Martínez-Soto[1], Shira Milo-Cochavi[1], Dilay Hazal Ayhan[1], Yong Zhang[1], Igor Grigoriev[2], Li-Jun Ma[1] 1) Department of Biochemistry and Molecular Biology, University of Massachusetts Amherst, Amherst, MA 01003, USA. ; 2) Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, University of California Berkeley, Berkeley, CA 94720, USA..

The cross-kingdom fungal pathogen *Fusarium oxysporum* species complex (FOSC) includes plant pathogens that cause severe vascular wilt diseases on many agricultural important plants and human pathogens that pose serious threats to public health. Each *F. oxysporum* genome can be divided into a core and an accessory region. While the core, organized in core chromosomes (CCs), executes essential housekeeping functions, the accessory genomic compartment, organized in accessory chromosomes (ACs), contributes to the host-specific adaptation. How ACs and CCs coordinate their gene function to accomplish the host-specific pathogenicity within the same genome is an intriguing question. This study probes into this question by investigating the conservation and diversification of transcription factors (TFs), key regulators that coordinate gene expression and probably coordinate the crosstalk between these two compartments. The comparison of the repertoire of TFs (*i.e.* TFome) among 15 *F. oxysporum* and 15 other ascomycete fungal genomes reveals a clear positive correlation ($y = 0.07264x - 190.9$, $r^2 = 0.9361$) between the number of genes (x) and TFome size (y) of an organism. Primarily due to the acquisition of ACs, we observed increased TFome sizes among FOSC genomes. Among a total of 48 families, 14 families (e.g. TATA-box binding protein) are highly conserved. In contrast to these remarkably stable TFs, 30 families are clearly expanded in various degree among FOSC genomes. Most interestingly, we observed the expansion of members of Zn2-C6 fungal-type TFs (eg. *FTFs* and *EBR*s; *Znf1*, *Ctf2* and *Fow2* homologs) and members of Zinc Finger C2H2

TFs (eg. *Cos1* and *PacC* homologs) among FOSC genomes. Unique expansion of some TFs, driven by ACs, may provide clues to host-specific interactions. For example, *Ftf1*, the TF involved in the tomato pathogenicity is most significantly expanded (10 copies of accessory *FTF*s) in the tomato pathogen Fol4287 genome and the expression of eight out of 10 were induced during plant colonization. Differently, the TF *Ren1* is most significantly expanded (seven copies of accessory *REN*s) in the Arabidopsis pathogen Fo5176 genome and two of them were induced during plant colonization. Collectively, this comparative study highlights potential novel regulatory mechanisms or modifications of existing regulatory pathways by acquiring additional TFs. The repeated and independent expansion of certain TF families among FOSC genomes may suggest a fine-tuning of regulatory networks in the environmental adaptation of this group of diverse organisms to engage in complex cross-kingdom interactions with different hosts.

473W ***Comparative QTL Mapping of Predation Resistance in a Microbial Predator-Prey System*** *Thomas Sauters*[1], Cullen Roth[1], Debra Murray[1], Sheng Sun[1], Anna Floyd-Averette[1], Chinaemerem Onyishi[2], Robin May[2], Joseph Heitman[1], Paul Magwene[1] 1) Duke University, Durham, NC; 2) University of Birmingham, Birmingham, UK.

Many fungal pathogens are hypothesized to have an origin of "accidental pathogenesis" in large part driven by interactions with environmental predators such as amoebae. To investigate this hypothesis, we developed a quantitative assay that measures resistance to amoeboid predation. Using this assay, we QTL (quantitative trait locus) mapped two genetically diverse crosses of *Cryptococcus neoformans* and *Cryptococcus deneoformans* and found a homologous major effect QTL that explains over 50% of the variation for amoeba resistance. This QTL was also found to be pleiotropic with the important virulence factor, melanization, in the *C. neoformans* background. Prior work in the field has highlighted that both amoeba resistance and ability to melanize are indicative of virulence capacity; however, our experiments that tested the correlation between resistance to amoeba, resistance to mammalian macrophages, and virulence in mice have yielded a lack of correlation. Given these results, the story behind the "accidental pathogen hypothesis" may be more complicated than originally believed, and commonly held beliefs about the connection between amoeba, macrophages, and mice may change as more diverse isolates of *Cryptococcus* are studied.

474T **Genome-wide identification of sexual-reproduction genes in fission yeast via transposon-insertion sequencing** *Blake Billmyre*[1], Michael Eickbush[1], Caroline Craig[1], Jeffrey Lange[1], Christopher Wood[1], Rachel Helston[1], Sarah Zanders[1,2] 1) Stowers Institute for Medical Research, Kansas City, MO; 2) University of Kansas Medical Center, Kansas City, KS.

Sex and meiosis genes are poorly conserved and and thus identifying genes involved in sex based on homology is often highly difficult to impossible. Using the model fission yeast *Schizosaccharomyces pombe*, we have developed an assay utilizing massively parallel in vivo transposition coupled with high-throughput targeted sequencing (TN-seq) to quantitatively measure the contribution of every *S. pombe* gene to sexual reproduction. We saturated a library of cells with a single transposon insert per cell at extremely deep resolution (1 unique insert every 35 bases in the genome on average) and determined the location and frequency within the pool of all inserts via sequencing. We then self-mated our library of cells, collected only viable spores (gametes), and re-measured the location and frequency of transposon inserts. Using this assay, we identified 532 candidate genes whose disruption results in quantitatively less productive sex. More than 200 of these candidates have not been previously annotated to be involved in sex, meiosis, or sporulation. From our set of sexual reproduction mutants, we observed an unexpected phenotype of poor gamete health exemplified by two mutants in our screen (*plb1* and *alg9*). *plb1*Δ and *alg9*Δ mutants produced normal quantities of viable meiotic products but those gametes appeared to be less healthy than wildtype and were rapidly outcompeted by wildtype competitors after germination, despite exhibiting normal growth prior to sex. We also identified an unnamed gene that was required for growth at low cell density, which we have dubbed *sdg1*(**s**ocial **d**istancing **g**ene). This approach will be valuable in exploring evolution of sex and meiosis genes across fungi and more broadly in understanding conservation and loss of sex genes.

475F **Segmental duplication, repeat-induced point mutation, and chromosome relocation in Neurospora crassa: non-coding regions are junkyards for de novo elements and factories for evolution** *Rudy Diaz*[1], Zheng Wang[1], Jeffrey P. Townsend[1,2] 1) Department of Biostatistics, Yale School of Public Health, New Haven, Connecticut 06511, USA; 2) Department of Ecology and Evolutionary Biology, Program in Microbiology, and Program in Computational Biology and Bioinformatics, Yale University, New Haven, Connecticut 06511, USA.

Orphan genes lack homology with genes from other evolutionary lineages. Their origins have been attributed to gene duplication followed by fast divergence, horizontal gene transfer, relocation and rearrangement, and long repeats abundant in non-coding sequences. The fungus Neurospora crassa is known to lack recent gene duplications due to its repeat-induced point-mutation defense system. However an in-depth analysis for orphan genes has not been performed. Therefore, we identified 636 orphan genes in Neurospora crassa and investigated their roles in development and ecology. The orphan genes are often clustered within extensive non-coding regions that exhibit condensed chromosome arrangement, gene duplications, and relocations; more than 60% of orphan genes aggregate in clusters adjacent to telomeres. To infer possible functional roles of orphan genes in metabolic regulation, we analyzed their transcriptional activity across the N. crassa life cycle under a variety of growth conditions. Most orphan genes appear to be non-essential in metabolism, and are not components of core regulatory networks. However, 299 orphan genes that were expressed during the sexual stage were also dynamially regulated during asexual growth; among these, 64% (190) were differentially regulated in response to distinct carbon resources. Previous data were reanalyzed for N. crassa grown on 16 different carbon supplies, including no carbons, artificial media with simple sugars such as sucrose, fructose, and maltose which promote asexual growth, complex carbohydrates such as cellulose, xylose, and furfural which promote sexual development, and natural media like maple sap agar and carrot medium which support both sexual and asexual growth. 179 orphan genes (60%) were regulated in response to non-preferred carbon sources like furfural, a wildfire-produced compound. Accordingly orphan genes may play roles in sexual adaptation and in mediating fundamental, developmental regulatory processes such as the asexual-sexual switch. Gene duplication, chromosomal rearrangement, and fast divergence introduced by repeat-induced point mutation likely play key roles in the origin of orphan genes in Neurospora crassa. Furthermore, segmental clustering and relocation may contribute to the functional renewal and integration of de novo genes into pre-existing networks.

476W The C_2H_2 transcription factor SltA is involved in conidial germination and hyphal elongation in *Aspergillus fumigatus* *Tim Baltussen*[1], Norman van Rhijn[2], Paul Verweij[1], Michael Bromley[2], Willem Melchers[1] 1) Department of Medical Microbiology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, the Netherlands; 2) Manchester Fungal Infection Group, Division of Infection, Immunity and Respiratory Medicine, Faculty of Biology, Medicine and Health, University of Manchester, Manchester.

Aspergillus fumigatus is a filamentous saprophytic fungus that produces multinucleate tubular cells termed hyphae. Hyphal tip extension occurs through the synthesis and addition of new cell wall and membrane at the apical plasma membrane. This highly polar extension of the tip helps *A. fumigatus* to penetrate and invade blood vessels and tissue which results in invasive aspergillosis (IA). Before the fungus grows in a highly polarized manner, the conidium breaks dormancy and the reactivated cell expands isotropically before it undergoes localized expansion of the cell membrane which leads to a tubular outgrowth. Potential regulators of germination and early growth remain largely unexplored. We selected fourteen transcription factors (TFs) upregulated during isotropic and/or polarized growth using transcriptomic data from our previous study. TF knock out mutants were generated in the parental strain MFIG001 (WT), which is deficient in non-homologous end joining. We utilized bright-field and fluorescence microscopy to examine conidial germination of the TF null mutants and WT temporally (0 to 12h). We observed a markedly distorted hyphal elongation morphology in the *ΔsltA* mutant that is not apparent in WT strain and other TF null mutants used in this study. The *ΔsltA* mutant had a germination rate almost two times higher after 6 h compared with the WT and Δ*sltA*rec strain. Germination rate was similar after 8 h of growth and reached around 95% in all strains. Hyphae of the *ΔsltA* mutant hyperbranched and some of the branched hyphae annihilated tubular elongation in RPMI-1640 medium. After 72 h the *ΔsltA* strain showed reduced colony growth on Aspergillus Minimal Medium when compared with the WT and *ΔsltA*rec strains. However, when exposed to cell wall stress agents (calco fluor white, congo red and caspofungin) the relative colony size increased in the Δ*sltA* strain compared with WT and Δ*sltA*rec strain. This suggests a role for *sltA* in cell wall biosynthesis and membrane stability. Altogether, we identified a role for the transcription factor *sltA* in germination and tubular growth of the hyphal tip. Additional experiments will be performed to analyze the molecular mechanisms underlying the distorted hyphal elongation phenotype in the *ΔsltA* strain.

477T Characterizing the role of anaerobic fungi in lignocellulolytic microbial communities and the gut mycobiome of herbivorous non-human primates *Katharine Dickson*[1], Michelle O'Malley[1] 1) University of California - Santa Barbara, Santa Barbara, CA.

The gut microbiome plays a critical role in the health of herbivorous non-human primates (NHPs), and its composition is linked to features of their ecology, behavior, and responses to habitat destruction. Little is understood about the plant-degrading activities of fungi in the gut microbiomes of herbivorous NHPs. Historically, research on the gut microbiomes of herbivorous NHPs has focused on their prokaryotic members, or employed methods of surveying their eukaryotic members that do not fully resolve the fungal population of the microbial community (the mycobiome). In particular, these methods often fail to identify anaerobic gut fungi of the phylum Neocallimastigomycota, which are widely recognized to constitute the majority of the gut fungal population in large herbivores, where they secrete an arsenal of carbohydrate active enzymes (CAZymes) to degrade ingested plant biomass. Anaerobic gut fungi have been identified in fecal samples of the Western lowland gorilla (*Gorilla gorilla gorilla*), and are likely to be distributed more broadly across highly herbivorous primates, particularly the foregut-fermenting members of subfamily Colobinae, who possess chambered stomachs and whose gut microbiota exhibit characteristics convergent with those of ruminants. To characterize the mycobiome of these primates, fecal samples have been obtained from wild mantled howler monkey (*Alouatta palliata*) and black howler monkey (*Alouatta pigra*), and will be collected from captive *G. gorilla gorilla*, black-and-white colobus (*Colobus guereza*), and Francois' langur (*Trachypithecus francoisi*). Our work seeks to test the hypothesis that anaerobic fungi can be cultivated from *G. gorilla gorilla*, *C. guereza*, and *T. francoisi* fecal samples, which would suggest a functional role for these fungi in the primate digestive tract. Shotgun sequencing of genomic DNA in samples from all species will be employed along with ITS and LSU amplicon sequencing to probe fungal metagenomes and characterize their CAZyme repertoire. Full characterization of the community membership, population dynamics, and lignocellulolytic capacities of anaerobic fungi in herbivorous NHP mycobiota will generate critical insights about the microbial ecology of herbivory in primates that are likely to not only play a central role in conserving these vulnerable species, but also lead to a fuller understanding of the evolutionary trajectory of herbivory among all primates, including humans.

478F Transcriptomic analysis of *Schizophyllum commune* in response to the lignin treatment *Jaehyuk Choi*[1], Ha Thi Kim Nguyen[1], Junhyueok Nam[1], Hyon Jin Park[1] 1) Incheon National University.

White-rot fungi have potentials to degrade complex chemicals such as lignin by virtue of their extracellular enzymes. The basidiomycete *Schizophyllum commune* is a white-rot fungus which show wide varieties of enzyme secretion in decolorizing synthetic dyes. Through series of screening and mating, a pair of monokaryotic strains of *S. commune* were chosen. In this study, we sequenced the whole genome for one of the pairs, *S. commune* IUM1114-SS13 which can decolorize at least ten synthetic dyes. Its genome size was estimated as 40.5 Mb and 14,231 coding sequences were annotated. To explore genes of lignin degradation, RNA-Seq was performed for the samples harvested at 24 and 36 hours post treatment of lignin, respectively. Differential gene expression analysis found that 18 and 25 genes were up-regulated in response to the lignin treatment. Among these genes, 13 genes including oxidoreductase and monooxygenase genes were found in common. Those genes are thought to be involved in initial setup for lignin modification in *S. commune.*

479W The active microbial communities of oil degradation – exploring bioremediation of mine waste water *Petter Madsen*[1], Maliheh Mehrshad[3], Anna Rosling[2], Hanna Johannesson[1] 1) Uppsala University Department of organismal biologi; 2) Uppsala University Department of ecology and genetics; 3) SLU Department of Aquatic Sciences and Assessment.

Crude oil and petroleum products are of specific concern in pollution studies due to their structural complexity, slow biodegradability, biomagnification potential, and the serious health hazards associated with their release into the environment. Hence, strategies for cleaning up of petroleum hydrocarbon (PHC) pollutants from the environment is a priority.

Bioremediation, which makes use of natural microbial biodegradation activity, is an attractive alternative and/or addition to physico-

chemical methods for restoration of PHC polluted environments, since it is more environmentally friendly and less resource demanding. However, for the development of effective bioremediation methods we need better understanding of the indigenous microbial community that use these compounds as an energy resource in the polluted areas, what controls their growth and activity, and what decay products are formed in the process. In this project, microbial community samples were collected in the underground mining facility in Kiruna underground mine (KUJ), in Lappland, Sweden, which is contaminated with pollutants due to the use of petroleum products for maintenance of machinery.

Microbial communities were characterized using metagenomics and metatranscriptomics of water and sediment samples collected throughout KUJ and the outflowing water from its tailing ponds, to describe the diversity of the indigenous microbial community and the expression of PHC degrading genes. Further, water samples were collected for characterization of the chemical reactivity of contaminants. This allows us to explore whether microbes in KUJ are primed towards PHC-degradation, and if there is a succession in the PHC degrading microbial community of KUJ depending on residence time of the circulating water. And finally, if the microbial community in KUJ is better at breaking down PHC when compared to previously described similar but pristine areas.

In parallel, we are conducting a large-scale cultivation effort to isolate individual strains and species of the community, that are able to utilize oil as sole carbon source. We have so far isolated *Fusarium oxysporum*, and are currently isolating and identifying additional strains and species. These will be used to specifically test decay capacity and optimal remediation conditions.

This project will build the foundation for developing local *in situ* bioremediation of KUJ.

480T Integrating multifaceted genetic tools to gear up the discovery of fungal mechanisms of wood decay *Jiwei Zhang*[1], Weiran Li[1], Jonathan Schilling[1], Hugh D Mitchell[2], Lye Meng Markillie[2] 1) University of Minnesota; 2) Pacific Northwest National Laboratory.

Fungi evolved efficient ways to degrade and recycle carbons sequestered in woody biomass. Their degradative mechanism offers industrially-relevant toolkits for developing green technologies for plant biomass conversions. Among these, brown- and white-rot fungi are two distinctive classes of wood decomposers that dominate the carbon degradation in the forest system. During wood decomposition, white-rot fungi can completely degrade and consume all formats of carbons in lignocellulose, while brown-rot shifted its strategy to first depolymerize the lignocellulose structures and then selectively utilize carbohydrates for fungal metabolism, but leave lignins as residues. We have known that the brown-rot strategy causes a faster depolymerizing rate than white-rot, and the biochemical analysis indicated that this is largely due to the use of reactive oxygen radicals (ROS) for intensive polymer deconstruction. However, we haven't known the genetic bases driving the ROS mechanism for this brown-rot efficacy. Genomic analyses implied that brown-rot fungi have adapted special genetic inventories, at both gene and gene regulation levels, to implement and manage ROS attacks during wood decay, but their genetic functions haven't been validated. Also, the regulatory systems controlling brown-rot genes expression remain uncharacterized. One of the main obstacles to this research is the lack of available genetic tools in those multiploidy, basidiomycete brown-rot variants that are difficult to genetically manipulate. To fill these gaps to facilitate the validation/investigation of the distinctive brown-rot mechanisms, our recent work has been focusing on combining systems biology and genome-editing for large-scale brown-rot phenotypic screening. In this talk, we will report our recent progress towards building the brown-rot genetic platform and then using it to dissect the fungal mechanisms involved in fast wood biomass decomposition.

481F Prevalence of aromatic lignin monomer metabolism phenotypes in a collection of wood-inhabiting fungi and characterization of putative metabolic pathways *Leon Rogers*[1], Gerald Presley[1] 1) Oregon State University.

Despite abundant sources of lignin in waste streams and broad interest, there is currently no commercially viable path for the conversion of lignin to value-added chemicals. Fungi are by far the most ecologically significant source of lignin degradation in nature, yet many aspects of lignin modification are unknown, particularly fungal pathways for aromatic and phenolic lignin monomer decomposition. In this study 114 fungal isolates (88 species) were inoculated into liquid minimal-media containing one of five lignin-derivable monomers (LM) as sole carbon sources; vanillate, syringate, para-hydroxy benzoate, catechol or protocatechuate. Reduction in carbon substrate concentration were tracked through UV-spectrophotometry. After 7 weeks growth only 14 isolates caused 25% reductions in catechol, and every fungus reduced at least one substrate by 25%.
Screened fungi were basidiomycetes (55 genera) and ascomycetes (12 genera) from the OSU Biodegradation Lab wood-decay culture collection. While the white-rot and brown-rot decay fungi produced consistent LM reductions, they were far less active than certain ascomycete non-decay fungi. Many ascomycetes with mild to insignificant decay potential had the highest LM activity.
Top performing fungi were re-cultured to better analyze metabolites in solution, and accumulations in their biomass using HPLC and GCMS. Genetic analysis of screened fungi is currently being conducted by comparing protein sequences derived from genomes to known enzymes involved in aromatic compound decomposition pathways. Also, protein sequences for fungi that successfully grew on aromatic substrates and those that did not will be collated separately, then compared to a relativized matrix of percent reductions in carbon source. This may indicate expressible enzymes with the highest correlation to specific aromatic activity rather than all genes and metabolic pathways.
These results indicate catabolic specialization with paraphyletic development among distantly related fungi and a likely mechanism for synergies among decay communities. Genomic analysis overlaid with phenotypic characterization will help identify novel gene targets to improve the biological valorization of lignin. While the gene targets indicated must be verified through insertions or knock-out editing, these findings should provide likely sources for novel enzymatic biosynthesis in the future.

482W The extrachromosomal circular DNAs of the rice blast pathogen *Magnaporthe oryzae* contain a wide variety of LTR retrotransposons, genes, and effectors *Pierre M Joubert*[1], Ksenia V Krasileva[1] 1) University of California, Berkeley.

One of the ways genomes respond to stress is by shedding extrachromosomal circular DNAs (eccDNAs). EccDNAs can contain genes and dramatically increase their copy number. They can also reinsert into the genome, generating structural variation. They have been

shown to provide a source of phenotypic and genotypic plasticity in several species. However, whole circularome studies have so far been limited to a few model organisms. Fungal plant pathogens are a serious threat to global food security in part because of their rapid adaptation to disease prevention strategies. Understanding the mechanisms fungal pathogens use to escape disease control is paramount to curbing their threat. We present a whole circularome sequencing study of the rice blast pathogen *Magnaporthe oryzae*. We find that *M. oryzae* has a highly diverse circularome containing many genes and showing evidence of large LTR retrotransposon activity. We find that genes enriched on eccDNAs in *M. oryzae* occur in genomic regions prone to presence-absence variation and that disease associated genes are frequently on eccDNAs. Finally, we find that a subset of genes is never present on eccDNAs, which indicates that the presence of these genes on eccDNAs is selected against.

483T Heat adaptation in *Fusarium oxysporum* *Dilay Hazal Ayhan*[1], Kaito Hioki[1], Domingo Martínez-Soto[1], Serena Abbondante[2], Michaela Ellen Marshall[2], Cristina López Díaz[3], Neta Shlezinger[4], Eric Pearlman[2], Antonio Di Pietro[3], Li-Jun Ma[1] 1) University of Massachusetts Amherst, MA; 2) University of California Irvine, CA; 3) Universidad de Córdoba, Spain; 4) The Hebrew University of Jerusalem, Israel.

Fusarium oxysporum is a cross-kingdom pathogenic fungus that can cause vascular wilt disease in many plant species and can infect animals and cause local or disseminated fusariosis in humans. To be able to infect humans, fungal pathogens must overcome some biotic and abiotic stresses, such as adapting to elevated body temperature.
Focusing on temperature adaptation, we conducted comparative evolution experiments using a plant pathogenic isolate (*F. oxysporum* f. sp. *lycopersici* Fol4287) and a keratitis strain isolated from the cornea of a patient (MRL8996). Both strains were passaged 10 times through media plates with minimal or rich nutrients, at 28°C or 34°C, with 5 independent replicates.
Belonging to the same species complex, Fol4287 and MRL8996 share a core genome with an average 98% nucleotide identity. However, each genome has its own distinct accessory chromosomes (ACs) with different gene ontology enrichments and transposable element (TE) contents. At the start of the short-term experimental evolution study, the human pathogenic strain MRL8996 exhibited higher fitness at elevated temperatures, while the plant pathogen Fol4287 had more tolerance to the osmatic and cell wall stress conditions.
After 10 passages, we observed the most significant phenotypic difference among Fol4287 populations evolved under elevated temperature, showing significant improvement in heat tolerance when compared to the ancestor. As a trade-off, these populations showed a reduced growth rate in some of the other stress conditions, such as oxidative and osmotic stresses.
Sequencing of the final populations revealed signatures of weak selection in the evolved MRL8996 populations with low-frequency TE insertion events by a hyper-active Foxy element. While TEs were also highly active in Fol4287, different patterns and TE families were involved.
Strikingly, an uncharacterized accessory chromosome gene was mutated by a DNA transposon, Hormin, in 8 out of 10 34°C-passaged Fol4287 populations that also showed increased heat tolerance. The mutation site is located in between a gene block that is upregulated at elevated temperature and a gene block that is up-regulated in plant infection.
Overall, our study demonstrated although similar mechanisms were employed in different *F. oxysporum* strains, the adaptation to elevated temperatures was distinct and ACs played an important role.

484F Exploring the genomes of *Phyllosticta*, a genus with multiple lifestyles *Valerie Buijs*[1, 2], Johannes Groenewald[1], Sajeet Haridy[3], Kurt LaButti[3], Anna Lipzen[3], Francis Martin[4], Igor Grigoriev[3, 5], Pedro Crous[1, 2], Michael Seidl[6] 1) Evolutionary Phytopathology, Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; 2) Laboratory of Phytopathology, Wageningen University and Research, Wageningen, Netherlands; 3) US Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA; 4) Institut National de la Recherche Agronomique, UMR INRA-université de Lorraine, Champenoux, France; 5) Department of Plant and Microbial Biology, University of California Berkeley, Berkeley, CA, ; 6) Theoretical Biology & Bioinformatics, Utrecht University, Utrecht, Netherlands.

Members of the fungal genus *Phyllosticta* can colonize a variety of plant hosts, including several *Citrus* species such as *Citrus sinensis* (orange), *Citrus limon* (lemon), and *Citrus maxima* (pomelo). Some *Phyllosticta* species have the capacity to cause disease, such as Citrus Black Spot, while others have only been observed as endophytes. Thus far, genomic differences underlying lifestyle adaptations of *Phyllosticta* species have not yet been studied. Furthermore, the lifestyle of *Phyllosticta citrichinaensis* is ambiguous, as it has been described as a weak pathogen but Koch's postulates may not have been established and the presence of this species was never reported to cause any crop or economic losses. Here, we examined the genomic differences between pathogenic and endophytic *Phyllosticta* spp. colonizing *Citrus* and specifically aimed to elucidate the lifestyle of *Phyllosticta citrichinaensis*. We found several genomic differences between species of different lifestyles, including groups of genes that were only present in pathogens or endophytes. We also observed that species, based on their carbohydrate active enzymes, group independent of their phylogenetic association, and this clustering correlated with trophy prediction. *Phyllosticta citrichinaensis* shows an intermediate lifestyle, sharing genomic and phenotypic attributes of both pathogens and endophytes. We thus present the first genomic comparison of multiple citrus-colonizing pathogens and endophytes of the genus *Phyllosticta*, and therefore provide the basis for further comparative studies into the lifestyle adaptations within this genus.

485W Factors driving genome evolution of *Anisogramma anomala,* the Eastern Filbert Blight fungus, reveal lifestyle and pathogen biology *Alanna Cohen*[1], Guohong Cai[2], Dana Price[1], Limei Du[1], Ning Zhang[1], Thoms Molnar[1], Bradley Hillman[1] 1) Department of Plant Biology, Rutgers The State University of New Jersey, New Brunswick NJ.; 2) USDA-ARS, Perdue University, West Lafayette, IN..

Eastern Filbert Blight (EFB) is a devastating disease of European hazelnut (*Corylus avellana*), limiting commercial production of hazelnut in the United States. *Anisogramma anomala,* the causal agent of EFB, is a homothallic ascomycete in the Diaporthales. It has remained poorly understood due to experimental constraints with growing the pathogen in a laboratory setting. Here we report the annotated draft genome of *A. anomala* and investigate factors that are major drivers of genome evolution. The *A. anomala* genome is 350 Mbp, over 7x larger than genomes of related ascomycetes. However, *A. anomala* has 10% fewer protein coding genes compared to related pathogens. This massive genome expansion is driven by proliferation of repeat elements, for the most part identifiable transpos-

able elements, that constitute approximately 88% of the genome. In addition to transposon-mediated genome evolution, anti-transposon genome defense mechanisms were investigated for their effect on the *A. anomala* genome. Repeat-induced point (RIP) hypermutation recognizes locally duplicated sequences and induces C --> T point mutations through a methyl-transferase. The *A. anomala* genome encodes homologues of the genes involved in RIP, including *rid* and *dim1* in *Neurospora crassa.* Dinucleotide frequencies reveal evidence of RIP in approximately 50% of sequences distributed throughout the genome. Together, transposon and RIP activity have contributed to shaping the genomic landscape of *A. anomala* resulting in a "two-speed" genome. This is displayed as alternating blocks of non-repetitive, gene-rich regions and highly repetitive gene-poor regions with high instance of RIP activity. The non-repetitive, GC-equilibrated regions account for 7% of the genome and encode approximately 90% of protein coding genes, including highly conserved housekeeping genes involved in fungal growth and metabolism. The other 93% of the genome comprises the repetitive, highly adaptable regions that encode genes predicted to be involved in virulence and host-pathogen interactions. These AT-rich genomic regions account for less than 10% of protein coding genes, but 30% of predicted effector genes. The genomic compartmentalization of these genes supports the hypothesis of an evolutionary arms race involving gene-for-gene interactions between *A. anomala* and its *Corylus* tree host. Ultimately, the drivers of genome evolution discussed here provide insight to the lifestyle of *A. anomala* as an obligately biotrophic pathogen.

486T Has *Cercospora kikuchii* vanished in the U.S.? Comparative genomics provides new clues *Burton Bluhm*[1], Alex Zaccaron[2], Kona Swift[1], Ahmad Fakhoury[3] 1) University of Arkansas System Division of Agriculture; 2) University of California, Davis; 3) Southern Illinois University.

Cercospora leaf blight (CLB) is one of the most prevalent foliar diseases of soybean in the U.S. Although the causal agent of CLB was originally described as *Cercospora kikuchii*, recent studies have identified two other pathogens associated with CLB: *Cercospora cf. flagellaris*, and *Cercospora cf. sigesbeckiae*. Intriguingly, recent surveys of pathogens associated with CLB across the U.S. failed to detect *C. kikuchii*. This has led to the question of whether *C. kikuchii* fell victim to interspecific competition from *C. cf. flagellaris* and *C. cf. sigesbeckiae*, or if there are other genetic or epidemiological mechanisms underlying this apparent shift. To address these questions, we sequenced the genome of a historical isolate of *C. kikuchii* (isolated in the late 1990s from soybean in Indiana), and two isolates of *C. cf. sigesbeckiae* (isolated from soybean in Louisiana in 2012, and Arkansas in 2017). High quality genome assemblies were obtained for all three strains. Surprisingly, the majority of the genomes (>70%) of all three strains were virtually identical, which indicated an extremely close taxonomic relationship. Intriguingly, the portion of the genomes that differed the most between *C. kikuchii* and *C. cf. sigesbeckiae* were clustered into distinct regions that were spread broadly across the assembly scaffolds (and thus presumably the chromosomes). The regions that distinguished *C. kikuchii* and *C. cf. sigesbeckiae* were highly conserved in the two *C. cf. sigesbeckiae* strains despite the amount of time and physical distance distinguishing their original collections. Together, these factors suggest that the genomic polymorphisms distinguishing *C. kikuchii* and *C. cf. sigesbeckiae* are introgressions resulting from interspecific hybridization. The high level of genomic identity among the three strains suggests that the hybridization event was relatively recent. Furthermore, the extremely high level of genomic identity between the two *C. cf. sigesbeckiae* strains implies that sexual reproduction is either uncommon or nonexistent in this species. In sum, these findings indicate that *C. cf. sigesbeckiae* is a hybrid derived from *C. kikuchii* and another as-yet unknown *Cercospora* species, and it has a propensity for clonal propagation. These results suggest that species barriers among *Cercospora* spp. are semi-permeable to the exchange of genetic information, which will be an important consideration for breeding strategies to control *Cercospora* diseases of soybean.

487F Complete Genome Sequence of Newly Reported *Fusarium solani* Sugarbeet Pathogen *Abbeah Mae Navasca*[1], Jatinder Singh[1], Viviana Rivera-Varas[1], Upinder Gill[1], Thomas Baldwin[1], Gary Secor[1] 1) North Dakota State University, Department of Plant Pathology, Fargo, ND.

Diseased sugarbeets from Wilkin County, MN, USA were reported with symptoms of vascular wilt and the first report of unusual darkened nodes or galls attached to the sugarbeet roots. Culturing of the suspected darkened nodes/galls produced an unknown *Fusarium* species prolific in microconidia production from phialides and with macroconidia containing 7-9 septum. Initial genetic characterization of the *Fusarium* culture by Nanopore long-read sequencing followed by assembly resulted in a draft genome similar in size to other *Fusarium* spp. Identification based on 16 housekeeping genes placed the unknown *Fusarium* in the clade of *Fusarium solani* species complex (FSSC) using Fusarioid-ID (http://www.fusarium.org). Here we report the genome sequence with greater than 50x coverage by Nanopore sequencing paired with ProxiMeta high-throughput chromatin conformation capture (Hi-C) and sequenced on an Illumina NovaSeq system with 150-bp paired-end reads for chromosome-level assembly. These results highlight the unique aspects of the new *Fusarium solani* genome compared to other published *Fusarium* genomes.

488W Mini-chromosomes as drivers of genetic diversity and host-adaptation in the blast fungus *Magnaporthe oryzae* *Thorsten Langner*[1], Angus Malmgren[1], Cristina Barragan[1], Adeline Harant[1], Joe Win[1], Sophien Kamoun[1] 1) The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, Norwich, UK.

Cereal blast is one of the most threatening plant diseases worldwide and impacts the most common cereal crops, including rice, wheat, and barley. Despite a prevalent lack of sexual recombination in most natural populations, the blast fungus continuously adapts to its host plants which can lead to host-jumps and recurrent pandemics. A major driver of host-adaptation is rapid genomic changes that lead to a gain and loss of effector genes. However, the molecular details that enable rapid genomic changes are not well understood. We established a state-of-the art, multidisciplinary approach to analyse structural variations including mini-chromosomes and identify mini-chromosome associated genes that likely contribute to virulence of the blast fungus. Mini-chromosomes in wheat- and rice-infecting blast fungus isolates contribute to their genomic diversity by horizontal mini-chromosome transfer and inter-chromosomal recombination with core-chromosomes leading to lineage- or isolate-specific genome arrangements that involve effector candidates. These structural changes in the genome facilitate gene gains or losses of virulence factors and ultimately enable rapid adaptation to varying host conditions. Using this approach, we identified several effector candidates that differentiate two sub-lineages of a pandemic wheat blast lineage. Our results suggest that mini-chromosomes are major drivers of genetic diversity and host adaptation in the blast fungus. We will leverage this knowledge to identify novel virulence genes and potential sources of resistance against the blast disease.

489T A putative transcriptional activator from the *PEP* cluster in *Fusarium vanettenii* contributes to virulence on pea *Ambika Pokhrel*[1], Jeffrey Coleman[1] 1) Auburn University, Auburn, AL.

Fusarium vanettenii (*Fv*), previously known as *Nectria haematococca* MPVI and *Fusarium solani* f. sp. *pisi*, is a soil borne filamentous fungus that causes root rot disease on garden pea. Of the seventeen total chromosomes from the first sequenced genome from a representative in this fungus, three chromosomes (14, 15 and 17) are characterized to be supernumerary chromosomes, and at least one encodes host specific virulence factors. Studies have found that genes involved in pea pathogenicity (the *PEP* genes) are clustered on chromosome 14 (the *PDA1* chromosome) of *Fv*. This *PEP* cluster contains five genes, *PEP1*, *PEP2*, *cDNA3*, *PDA1* and *PEP5* wit hin a ~20 Kb locus in the *PDA1* chromosome. Among these genes *cDNA3* is particularly interesting due to its presence only in highly virulent *Fv* field isolates and its unknown function. Therefore, this research aims to elucidate the contribution of *cDNA3* in virulence and explore its function during infection. To assess the role of *cDNA3* in virulence, a *cDNA3* mutant was generated and assessed in a stem lesion assay on garden pea and revealed that virulence on pea was significantly reduced in the *cDNA3* mutant when compared to the wild type isolate. The expression of the *PEP* genes both in the presence and absence of *cDNA3* was evaluated through *in-vitro* and *in-planta* gene expression studies and revealed that cDNA3p significantly alters the expression of other *PEP* genes and could serve as a transcriptional activator. The last phase of this work involves evaluation of the subcellular localization of cDNA3p during the infection process through GFP tagging which may provide further insight on its role as a transcriptional activator.

490F Transcriptome analysis of *ras2* knockout mutant and wild type *Fusarium circinatum* strains: molecular insights into growth, development and virulence Mmatshepho Phasha[1], Mike Wingfield[1], Brenda Wingfield[1], Martin Coetzee[1], *Emma Steenkamp*[1] 1) Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Fusarium circinatum causes pitch canker of *Pinus* species in various countries across the globe. Despite its socioeconomic importance, knowledge is limited regarding the molecular basis and mechanisms governing complex traits in this fungus. Previous work on other fungal pathogens showed that growth, development and virulence/pathogenicity are regulated by Ras2 in a manner that is dependent on mitogen-activated protein kinase (MAPK) and/or cyclic adenosine monophosphate (cAMP) signaling pathways. The aim of our study was therefore to identify genes under the control of RAS2 by sequencing and analyzing the transcriptomes of a *ras2* knockout mutant (ΔFcras2) and a wild-type strain of *F. circinatum*. Comparison of the two transcriptomes showed that RAS2 regulation happens in a MAPK-dependent manner. These data further revealed down-regulation in the ΔFcras2 mutant of more than 200 genes, of which many have putative functions in cytokinesis, primary and secondary metabolism, and in virulence. Particularly interesting genes identified were those encoding a putative LaeA transcriptional regulator (potentially involved in fungal development and secondary metabolism) and a putative ankyrin repeat protein (potentially involved in cell structure and shape). We also identified various genes involved in the biosynthesis of secondary metabolites, such as fusaric acid and fusarin C, and in virulence (e.g., salicylate 1-monooxygenase and trypsin). Collectively, these results will significantly improve our knowledge of the molecular basis and mechanisms underpinning growth, development and virulence in *F. circinatum* and possibly related fungi.

491W Elucidating the obligate biotroph lifestyle of *Phyllachora maydis* *Emily Roggenkamp*[1], Joshua MacCready[1], Martin Chilvers[1] 1) Michigan State University, East Lansing, MI.

Phyllachora maydis is a fungal pathogen of *Zea mays* that causes the disease tar spot of corn. Since its identification in the United States in 2015, *P. maydis* has remained largely understudied due to difficulties surrounding its obligate nature. Indeed, not only has culturing not been possible, but also greenhouse inoculation procedures have not been replicable. Thus, since samples must be collected from the field environment, a low amount of molecular work has been performed on this pathosystem. Recently, the first draft genome of *P. maydis* was sequenced using Illumina technology. However, although this genome was an excellent resource, it is largely incomplete, contains a high number of contigs, lacks annotation, and possesses a relatively low BUSCO score. Therefore, in this study, we sought to increase the quality of the *P. maydis* genome assembly and provide annotation using multi-point transcriptomic data. Genomic DNA was sequenced using both Oxford Nanopore and Illumina sequencing to achieve a high-quality assembly in 20 contigs with a total length of 65 Mb, N50 of 5.4 Mb, and 98.6% fungal BUSCO. *In silico* annotation predicted 7,393 protein coding genes. Transcriptome data from various *P. maydis* life cycle stages will be used to validate this annotation. This higher quality *P. maydis* assembly and annotation will then be analyzed to identify candidate genes necessary for infection and colonization of *Z. mays.* Furthermore, genome comparisons will be conducted to better understand the obligate lifestyle of *P. maydis.* For example, the metabolic capacity of *P. maydis* will be modeled and compared to those of non-obligate plant pathogens in the *Fusarium* and *Magnaporthe* genera as well as closely related fungal species. Collectively, our study aims to establish a strong foundation upon which *P. maydis* obligate biotrophy can be better studied with the goal of identifying the nutrients necessary for culturing *P. maydis.*

492T Comparative analyses of effector and CAZyme profiles in *Rhizoctonia* species *Juanita Gil*[1], Alejandro Rojas[1] 1) University of Arkansas, Fayetteville, AR.

The *Rhizoctonia* species complex in the family Ceratobasidiaceae is commonly associated with its pathogenic lifestyle causing diseases in crops of economic importance. However, this family comprises saprobes and mycorrhizal symbionts associated with orchids. Based on nuclear content and hyphal recognition, anastomosis groups are distinguished within 2 major groups: multinucleate and binucleate, which differ in genome sizes, host specificity and number of secreted proteins, suggesting there might be differences in pathogenesis or their plant associations. Here, we present the comparative analyses of the profile of secreted effector proteins and carbohydrate active enzymes (CAZymes) in *Rhizoctonia* species from Arkansas, Alabama and Louisiana obtained from rice, maize, soybean, and soil, to investigate their interactions with different hosts and understand their pathogenicity mechanisms. Based on draft genome assemblies obtained using long Nanopore reads, proteins were predicted and functionally annotated. This was followed by the prediction of the secretome based on the presence of signal peptides, presence or absence of transmembrane domains, absence of mitochondrial target sequences, and identification of domains of interest. CAZymes were directly predicted from the proteome based on homology search. First, completeness of the genomes was determined based on BUSCO content recovering >75% complete ortholog genes. Between 17,223 to 19,452 genes with average length of 2,108 nucleotides were annotated, from which 7.8 and 9.2%

are secreted by our tested isolates, corresponding to around 1,600 proteins. These were used to identify potential apoplastic and cytoplasmic effectors. About 10,000 CAZymes were identified in each of the six *Rhizoctonia* isolates with 15% corresponding to plant cell wall degradation and 2% associated to the degradation of fungal cell wall. These results contribute to the understanding of the genome diversity of the *Rhizoctonia* species complex and shed light on the host range and virulence differences between different isolates.

493F Signatures of necrotrophy in genomes of the Eurotiales *Tristan Wang*[1], Elena Baugh[2], Jennifer Gonzalez[2], Kathie Hodge[1] 1) Cornell University; 2) Nazareth College.

The Eurotiales are a diverse and economically important group that include industrially relevant fungi, food spoiling agents, and plant pathogens. Mapping trophic lifestyles onto fungal phylogeny in this group suggests multiple independent origins of the different lifestyles, and in particular, necrotrophy. Multiple models in literature of necrotrophy have emerged including overwhelming host defenses by deploying various lytic enzymes, effectors, and phytotoxic secondary metabolites, and interacting with hosts on a gene-for-gene basis as observed with many pathogens with narrow host ranges. To better understand the genetic factors that allow for specialized ecology, we performed comparative analyses of publicly available genomes to attempt to characterize a genetic toolbox for necrotrophy. We hypothesize that a core functionality and genetic signature of necrotrophy within the Eurotiales is an expanded toolset to disable plant host defenses and persist in unfavorable host environments. We present a broad sampling of 41 species within the Eurotiales to determine whether genetic features important for necrotrophic lifestyle in other taxa are also important within this order. Our investigation includes secondary metabolite gene clusters, and protein orthogroups specific to clade and lifestyle, plant cell wall degrading potential though expansion of key CAZyme families, and repertoire of putative effectors, which are important in plant pathogenesis. Gains and losses of key genes suggest there are multiple ways to succeed as a necrotroph within the Eurotiales.

494W A near-complete genome assembly of the tomato pathogen *Cladosporium fulvum* reveals a compartmentalized genome architecture and the presence of a dispensable chromosome *Alex Zaccaron*[1], Li-Hung Chen[1,2], Anastasios Samaras[1], Ioannis Stergiopoulos[1] 1) University of California, Davis, CA; 2) National Chung Hsing University, Taichung, Taiwan.

The tomato pathogen *Cladosporium fulvum* has been extensively used as a model species to study plant-microbe interactions. The first reference genome of this pathogen, released a decade ago, has been a valuable resource to provide insights into the biology of the fungus. However, its high repetitive DNA content prevented a contiguous assembly and further prohibited the analysis of its genome architecture and the mapping of its genes to chromosomes. In this study, we combined long-read sequencing technology with the Hi-C chromatin conformation capture technique to produce a high-quality and near complete genome assembly and gene annotation of a race 5 isolate of *C. fulvum*. The resulting genome assembly contains 67.17 Mb organized into 14 chromosomes, all of which were assembled telomere-to-telomere. Notably, chromosome 14 is only 460 kb in size and contains a total of 25 genes that all encode hypothetical proteins. PCR assays revealed that chromosome 14 was absent in 19 out of 24 isolates of a world-wide collection of *C. fulvum*, indicating that this chromosome is dispensable, thus making *C. fulvum* currently the second species of Capnodiales shown to harbor dispensable chromosomes. A total of 14,690 genes were predicted in the genome of *C. fulvum* Race 5 with an estimated completeness of 98.9%, currently one of the highest among the Capnodiales and a considerable improvement over the previous reference genome of *C. fulvum* strain 0WU with 95.9% completeness. Genome structure analysis revealed a compartmentalized architecture composed of gene-dense and repeat-poor regions interspersed with gene-sparse and repeat-rich regions. Significant enrichment of genes encoding candidate effectors located in gene-sparse regions was observed, which is in accordance with the “two-speed genome” model of evolution. Finally, the new reference genome of C. *fulvum* presents several notable features and is a valuable resource for studies in plant pathogens.

495T Assembly and annotation of the mitochondrial genomes of four powdery mildew pathogens reveals remarkable variation in size and nucleotide composition *Alex Zaccaron*[1], Jorge De Souza[1,2], Ioannis Stergiopoulos[1] 1) University of California, Davis, CA; 2) Federal University of Lavras, Brazil.

Powdery mildews represent a large and diverse group of economically important obligate biotrophic pathogens that cause disease in a wide range of monocots and dicots. Despite their importance, limited information exists on their genomes, particularly on their mitochondrial genomes. In this study, we assembled and compared the mitochondrial genomes of the powdery mildew pathogens *Blumeria graminis* f. sp. *tritici*, *Erysiphe* pisi, *Erysiphe* necator, and *Golovinomyces cichoracearum*. Gene content and organization were similar among the mitochondrial genomes of the four Erysiphales. However, they exhibited large variation in size, ranging from 109800 bp in *B. graminis* f. sp. *tritici* to 332165 bp in *G. cichoracearum*, which possessed the largest mitochondrial genome of a fungal pathogen reported to date. Difference in mitochondrial genome sizes was mostly caused by differences in number and size of introns, with sizes ranging from 501 bp to 11605 bp. In contrast to typical fungal mitochondrial genomes that have low GC content, comparative genomic analysis revealed that the mitochondrial genomes of *B. graminis* f. sp. *tritici* and *G. cichoracearum* contain atypical GC-rich isochore-like regions that intersperse GC-poor regions. This organization results in an unusual bimodal GC content distribution in the mitochondrial genomes of *B. graminis* f. sp. *tritici* and *G. cichoracearum* that was not previously reported in other fungi. Moreover, the cytochrome *b* (*cob*) genes of *E. necator*, *E. pisi*, and *G. cichoracearum* were rich in introns, which harbored open reading frames encoding reverse transcriptases not commonly observed in fungi that were likely acquired via horizontal transfer. Comparison among 703 fungal *cob* genes revealed that *G. cichoracearum* had the longest *cob* gene (45 kb) among fungi due to large number of introns (n=13) and their size (average = 3397 bp). Collectively, these results provide novel insights into the organization of mitochondrial genomes of powdery mildew pathogens and represent valuable resources for population genetic and evolutionary studies.

496F Exploring the Carbohydrate-active enzyme profiles of Ophiostomatoid fungi *Kamaldeep Bansal*[1], Sarah Barthle[1], Nemat Keyhani[1] 1) Department of Microbiology and Cell Science, University of Florida, Gainesville, FL..

Ophiostomatoid fungi are a polyphyletic group of ascomycetes from orders Ophiostomatales and Microascales. These fungi form symbiotic associations with bark and ambrosia beetles, some of which serve as tree pests and cause significant economic losses. While the bark beetles feed primarily on phloem tissues of host trees, ambrosia beetles typically attack diseased trees to establish fungal gardens

within the sapwood of host plants. The fungal gardens produce conidia, which serve as the primary source of nutrition for beetles. To fulfill their nutritional requirements, both bark and ambrosia beetles depend on fungi as their key obligate nutritional symbionts. To gain insight into the nature and extent of fungal and plant cell wall degrading capabilities of symbiotic fungi, we generated and compared the carbohydrate-active enzyme profiles (CAZomes) of fourteen Ophiostomatoid fungi. Among the fourteen Ophiostomatoid genomes, the CAZome repertoire accounts for 2.5-4.2 percent of the total genes. Comparative analysis of these CAZOmes revealed specialized polysaccharide degrading capabilities of Ophiostomatoid fungi, with glycoside hydrolases being the largest and polysaccharide lyases being the smallest enzyme classes. In addition, carbohydrate enzyme profiling using various substrates revealed enhanced utilization of various substrates by the laurel wilt pathogen, *Raffalea lauricola* as compared to the related but non virulence species, *R. arxii*.

497W The latent pine pathogen *Diplodia sapinea* contains two dispensable chromosomes with distinct genomic characteristics Preston Shaw[1], Benoit Laurent[2], Brenda D. Wingfield[1], Bernard Slippers[1], Michael J. Wingfield[1], Benjamin Penaud[2], Pedro W. Crous[3], Wubetu Bihon[1,4], *Tuan A. Duong*[1] 1) University of Pretoria, Pretoria, South Africa; 2) French National Institute of Agronomy, Univ. Cestas, France ; 3) Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; 4) World Vegetable Center, Eastern and Southern Africa, ILRI Campus, Addis Ababa, Ethiopia.

Diplodia sapinea (Dothideomycetes) is an opportunistic fungal pathogen of mainly *Pinus* spp., which is found in many parts of the world where these trees are native or planted as exotics. The fungus can remain in infected plants as an endophyte in the absence of symptoms for long periods of time, but can cause serious disease including shoot blight, stem cankers and tree death. The aggressiveness of *D. sapinea* has been found to vary amongst isolates, but the genetic mechanisms that underpin this characteristic is unknown. We sequenced and assembled the genomes of three *D. sapinea* isolates (CMW39103, CMW190 and CMW45410) using a combination of Nanopore and Illumina sequencing technologies. These genomes were assembled to a high level of contiguity and completeness and were found to have 14, 15 and 16 putative chromosomes, respectively. Comparing three assemblies revealed the presence of two dispensable chromosomes (DCs) of 0,46 Mb and 0.64 Mb that encoded for 80 and 152 proteins, respectively. The genomic characteristics of the DCs were remarkably different to those of the core chromosomes in that they had lower GC content, lower gene density and a greater proportion of transposable elements. Sequence homology analyses suggested that the DCs were likely acquired horizontally. Low-coverage Illumina resequencing of seven additional isolates from six countries indicated that one of the DCs was common (present in 9 out of 10 isolates), only one isolate had the second DC and this isolate also had the first DC, and one isolate had neither of these DCs. Sequence analyses indicated that genes on the DCs are rapidly evolving suggesting that they serve as evolutionary hotspots in the genome of *D. sapinea*. Pathogenicity trials conducted on *Pinus patula* seedings showed no apparent correlation between the presence of DCs and isolate aggressiveness and further work is necessary to determine their functions.

498T Study of the transcriptional regulation of the host-pathogen interaction between *Ulmus americana* and *Ophiostoma* spp *Thais Campos de Oliveira*[1,2,3,4], Nastasia Freyria[2,4], Jorge-Luis Sarmiento-Villamil[1,2,4], Ilga Porth[1,2,4], Philippe Tanguay[3,4], Louis Bernier[1,2,4] 1) Centre d›étude de la forêt , CEF, Québec, QC; 2) Institute of integrative biology and systems, IBIS, Québec, QC; 3) Centre de foresterie des Laurentides, CFL , Québec, QC; 4) Université Laval, Québec, QC.

American elm (*Ulmus americana*), highly prized for its ornamental value, has suffered two successive outbreaks of the vascular wilt known as Dutch elm disease (DED) caused by two closely related ascomycete fungi. The first pandemic was caused by *Ophiostoma ulmi* and lasted from *ca* 1920 to 1970. The second, ongoing pandemic started in the 1960's and has been even more devastating than the first one. The causal agent is *O. novo-ulmi* . In order to identify genes linked to the pathogenicity of different *Ophiostoma* taxa, as well as genes linked to the immune response of elm following infection, we inoculated *U. americana* saplings with seven strains representing the moderately aggressive *O. ulmi*, three genetic lineages within the highly aggressive *O. novo-ulmi*, the related pathogen *O. himal-ulmi* which occurs in natural balance with Himalayan elms, and the saprobe *O. quercus*. Total RNA was extracted from elm tissue at 3 and 10 days post infection (dpi) and subjected to RNASeq. Transcripts were assigned to either elm or fungus by mapping to *Ulmus americana* or *Ophiostoma novo-ulmi* reference genomes. Differential expression analyses were performed on a total of 8 640 *Ophiostoma* genes and 23 435 *Ulmus americana* genes. Gene expression in the two organisms differed depending on the strain inoculated and the length of infection. Genes overexpressed in the more virulent strains of *Ophiostoma* included genes that encode hydrolases that possibly act synergistically, as well as genes for effector-like proteins (cytoplasmic effectors). A deletion mutant of *O. novo-ulmi* lacking the Ogf1 transcriptor factor did no longer express the gene encoding cerato ulmin, a protein considered to be a parasitic fitness factor. In elms, overexpressed genes were linked to the synthesis of secondary metabolites in the presence of the most virulent strains of *Ophiostoma*, as well as genes linked to the degradation of xenobiotics. Elms also had a different transcriptional behavior when infected by the most virulent species at 10 dpi. Based on analysis of fungal transcripts in planta, we selected 18 *Ophiostoma* candidate pathogenicity genes for further functional analysis based on the recovery, inoculation and phenotyping of CRISPR-Cas9 targeted deletion mutants.

499F Decoding wood decay mechanisms in *Armillaria* species using new genomes *Neha Sahu*[1], Zsolt Merényi[1], György Sipos[2], László G. Nagy[1] 1) Synthetic and Systems Biology Unit, Biological Research Center, Szeged, Hungary; 2) Functional Genomics and Bioinformatics Group, Research Center for Forestry and Wood Industry, University of Sopron, Sopron, Hungary.

Agaricomycetes exhibit diverse wood-decay strategies, making them important players in forest ecosystems as well as in biotechnology. Previous studies classified wood-decaying Agaricomycetes as white-rot or brown-rot based on their patterns of lignin-decay. However, recent fungal -omics data have revealed species that do not clearly fall into this dichotomous classification. Here, we focus on *Armillaria* species, which include wood-decaying saprotrophs as well as forest pathogens, and are reported to be white-rot fungi. Our previous results from wood-decay -omics in two *Armillaria* species revealed an atypical white-rot behavior, resembling soft rot, a decay-type restricted to Ascomycota. To explore the lignocellulose degrading toolkit of these fungi, we assembled a dataset of 132 fungi from various nutritional lifestyles across Basidiomycota and Ascomycota, including 7 newly sequenced *Armillaria* genomes, along with 7 previously published *Armillaria* genomes and 6 other Physalacriaceae species. Using this dataset we inferred gene gain/loss patterns and investigated evolution of wood-decay strategies. Phylogenetic PCAs based on gene copy numbers of plant cell wall degrading enzymes (PCWDEs) such as cellulases, hemicellulases, pectinases and ligninases showed a clear separation of *Armillaria* species

from typical white-rot fungi. *Armillaria* species have larger genome sizes relative to their sister taxa, due to duplications, especially PCWDEs. We noticed that apart from genome expansion, these species also exhibit shared gene families with Ascomycota, which otherwise were reduced in typical white-rot fungi. These shared gene families included not only PCWDEs, but also families related to aromatic compound degradation, pathogenicity and transport. This suggests that the wood-decay machinery in *Armillaria* species was modified during evolution, resulting in genomic traits resembling soft-rot type decay. Our findings can aid in explaining the diversity and evolution of unusual wood-decay apparatus in Agaricales, as well as define new decay lifestyles that are similar yet distinct from the existing classification.

500W Uncovering long non-coding RNA associated with drug response in *Aspergillus fumigatus* Danielle Weaver[1], *Harry Chown*[1], Takanori Furukawa[1], Fabio Gsaller[1], Daniella Delneri[1], Paul Bowyer[1], Michael J. Bromley[1] 1) Manchester Fungal Infection Group, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester Academic Health Science Centre, Core Technology Facility, Manchester, UK.

Our understanding of the non-coding RNA (ncRNA) repertoire in the pathogenic fungus Aspergillus fumigatus is limited. Excluding housekeeping ncRNA, less than 20 ncRNAs have been identified in the sequenced type strain Af293, with the majority of these being small ncRNA. Long non-coding RNAs (lncRNAs) have emerged as important regulatory elements in many organisms and we hypothesised that they could influence the way A. fumigatus responds to antifungal drugs. RNAseq data from 6 drug exposure experiments were used to generate a novel A. fumigatus transcriptome assembly and identify lncRNA candidates. Using this assembly, we performed differential expression analysis to discover over 200 candidates which are associated with response to the antifungal, Itraconazole. Of these, we show that some lncRNA are found flanking protein-coding genes in the genome, hinting towards similar azole-resistant roles. This study has revealed novel putative lncRNA in A. fumigatus which may contribute to, and inform our understanding of, the mechanisms of drug resistance in this pathogen

501T Sifting noncanonical Basidiomycete biosynthetic gene clusters from shared genomic regions *Zachary Konkel*[1], Jason Slot[1] 1) The Ohio State University.

Fungal secondary metabolites (SMs) are a bountiful source of drug candidates, agricultural applications, and insight into the chemical ecology of their producers. The bulk of SMs are undescribed, particularly in understudied phyla. Fungal SMs are commonly derived from biosynthetic gene clusters (BGCs) comprised of colocalized genes with concerted functions. Because BGCs encode SM biosynthetic pathways within a single locus, genomic-based BGC detection algorithms have emerged as important tools in unveiling SM production. These algorithms commonly identify BGCs via function-centric approaches that search for protein family domains traditionally associated with SM production. Genomic BGC screening has been implemented extensively throughout Fungi, and Ascomycota in particular. Indeed, Ascomycete BGCs comprise approximately 95% of the fungal Minimum Information about a Biosynthetic Gene Cluster (Mi-BiG) database. Though relatively overlooked, Basidiomycota also produce SMs from BGCs, such as the neuroactive SMs muscimol and psilocybin. Basidiomycete SM repertoires are relatively understudied in part because these organisms are generally more recalcitrant to culturing in laboratory conditions than filamentous Ascomycetes. Furthermore, some Basidiomycete SMs are only produced in sporocarps or conditions unbeknownst to the researcher. To this end, genome-based screening holds promise for efficiently screening Basidiomycete SM production from individual samples. However, Basidiomycete SMs are often produced by noncanonical, lineage-specific BGC classes that function-centric software cannot detect *de novo*. To detect noncanonical BGCs, we implement a function-agnostic algorithm that identifies loci under selection for gene colocalization and subsequently filters these genomic regions using phylogenetic correlates of BGC evolution. We identify shared genomic regions that are widely distributed across the phylum, explore the functions associated with these regions, and present our approach for mining noncanonical BGCs from shared genomic regions.

502F Telomere-to-telomere genome assemblies for *Fusarium circinatum* *Lieschen De Vos*[1], Magriet van der Nest[1,2], Quentin Santana[1], Stephanie van Wyk[1], Kyle Leeuwendaal[1], Brenda Wingfield[1], Emma Steenkamp[1] 1) Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; 2) Biotechnology Platform, Agricultural Research Council, 100 Old Soutpan Road, Onderstepoort, Pretoria, South Africa.

Several genome assemblies for the pine pitch canker pathogen, *Fusarium circinatum*, is available publicly, but none of these are complete. Here we used the Oxford Nanopore Technologies (ONT) MinION sequencing platform to generate the near-complete telomere-to-telomere chromosome assemblies for two *F. circinatum* strains (FSP34 and KS17), which differ in their geographic origin, cultural growth and virulence to *Pinus* species. We observed a level of high synteny between the 11 core chromosomes of the two strains. For chromosome 12, which is apparently dispensable in *F. circinatum*, a striking length polymorphism was observed, with FSP34 missing large portions from the distal and proximal portions of the chromosome. The corresponding regions in KS17 were gene poor, repeat-rich and had lower G+C content, relative to the pattern observed for the syntenous portion shared between chromosome 12 of the two strains, as well as for the core chromosomes. Also, the location of centromeres for some chromosomes differed between the strains. In chromosome 12 of FSP34, the centromere was located telocentric (distally) and in KS17 it was subtelomeric, indicating that loss of the distal portion in FSP34 or its gain in KS17 occurred in close proximity to the centromere. The latter could have been facilitated transposable elements in KS17 that were not found in FSP34. Furthermore, the characteristic reciprocal translocation between chromosome 8 and 11 was positioned over the centromeric region, which also points to a role for centromeres in chromosome polymorphism. Our findings thus showed that the genomes of *F. circinatum* strains can differ substantially, not only in sequence but also in overall chromosome architecture, and that diverse processes might underly the observed differences. These improved genomic assemblies thus provide essential resources to aid future research into the ecology and biology of *F. circinatum* and *Fusarium* in general.

503W A reciprocal chromosome translocation within the American clade of the *Fusarium fujikuroi* species complex (FFSC) *Lieschen De Vos*[1], Kyle Leeuwendaal[1], Magriet van der Nest[1,2], Stephanie van Wyk[1], Emma Steenkamp[1], Brenda Wingfield[1] 1) Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; 2) Biotechnology Platform, Agricultural Research Council, 100 Old Soutpan Road, Onderstepoort, Pretoria,

South Africa.

The *Fusarium fujikuroi* species complex (FFSC) is a large and well-studied lineage that include many known pathogens. However, the evolution and development of fungal species is typically unpredictable, which in turn limits efforts to accurately assess risks associated with emerging pathogens. In the current study, we examined whether chromosome rearrangement might impact evolution by using the available genome sequences for taxa in the FFSC. Our analysis indicated that all species in the so-called American Clade of the FFSC are characterized by a reciprocal translocation involving chromosome 8 and 11, which was confirmed in species with genomic resources organized in chromosomal scaffolds. We then characterized the genomic regions in which it occurs by specifically focusing on genomic context and location, gene coding and organization, and basic sequence properties. These analyses demonstrated that the breakpoints of this translocation were associated with centromeric regions on each chromosome, suggesting that a recombination event occurred between chromosome 8 and 11 at this position. However, the >40kb base pair region surrounding the breakpoint were gene poor and GC depleted compared to the remainder of the respective chromosomes. Up to the breakpoint, both chromosome 8 and 11 were highly syntenic to their respective homologues in the American Clade and the remainder of the FFSC. This was also true for the portion of chromosome 8 that was translocated to chromosome 11, but not for the portion translocated to chromosome 8; this translocated portion of chromosome 11 contained multiple interspecies rearrangements. Conceivably rearrangement of chromosome 8 and 11 could led to formation of the American clade because its ancestor likely would have been reproductively isolated following the translocation event. Our future research will explore possible correlations between population structure and chromosome-level rearrangements, which will ultimately improve our understanding of how chromosome architecture and plasticity may underpin evolution in the FFSC.

504T Aspergillus as model for analyzing the fungal digestive enzyme profile -to be included in species description and classification? *Lene Lange*[1], Kristian Barrett[2], Anne S Meyer[2], Jens Christian Frisvad[2] 1) BioEconomy, Research & Advisory; 2) Bioengineering, Technical University of Denmark, Lyngby, Denmark .

Species of *Aspergillus* are rich in carbohydrate active enzymes (CAZymes) and also efficient secreters; array of CAZymes is basis for invasive power and reflects specialized habitat and substrate affinity. *Aspergillus* was chosen as model for analyzing evolution of the fungal secretome. Despite its importance for fitness, enzyme secretome is generally not used, neither in description of fungal species nor fungal classification, since overarching relationship between CAZyme profiles and fungal phylogeny/taxonomy has not been established. For robust, high precision/high sensitivity prediction of function, we used CUPP, Conserved Unique Peptide Pattern, a new, automated and validated method. Evolutionary pressure selects for having i) the needed functions, ii) found in the optimal type of proteins; the latter being important for steric accessibility, protein stability, pH & temperature optimum. Thus, to annotate, closely mimicking fitness relevance, we based our annotation on recognizing integrated "Function;Family observations". Our hypothesis, that "F;F"-observation-based annotation of digestive *Aspergillus* secretome is congruent with the phylogeny of the species was confirmed. The phylogenetic tree of *Aspergillus* is a stunning match to the dendrogram of a Yule dissimilarity calculation of "F;F" observations of the secretomes! *Identifying Global CAZyme Hotspots*: For each genome a summing-up was done of number of F;F observations, in order to rank species according to capacity and diversity of digestive enzymes. Of approx. 2000 genome sequenced fungi, the 103 *Aspergillus* species/strains ranked from topmost, *A. latus* #5, to the species with the weakest digestive secretome, *A. cejpii,* ranking #1109. For comparison, *Penicillium* ranked a bit lower, highest of 48 *Penicillium* species, was *P. sp.* #61 (unidentified, unfortunately); lowest ranking in biomass degrading capacity, *P. decumbens,* #1258. The F;F-observations-based Hotspot analysis of genome-sequenced *Aspergillus,* distinguishes the species with highest total capacity in digestive enzymes and the ones with the richest function specificity diversity. And it identifies top-degraders of cellulose, xylan, lignin or pectin. Thus, providing a short cut for enzyme discovery! Next step is to analyze the secretome of *Aspergillus (*and *Penicillium*) for both mycotoxins and enzymes. Hypothesis: Patterns of integrated evolution exist between the two, major fungal secretome components, mycotoxins and digestive enzymes.

505F Fungal digestive enzyme profile: Essential for fitness and integrated part of speciation and evolution *Lene Lange*[1], Anne S. Meyer[2], Kristian Barrett[2] 1) BioEconomy, Research & Advisory; 2) Bioengineering, Technical University of Denmark, Lyngby, Denmark .

Fungal growth impacts the environment significantly by its invasive power; made possible by the hyphal enzyme secretome. Therefore, analysis of evolution, function and composition of secretome gives valuable insight into fungal biology *per se.* Robust prediction of enzyme function, using the CUPP method, (Conserved Unique Peptide Pattern), opened for developing and testing a hypothesis for how the fungal digestive enzyme secretome evolved during evolution. Hypothesis: Increased fitness of fungal species is achieved by the fungi having *the right type of protein* (stability, pH and temperature optimum steric substrate accessibility etc) with *the right functions* for efficient degradation of available and accessible substrate. An evolutionary, fitness-relevant genome annotation can be achieved by annotating not to protein family and to function separately, but by using "EC-Function;Protein-Family" as one integrated observation, distinguishing and counting the type of specific type of protein with a specific function. Hereby also capturing the frequent occurrence in strong biomass degrading fungi, having several different types of enzyme protein with the same function. The hypothesis was tested by a Yule calculation for relatedness of "F;F"-observation profiles of all *Penicillium* and *Aspergillus* species. (Notably, Yule gives equal weight to "shared-presence" and "shared absence" of CAZyme "F;F" observations. The hypothesis was confirmed for these two genera, as the resulting "F;F"-relatedness dendrogram was stunningly similar to the organismal phylogenetic tree. This indicates that fungal digestive enzyme profile is an integrated part of speciation and evolution. *Global Hotspots of fungal CAZymes.* Summing up "F;F"-observations for 1.932 genomes demonstrated that fungal enzyme hotspots are found in species of very different taxonomy, lifestyle, ecology, physiology and substrate/host affinity. Surprisingly, most CAZyme hotspots are found in enzymatically understudied and unexploited species and the most well-known fungal enzyme producers, industrially exploited are not found to be among the top-ranking. The results contribute to elucidating the evolution of fungal substrate-digestive CAZyme profiles, ecophysiology, and habitat adaptations, and expand the knowledge base for novel and improved biomass resource utilization

506W A diversified metabolic toolkit in budding yeasts linked to ecological adaptation *Carla Gonçalves*[1,2,3], Chris Todd Hittinger[4], Antonis Rokas[1], Paula Gonçalves[2,3] 1) Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee, USA; 2) Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de

Lisboa, 2829-516 Caparica, Portugal; 3) UCIBIO – Applied Molecular Biosciences Unit, Department of Life Sciences, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 4) Laboratory of Genetics, J. F. Crow Institute for the Study of Evolution, Wisconsin Energy Institute, DOE Great Lakes Bioenergy Research Center, Center for Genomic Science Innovation, University of Wisconsin-Madison, Madison, Wisconsin, USA.

The non-conventional budding yeasts belonging to the *Wickerhamiella* and *Starmerella* genera (W/S clade, subphylum Saccharomycotina) are usually found in association with the sugar-rich floral niche, where sucrose, fructose and glucose are the main sugars available.

Contrary to the trend of glucose being the preferred source of carbon and energy, W/S-clade yeasts are generally not avid glucose utilizers especially when fructose is present. In the presence of both sugars, fructose is consumed more efficiently, a relatively rare metabolic attribute referred to as fructophily. We showed that fructophily is associated with both the loss of the alcoholic fermentation pathway, otherwise ubiquitous in yeasts, as well as with the horizontal acquisition of a gene encoding a high-capacity fructose transporter with an unusual evolutionary trajectory placing it outside the sugar porter family. Additional horizontal gene transfer events from bacteria were shown to impact the utilization of the other predominant sugars present in the floral environment: the reinstatement of alcoholic fermentation accomplished with bacterial alcohol dehydrogenases improved glucose metabolization, whereas the acquisition of a gene encoding an extracellular sucrose hydrolase of bacterial origin (*SUC2*) enabled sucrose utilization in most *Starmerella* species. On the other hand, some *Wickerhamiella* species evolved a different strategy for sucrose utilization involving a sugar proton symporter and an alpha-glucosidase. The two genes are organized in gene clusters that were formed *de novo* and we showed that they are involved in the utilization of sucrose but also of other alpha-glucosides found in floral nectar such as maltose and melezitose. Moreover, we demonstrated that the expression of these genes dispensed the activator usually present in similar yeast clusters and escaped glucose repression, allowing the co-utilization of glucose and sucrose. The different modes of disaccharide utilization uncovered in the W/S-clade species examined also includes a version in which alpha-glucoside transporters are absent and both sucrose and maltose are hydrolysed extracellularly. Investment of cellular energy in active alpha-glucoside transport is a hallmark of non-fermenting *Wickerhamiella* species. Hence, the W/S clade emerges as a framework in which to study how the interplay between (energy) metabolism and various ecological factors may contribute to shape the genetic toolkit used for sugar catabolism.

507T Three-dimensional chromatin organization determines the evolution of adaptive genomic regions in the plant pathogen *Verticillium dahliae* *David E Torres*[1,2], Martin H Kramer[1], Vittorio Traccana[3], Gabriel L Fiorin[1], David E Cook[1,4], Michael F Seidl[2], Bart PHJ Thomma[1,3] 1) Laboratory of Phytopathology, Wageningen University and Research, The Netherlands; 2) Theoretical Biology & Bioinformatics Group, Department of Biology, Utrecht University, The Netherlands; 3) Institute for Plant Sciences, Cluster of Excellence on Plant Sciences (CEPLAS), University of Cologne, Germany; 4) Department of Plant Pathology, Kansas State University, USA.

The spatial organization of the eukaryotic nuclear genome is intimately linked to its biological functions. Beyond the linear organization of genetic elements in the DNA, chromatin folding and organization play an important role in the regulation of gene expression and genome evolution. In fungi, the 3D organization of the genome is still under-investigated. Therefore, the role of 3D organization in transcriptional regulation, genome organization, and evolution remains unclear, particularly in fungal plant pathogens that are constantly challenged by the immune systems of their hosts. Such challenges necessitate genomic responses on short and long evolutionary time scales. *Verticillium dahliae* is a filamentous fungal plant pathogen that causes disease on hundreds of plant hosts. The *V. dahliae* genome contains designated plastic regions, known as adaptive genomic regions (AGRs), that are enriched in transposable elements and in *in planta*-induced genes that mediate pathogen aggressiveness during host infection. Here, we explore the *V. dahliae* chromatin conformation with DNA proximity ligation followed by sequencing (Hi-C) to uncover the spatial organization of the core genome and the AGRs. Our analysis reveals the presence of topologically associating domains (TADs) in *V. dahliae.* Interestingly, we observe that TAD boundaries are gene-rich regions that display quantitatively lower gene expression than the genome-wide average. Moreover, we observe enrichment of facultative heterochromatin in weak TAD boundaries within AGRs. Interestingly, TADs in AGRs cluster physically within the nucleus, suggesting a common 3D organization of AGRs. By comparing different *V. dahliae* strains and other *Verticillium* species, we show that TAD boundaries are depleted in genomic variation. Thus, our analysis demonstrates that the 3D organization is conserved within the *Verticillium* genus and indicates that this organization contributes to the evolution of AGRs in *V. dahliae.*

508F The GATA transcription factor NsdD governs development and metabolism via species-specific gene regulatory networks in *Aspergillus nidulans* and *Aspergillus flavus* *Heungyun Moon*[1], Mi-Kyung Lee[2], Junha Shin[1], Jin Woo Bok[1], Kap-Hoon Han[3], Nancy Keller[1], Jae-Hyuk Yu[1] 1) University of Wisconsin-Madison, Madison, WI; 2) Korea Research Institute of Bioscience and Biotechnology, Jeongeup-si, Republic of Korea; 3) Woosuk University, Wanju, Republic of Korea.

The *Aspergillus*-conserved GATA-type transcription factor (TF) NsdD regulates sexual/asexual development and secondary metabolism. To gain insight into the molecular and genomic bases of NadD-mediated regulation of cellular and metabolic developmental traits, we used diverse omics approaches., i.e., transcriptome, protein-DNA interactions, and secondary metabolism. We intended to reveal not only the molecular function of NsdD, but also the evolutionary transition of NsdD-mediated gene regulatory networks (GRNs) that result in the changes of development and metabolism in two distantly related species; *Aspergillus nidulans* and *Aspergillus flavus*. NsdD contains a highly conserved GATA-type IVb DNA-binding domain (DBD). The deletion of *nsdD* in *Aspergillus* species leads to common features such as hyperbranching hyphae, hyper-conidiation, and no sexual development, but also distinct phenotypes including different morphology of conidiophores and spores as well as mycotoxin production. Particularly in mycotoxin production, NsdD inhibits biosynthesis of sterigmatocystin (ST) in *A. nidulans* but is required for aflatoxin production in *A. flavus*. These suggest that despite of the conserved DBD, NsdD-mediated GRNs possibly differ in a species-specific manner.
To elucidate the GRNs in two species, RNA-seq and chromatin immunoprecipitation (ChIP) assay were performed. Three different cell types are selected for RNA-seq to understand cell type-dependent effects of NsdD: vegetative cell (Veg), asexually developing cell (Asex), and conidia. In *A. nidulans*, 23%, 43%, and 10% of 10,988 genes are differentially expressed in *ΔnsdD* in Veg, Asex, and conidia, respectively. In *A. flavus*, 3%, 9%, and 15% of 13,485 genes are affected by NsdD in Veg, Asex, and conidia, respective-

ly. Of note, 68% of DEGs in *A. nidulans* are core genes (conserved genes in both species) compared to 47% of DEGs in *A. flavus*. From ChIP-seq analyses of conidia, 505 and 674 possible direct targets of NsdD were identified including 24 & 41 TFs and several regulators. Some are well known to play crucial roles in development and SM such as *vosA*, *veA*, and *laeA*. All peaks from ChIP-seq were subjected to Multiple Em for Motif Elicitation (MEME) analysis and the predicted NsdD response element is 5'-GATC-3' in both species. In addition, from metabolomic analyses, both species showed significant amount changes of near 170 metabolites in Δ*nsdD*. These data suggest that NsdD forms distinct GRNs by directly regulating large numbers of TFs and regulators resulting in an extensive change of gene expression and metabolism.

509W Comparative transcriptomics to study stress induced morphological changes in fungi *Arpad Csernetics*[1], Torda Varga[1], Botond Hegedus[1], Benedek Szathmari[1], Neha Sahu[1], Hedvig Hegyi[1], Laszlo G. Nagy[1] 1) Institute of Biochemistry, Biological Research Centre, Szeged.

Fungi colonize a wide range of habitats and are exposed to diverse environmental stimuli including climatic, biological and chemical stressors. Fungi are able to survive under extreme conditions as stress response networks enable them to adapt to or escape from unfavorable environments. Stress responses include the modulation of physiology and metabolism and morphological changes, the latter in most cases provoked by high temperature or nutrient limitation. For example, thermally dimorphic fungi respond to temperature shifts by morphological transition between hyphae and yeast, which allow dissemination within and colonization of the host by pathogenic fungi.
Activation of stress response networks by different environmental stimuli and morphological changes involved in stress survival are still not well understood and comparative studies of differences in stress induced morphological changes are missing. In this study our aim was to compare the stress response networks in yeast and filamentous forms of dimorphic fungi with regard to conserved morphogenetic genes and decipher evolutionary differences between morphological changes provoked by environmental stress. Transcriptomic datasets of 4 dimorphic species (*Candida albicans, Ophiostoma novo-ulmi, Metarhizium acridum, Geotrichum candidum*), covering altogether 20 developmental stages were obtained. Published datasets of two species (*Histoplasma capsulatum, Blastomyces dermatitidis*) were also included in the comparative analysis. Differential expression analyses were performed between stressed and non-stressed conditions of the six species using the DESeq2 statistical framework and shared regulated genes were identified based on comparisons across orthologous groups. Our study gave deeper insight into the genetics of fungal stress avoidance via morphological plasticity.

510T Chromosomal responses to telomere dysfunction in *Pyricularia oryzae* are determined by subterminal sequence composition *Mostafa Rahnama*[1], Baohua Wang[1], Benjamin Peppers [1], Daniel Yackzan[1], Mark Farman [1] 1) University of Kentucky, Lexington, KY.

Telomeres are the sequences that occur at the ends of eukaryotic chromosomes and are responsible for binding nucleoprotein complexes that guard the chromosome termini from nucleolytic attack and the break-repair machinery. In most organisms, the telomeres comprise short, repeated sequence motifs that are added by the telomerase holoenzyme. Notable exceptions include certain Diptera which lack telomerase and, instead, have telomeres consisting of transposable element arrays. Telomeres and the adjacent chromosome regions are of special interest in the blast fungus, *Pyricularia oryzae,* because they are enriched in genes controlling host interactions, are highly dynamic, and exhibit extensive structural variation and presence/absence polymorphism among strains. In the most extreme cases, single spores from genetically-purified strains exhibit numerous telomere alterations from one generation to the next. While studying the molecular basis for such instability, we identified two transposons (MoTeRs) that insert specifically into the telomeres, forming arrays that resemble Dipteran telomeres. We thus hypothesized that MoTeRs might assume telomere functions in the absence of canonical telomere maintenance. To test this, we deleted the telomerase gene in select strains that had different numbers of telomeres with MoTeR insertions and examined the fate of individual chromosome ends to determine: i) if MoTeR presence could guard against the loss of terminal sequences that is expected to occur in the absence of telomere maintenance; and ii) if MoTeRs can transpose to telomeres that lacked them prior to telomerase deletion. We show that a favored pathway to telomere repair involves the invasion of short, internal telomere repeat motifs ($TTAGGG_{1.5+}$) by the deprotected telomeres, resulting in tandem amplification of the intervening MoTeR sequences at the chromosome ends. MoTeRs prevent the loss of terminal sequences because they contain telomere repeat motifs separating element copies. Next, we found that new MoTeR insertions can be acquired by telomeres that lacked them prior to the TERT KO experiments, allowing such telomeres to be rescued by the aforementioned pathway. Chromosome ends lacking internal telomere repeats were prone to loss of terminal sequences, presumably due to replicative attrition. These ends were ultimately repaired by break-induced replication using repeated sequences shared with other chromosome ends, or by non-homologous end-joining. Overall, we found that the sequence composition of individual chromosome ends was highly predictive of the mechanisms used for their repair.

511F High-throughput functional profiling of *Trichoderma atroviride* by RbTDNA-seq *Jose Manuel Villalobos Escobedo*[1,2], Catharine Adams[1,2], Lori Huberman[3], Ran Shi[1], Adam Deutschbauer Deutschbauer[2], Louise Glass[1,2] 1) University of California, Berkeley, CA 94720, USA.; 2) Environmental Genomics and System Biology/Biosciences Area, Lawrence Berkeley National Laboratory, Berkeley, Berkeley, CA 94720, USA.; 3) Cornell University›s College of Agriculture and Life Sciences, Ithaca, NY 14850, USA..

Trichoderma atroviride is a filamentous fungus that establishes a relationship with plants, produces compounds that promote their growth, and performs specific processes such as mycoparasitism. Until now, null mutants of some genes have been evaluated to determine their function. But to date, no high-throughput analysis has been performed to determine the function at the whole genome level.

In recent years, it has been shown that in unicellular organisms such as bacteria and yeasts, Rb-TnSeq and BAR-seq experiments allow determining the global function of genes under stress conditions. However, in filamentous fungi, this approach represents a challenge since these are multinucleated organisms and conidia that are uninucleate structures present a certain degree of recalcitrance to transformation. The objective of this work was to create a library of TDNA insertion mutants in *T. atroviride* with thousands of unique barcode codes inserted randomly throughout the genome and subsequently evaluate the fitness of each of the mutants in the pool by

Bar-seq.

The preliminary TDNA-seq in *T. atroviride* showed that more than 75% of the reads obtained from the genome have inserted barcodes and we did not observe bias due to %GC content or a clear enrichment of insertions in any contig. The barcodes analysis showed that 7,115 genes of the 11,816 in the genomes have at least one insertion. We performed a Bar-seq experiment under nutrient deficiency conditions. We observed that in glucose deficiency, strains with mutations in 67 genes showed lower fitness, while mutants in 36 genes have a positive fitness under these conditions. Under nitrogen starvation, mutants in 53 genes had a negative effect while 36 mutations had a positive effect on fitness. Under phosphate deficiency, only mutants in 13 genes had a significant change in fitness. These results suggest that there are more essential genes to cope with nitrogen and carbon deficiency than phosphate.

Our preliminary data in *T. atroviride* suggest that this technique also works in filamentous fungi to annotate the whole genome under different experimental conditions.

512W Functional analysis of the bZIP transcription factors AtfA and AtfB in *Aspergillus nidulans* Beatrix Kocsis[1], Mi-Kyung Lee[2], Jae-Hyuk Yu[3], *Istvan Pocsi*[1], Eva Leiter[1] 1) Department of Molecular Biotechnology and Microbiology, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, 4032, Debrecen, Egyetem tér 1., Hungary; 2) Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Jellobuk-do, 56212, Republic of Korea; 3) Department of Bacteriology, University of Wisconsin-Madison, 1550 Linden Drive Madison, WI 53706, USA.

Basic leucine zipper (bZIP) transcription factors (TFs) are conserved regulators found in all eukaryotic organisms and play a crucial role in many cellular processes including development and stress responses in fungi. We have been studying the two key bZIP TFs AtfA and AtfB in fungi. AtfA orchestrates the stress defense system of filamentous fungi by globally regulating expression of genes associated oxidative/osmotic stress responses, conidial heat stress tolerance, and secondary metabolism and asexual sporulation in various filamentous fungi. AtfB governs resistance to oxidative stress, controls gene expression during conidial development, and plays a role in the regulation of carbon and amino acid metabolisms in the Aspergilli.
To analyze further the physiological functions of these key TFs in *Aspergillus nidulans*, we constructed gene deletion and overexpression mutants in all combinations including the *ΔatfA, ΔatfB, ΔatfAΔatfB, ΔatfAatfBOE, ΔatfBatfAOE, atfAOE, atfBOE* and *atfAOEatfB OE* strains. The following phenotypes were observed on stress agar plates in the presence of oxidative, osmotic, heavy metal and cell wall stress generating agents: The *ΔatfA* mutant showed an oxidative stress sensitive phenotype in the presence of 0.8 mM *t*BOOH, 2 mM diamide and 0.08 mM menadione meanwhile the oxidative stress sensitivity of the *ΔatfB* mutant was comparable to that of a control strain. The *ΔatfAΔatfB* mutant was moderately sensitive to 0.8 mM *t*BOOH and highly sensitive to 2 mM diamide. The overexpression (OE) of neither *atfA* nor *atfB* compensated for the negative effects of *t*BOOH in the null mutants. However, OE of *atfB* alone protected the fungus against *t*BOOH stress. Moreover, OE of *atfB* either alone or together with *atfA* as well as the deletion of *atfA* resulted in heavy metal stress sensitive phenotypes while the *ΔatfB* mutant was moderately tolerant to $CdCl_2$. In addition, the *ΔatfB* mutant showed reduced growth on solid medium containing 1.5 M NaCl meanwhile this mutant was the most tolerant to 2 M sorbitol. The *atfAOEatfBOE* mutant showed an increased tolerance to NaCl. Only the *ΔatfA* mutant displayed moderate tolerance to the cell wall stress inducing agent, Congo Red. In summary, AtfA and AtfB play distinct roles in governing the fungal responses to various stresses.

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513T Genome-wide analysis of AtfA/AtfB-mediated menadione stress response in *Aspergillus nidulans* Beatrix Kocsis[1], Mi-Kyung Lee[2], Jae-Hyuk Yu[3], *István Pócsi*[1], Éva Leiter[1], Tamás Emri[1] 1) Department of Molecular Biotechnology and Microbiology, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, 4032, Debrecen, Egyetem tér 1., Hungary; 2) Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Jellobuk-do, 56212, Republic of Korea; 3) Department of Bacteriology, University of Wisconsin-Madison, 1550 Linden Drive Madison, WI 53706, USA.

The bZIP transcription factors (TFs) are important regulators of the oxidative stress response in filamentous fungi. In this work we carried out genome-wide expression studies employing Illumina RNAseq to understand the roles of the two bZIP TFs AtfA and AtfB in *Aspergillus nidulans*. Specifically, comparative analyses of transcriptomes of vegetative mycelium and conidiospores obtained from the surface cultures of the control, *ΔatfA*, *ΔatfB*, *ΔatfAΔatfB* mutant strains with/without menadione sodium bisulfite (MSB, a superoxide generating agent) treatment were carried out. Evaluation of transcriptomics data was carried out *via* Venn diagram comparisons of the global gene expression patterns gained for the four strains.
As both the presence of MSB and *ΔatfA* downregulated expression of *atfB*, AtfA can affect the AtfB-dependent genes *via* activating *atf B* expression. In untreated mycelia we have found a higher number of differentially expressed genes (DEGs) in *ΔatfA* than in *ΔatfB*. Compared to untreated ones, MSB treated mycelia showed a lower number of DEGs in *ΔatfA*, or *ΔatfB vs.* control. Only 9 AtfB-dependent genes were found in MSB treated mycelia and most of these were also AtfA-dependent. The observed differences in the responsive gene sets of the MSB treated and untreated cultures suggest that AtfA regulates distinct genes under different culture conditions.
In conidia, more DEGs were found in *ΔatfA* or *ΔatfB*, supporting the idea that AtfA and AtfB play more global regulatory roles in conidiospores than in vegetative tissues. Interestingly, MSB treatment of conidia did not lower *atfB* mRNA levels, and most of the *atfB* deletion DEGs were also found in *ΔatfA*. In general, DEGs in conidia showed low overlap with DEGs found in the corresponding mycelial samples.
Functional category analyses of DEGs have revealed that AtfA affects some glycolytic genes and iron-sulfur cluster assembly genes in general. Phosphorelay response regulator genes were enriched in all AtfA-dependent gene sets except the untreated mycelial samples. Genes that were regulated by AtfA under all experimental conditions include those encoding catalase, histidine-containing phosphotransfer proteins.

Only 23 genes were solely dependent on AtfB considering all transcriptomics data sets, including a putative a-glucosidase (*agdB*), a putative a-amylase, *calA* involved in early conidial germination and an alternative oxidase.
In summary, our genome-wide expression studies have revealed cell-type dependent distinct regulatory roles of AtfA and AtfB in *A. nidulans*.

This work was supported by the European Union and the European Social Fund through the project EFOP-3.6.1-16-2016-00022, by the Thematic Excellence Programme (TKP2020-IKA-04) of the Ministry for Innovation and Technology in Hungary, and the National Research, Development and Innovation Office with the grants NKFIH K119494 and NN125671.

514V Characterizing Genetic Mechanisms for Measuring Day-Length in Neurospora crassa *Sienna Casciato*[1], Kwangwon Lee[1] 1) Rutgers University.

There are predictable changes most of the organisms on Earth experience based on the daily rotation of the Earth and the rotation of the Earth around the sun. The biological rhythms with a period of about one day is called circadian rhythm and the seasonal rhythm with a period of one year is called circannual rhythm. Because ambient light condition is one of the strongest environmental cues, the predictable seasonal rhythm of a day length must play an important role in developing the circannual rhythm. Photoperiodism is a physiological response of an organism to changes of the ambient environment over a year and plays a major role in fitness of an organism in nature. For the past half century, the molecular mechanisms of the circadian clock have been characterized. However, the mechanisms of the circannual clock and the role of the circadian clock in the circannual clock have not been understood. We hypothesized that there are multiple genes that are involved in photoperiodism, and that the genes involved in the circadian clock might also be involved in photoperiodism. To test our hypotheses, we developed the protoperithecia assay (PPA). Protoperithecia is a female sexual reproductive structure in *N. crassa*. It is known that the number of protoperithecia changes in response to different day-length periods. We performed Quantitative Trait Loci (QTL) analysis on the change of the number of photoperiod-dependent protoperithecia as a trait in 91 F1 progeny of *N. crassa*. We found a major QTL on chromosome (Chr) 1 and characterized 17 knockout mutants in the target region on Chr 1. We identified a candidate QTL gene whose deletion had a significant deviation of the photoperiodic response from that of the parent genotype. We also performed PPA on 10 known classical circadian clock mutants and found that some of the clock genes showed altered photoperiodic responses. Our data support the view that the circadian clock is a part of the day-length measuring mechanism, and thus involved in the circannual clock.

515V Derivative Profiling to Determine Differential Analysis in -omic Datasets *Harley Edwards*[1], Joseph Zavorskas[2], Mark Marten[1], Ranjan Srivastava[2], Steven Harris[3] 1) University of Maryland, Baltimore County; 2) University of Connecticut; 3) Iowa State University.

This work establishes a method of analysis to determine significantly differentially regulated expression profiles from within -omic datasets. This method leverages variable timestep differentiation via LaGrange interpolation (Singh and Bhadauria 2009) to achieve estimates of the derivative throughout experiments that contain variable time point sampling. The population of derivative signals is regressed via a first order polynomial, and the slopes and y-intercepts of these regression results are used to infer meaning from the population of signals. Normalization options are tested to determine the optimal normalization factor. A Grubbs test for outliers is utilized to identify outliers and isolate them so that the remaining data fits a normal distribution. Using data which now fits within a normal distribution, statistical confidence about a profiles place in the distribution can be determined by the standard deviation from the mean. This can be done for both the slope and y-intercept. The results of this method are compared to established methods like MARS (Jekabsons 2011), Volcano (Cui and Churchill 2003), and FDR adjusted ratio over intensity analysis (Benjamini and Hochberg 1995). This is done for transcriptomic and phosphoproteomic expression profiles previously characterized in Aspergillus *nidulans* (Chelius, Huso et al. 2020). The advantage of this technique is that it is computationally inexpensive, does not require fold change calculations, and it is possible to achieve statistical confidence about a profile's behavior by using only one time course experiment, allowing independent analysis per bio-replicate. We demonstrate this method for transcriptomic and phosphoproteomic datasets, but there is no fundamental reason why this technique could not be applied to other types of data sets. To the best of the author's knowledge this is a novel approach to identify differentially regulated expression profiles from within -omic data sets.

516V Generation of Synthetic Time-Course Omics Data using Long Short Term Memory Networks *Joseph Zavorskas*[1], Harley Edwards[2], Ranjan Srivastava[1], Mark Marten[2], Steven Harris[3] 1) University of Connecticut; 2) University of Maryland, Baltimore County; 3) Iowa State University.

Omics analysis using statistical methods or machine learning requires multiple biological and technical replicates to be successful. These replicates are often expensive and time-consuming to generate. Gene expression/stimulus response experiments also require time-course data at multiple stimulant concentrations to measure dose-response. A long-short term memory (LSTM) neural network can be trained using stimulant concentration as the input and the respective time courses as the output. The trained neural network can be queried to generate synthetic data time-courses in the place of conducting further experiments. The network can also be queried at doses of perturbant that were not included in the original dataset. Interpolations on perturbant concentration are accurate, but other methods perform that analysis. The main strength of this method is its ability to perform extrapolations on the perturbant.

Case studies were performed in silico to demonstrate the LSTM's predictive capabilities. These in silico case studies were performed on the Lotka-Volterra predator/prey system, and a model for viral reproduction. The LSTM produced interpolations and extrapolations based on initial conditions whose fit to testing data produced R-values at or above 0.9 with only 4 total replicates. An in vitro case study will be performed on a transcription factor in Aspergillus nidulans, brlA. This transcription factor is known to be differentially expressed in response to the echinocandin antifungal agent, micafungin (Reese et. al., 2021). Quantitative PCR (qPCR) will be performed at various sublethal concentrations of micafungin, and an LSTM network will be trained on a subset of the time-course data. Data, reserved selectively, can ensure that the LSTM will be queried at doses that both interpolate and extrapolate on the training dataset. The results will be presented along with the in silico study. The utility of using LSTMs is their ability to recognize and reinforce patterns in data with few

replicates. The current standard in -omics experimentation is to generate 3 biological replicates to perform statistical analyses. These three replicates can be used to produce further synthetic replicates for further analysis.

517V Revealing the effector repertoire of the sweetpotato black rot fungal pathogen *Ceratocystis fimbriata* *Camilo H. Parada-Rojas*[1], Madison Stahr[2], Kevin Childs[3], Lina M. Quesada-Ocampo[1] 1) Dept. of Entomology and Plant Pathology, NC State University, NCSU, Raleigh, NC; 2) Sugarbeet and Bean Unit, USDA-ARS, East Lansing, MI; 3) Dept. of Plant Biology, Michigan State University, MSU, East Lansing, MI.

There are several fungal rot diseases of sweetpotatoes, but in terms of destructiveness, none compare to black rot caused by *Ceratocystis fimbriata*. In 2015, sweetpotato producing counties in the United States experienced one of the worst outbreaks of black rot recorded in history with up to 60% losses reported in field, packing houses, and at shipping ports. Chemical management restrictions combined with persistence and reemergence of *C. fimbriata* have prompted the exploration of host resistance as an essential management practice. However, lack of knowledge of *C. fimbriata* biology represents a critical barrier for the deployment of resistance to black rot in sweetpotato. Soilborne pathogens deploy diverse repertoires of secreted proteins known as effectors to modulate a plethora of host processes for their welfare. In this study, we sequenced, assembled and annotated the genome from *C. fimbriata* isolate AS236 using PacBio and Illumina reads. The resulting 31.7 Mb assembly yielded 18 contigs and 6,481 predicted genes. The secretome of AS236 was defined as the set of 477 proteins with signal peptides but without transmembrane domains as predicted by SignalP and TMHMM. A set of 224 putative effectors were identified among the set of secreted proteins using EffectorP v 2.0. To identify core candidate effectors, we performed differential expression analysis on a time-course of pre- and post-epidemic *C. fimbriata* isolates during infection on sweetpotato storage roots (cv. Covington). Our study revealed a catalog of candidate effector proteins that provide insight into *C. fimbriata* infection mechanisms and represent a core catalog to implement effector assisted breeding in sweetpotato.

518V Fungal mitochondrial genomics - insights and challenges *Steven Ahrendt*[1], Sajeet Haridas[1], Asaf Salamov[1], Stephen Mondo[1], Igor Grigoriev[1,2] 1) US Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA; 2) Department of Plant and Microbial Biology, University of California-Berkeley, Berkeley, CA.

Mitochondria are specialized organelles found within cells of nearly all Eukaryotes and are responsible for ATP generation through oxidative phosphorylation. It is generally accepted that mitochondria are derived from an ancient endosymbiotic event, and therefore possess their own genomes (mitogenomes). Fungal mitogenomes retain a set of genes related to electron transport and oxidative phosphorylation, translation, and tRNA processing. However, this gene set is substantially reduced relative to the mitochondrially-encoded gene set of many other Eukaryotes, with many functions having been transferred to the nucleus over the course of evolution. Fungal mitogenome annotation is challenging due in part to the large number of introns, which are primarily either group I or II and are self-splicing, often containing homing endonuclease genes. While there are increasing numbers of complete and annotated fungal mitogenomes, the relatively few existing sequences are largely from the Ascomycota and Basidiomycota. Using a newly developed annotation pipeline for fungal mitogenomes, we present here a summary of around 300 mitogenome annotations from across the kingdom Fungi. Single-scaffold fungal mitogenome assemblies range from 12 kb in the Cryptomycota to 1.3 Mb in the Pezizaceae. Across the Fungi, 14 genes encoding subunits from four oxidative phosphorylation complexes are conserved, with noticeable gene losses observed in related clades of Dothideomycetes. A phylogenetic comparison between mito- and nuclear genome trees using ete3 suggests that they share largely similar topology. We investigated tRNA distribution and codon usage potentially related to alternative translation strategies employed by fungi within the early-diverging lineages. Going forward, fungal mitogenomes annotated by the JGI will be presented with a new interactive analysis tab on individual MycoCosm genome portals and available for comparative analyses. This effort will expand the set of fungal mitogenome annotations, and work toward a better understanding of fungal mitochondrial genome structure and evolution.

519V Identification of structurral protein orthologs using predicted protein structures *Fred Dietrich*[1] 1) Duke University, Durham NC.

Over the past several years significant progress has been made in the prediction of protein sequences. This has the potential to open a new approach to the identification of orthologs between distantly related fungal species. For relatively closely related species, such as *Ashbya gossypii* and *Saccharomyces cerevisiae* orthologs can be determined by using a combination of protein sequence identity/similarity and gene order conservation. By this means it has been determined that the vast majority of proteins encoded in the *A. gossypii* genome have orthologs in *S. cerevisiae.* For more distantly related species gene order conservation is generally not applicable, and many proteins do not have homologs that can be recognized by sequence similarity. It has long been hypothesized that protein structure is more conserved than protein sequence. However, due to the cost and effort involved in experimental determination of protein structure, it has not been feasible to use protein structure to compare sets of proteins from fungal species. With the release of the source code for alphafold in July 2021, it is not possible to test whether protein structure prediction can be used to identify protein structure orthologs between distantly related fungal species. Using the set of protein structures determined using alphafold by the EBI for *S. cerevisiae*, and the set of protein structures we have predicted for *A. gossypii* using alphafold, the best structural matches for pairs of proteins between these species generally corresponds to the previously determined ortholgs. This is true even for orthologs with less than 30%. identity. Now that we have been able to confirm that the general concept works we are working on identifying structural orthologs for more distantly related species. The long term goal is to use experimental validation of conserved function to confirm ortholgs initially identified by. predicted protein structure.

520V No genes left behind: Associating phenotypes with genes in *Neurospora crassa* *Scott Baker*[1], Kevin McCluskey[2] 1) Pacific Northwest National Laboratory, Richland, WA; 2) Bolt Threads, Emeryville, CA.

Despite its role as a premier model organism for fungal biology studies, many classically identified genes in *Neurospora crassa* are “anonymous.” These genes have a phenotype that segregates in a cross and most have been mapped to a genetic region, but they have not been associated with physical location in the genome. As such there is no correlation between the genetic locus and any open reading frame in the genome. We have resequenced over 500 strains of *N. crassa* representing over 300 classically described

but otherwise anonymous genes. Using an in silico subtraction approach we are able to identify and therefore present candidate loci for many of these genes.

521V REPAINT – an artificial intelligence algorithm for the comprehensive phenotyping of conidial fungi Guan Pang[1], Feng Cai[2], Renwei Gao[1], Thomas Ebner[3], Michael Mayrhofer-Reinhartshuber[3], Philipp Kainz[3], Christian P. Kubicek[4], *Irina S. Druzhinina*[1] 1) Fungal Genomics Group (FungiG), Nanjing Agricultural University, Nanjing, China; 2) School of Ecology, Sun Yat-sen University, Shenzhen, China; 3) KML Vision GmbH, Graz, Austria; 4) Institute of Chemical, Environmental and Bioscience Engineering (ICEBE), TU Wien, Vienna, Austria .

In fungi, the genetic distances assessed from genomes are huge, and two seemingly similar molds from one genus can share only three-quarters of their genes, i.e., different as humans from fish. Understanding these genomic variants requires the consideration of fungal phenotypes. However, *in situ* studies of fungal biology are impeded by identification ambiguity and their diminutive bodies, which develop inside a substrate and have exceptional metabolic and ecological plasticity. Therefore, the interpretation of genomic traits frequently solely depends on *in vitro* phenotyping. The lack of quantitative measures can represent a bottleneck for understanding different phylotypes. This study aims to develop a high-throughput comprehensive phenotyping system for conidial fungi in pure culture (autecology). We consider that phenotypes should be assessed (*i*) at several stages of the life cycle (the development), (*ii*) in versatile nutritional conditions, (*iii*) exposed to a variety of abiotic stressors, and (*iv*) be quantitative. The commonly used Biolog FF MicroPlates containing 95 carbon sources and water offer a convenient platform for such phenotyping. These microplates can be inoculated with spore suspensions either in a semisolid Phytogel or water. In addition, the culturing system can be supplemented with xenobiotics or stress-generating compounds (e.g., ROS-producing chemicals or osmolytes) and exposed to different spectra of electromagnetic radiation, temperature, or other conditions. Fungal development in microplates can be divided into at least four stages and quantified. First, spore germination is best reflected by turbidity values at 490 nm corresponding to the respiration measured as intensity of the red formazan dye formed when the iodonitrotetrazolium violet is reduced by succinate dehydrogenase (citric acid cycle). Second, the development of feeding hyphae is assessed as hyphal density by turbidity values at 750 nm at multiple time points. Then, the reproductive potential of a fungus (aerial stages) is assessed by analyzing high-resolution digital images of the microplates. For this purpose, we developed the REproduction Potential Artificial INTelligence assay (REPAINT). Specifically, the image of every well of the microplate is automatically converted to numerical values corresponding to the percentages covered by hyphae or conidia (the third and fourth stages of the life cycle, respectively). In this presentation, we will report the results for *Aspergillus* spp*., Neurospora crassa, Trichoderma* spp., and a few other model fungi and show how phenotype microarrays (e.g., Biolog) and REPAINT can be combined to reveal different developmental strategies within one fungal genus, how specific carbon sources influence conidiation of most fungi, and how spore germination is determined by abiotic and genetic factors but is not influenced by nutrients.

522V Uncovering the fungal pangenome of *Penicillium* *Celine Petersen*[1], Trine Sørensen[1], Teis Esben Sondergaard[1], Jens Laurids Sørensen[1], Kåre L. Nielsen[1] 1) Department of Chemistry and Bioscience, Aalborg University, Denmark.

The *Penicillium* genus is a valuable collection of ascomycetous fungi that have widespread occurrence in natural environments such as soil. They have attracted much interest due to their ability to produce a wide range of secondary metabolites with importance in the food and pharmaceutical industry, but the full biosynthetic potential of these fungi is yet to be characterized.

A pangenome combines all the genetic information of a set of related organisms and divides this into a set of core genes (genes present in all individuals), soft core genes (genes present in ≥ 95% of all individuals), accessory genes (genes present in < 95% of all individuals, but still at least two individuals), and singleton genes (present in one individual only). Improvements in DNA sequencing technologies have resulted in high quality genome draft assemblies, which can be used to create pangenomes. Since many genes involved in the production of secondary metabolites have been observed to be part of the accessory genome, pangenome characterization is therefore highly useful to explore the biosynthetic potential to produce secondary metabolites.

High molecular DNA was extracted from 94 different *Penicillium* species and subsequently sequenced in-house using MinION sequencing to create high quality genome drafts. Together with additional ten genome assemblies from NCBI, the collection of species covers diversity of the genus of *Penicillium* well and forms the basis of a pangenome analysis of the *Penicillium* genus.

523V Comprehensive high-throughput phenotypic microarrays analysis of clinical diversity in Aspergillus fumigatus *Renad Aljohani*[1], Andrew Scourfield[2], Johanna Rhodes[3], Matthew Fisher[3], Darius Armstrong-James[1] 1) Department of Infectious Diseases, Imperial College London, London, UK; 2) Specialty Registrar in Clinical Pharmacology and Therapeutics and General Internal Medicine at Guys and St Thomas' NHS Foundation Trust; 3) MRC Centre for Global Infectious Disease Analysis, School of Public Health, Imperial College London, London, UK.

Aspergillus fumigatus is a ubiquitous filamentous mould associated with globally distributed pulmonary pathology in patients suffering from asthma, cystic fibrosis (CF), and immune deficiencies. It inhabits a variety of environments, and this contributes to genotypic and phenotypic variation among isolates. We investigated the phenotypic correlates of genomic diversity in triazole-resistant *A. fumigatus* clinical isolates by analysing the genomes and characterising the phenotypic profiles of 164 *A. fumigatus* isolates obtained from CF and non-CF patients at Royal Brompton Hospital (London, UK). Reference-based genome comparisons, using the number of SNPs of each isolate, grouped *A. fumigatus* isolates into two independent clades, A and B, distinguished by different drug resistant polymorphisms. The azole-resistant isolates that harboured TR_{34}/L98H were primarily clustered in Clade A, while wildtype/non-wildtype isolates were clustered in Clade B. Isolates from patients with CF were dispersed but mostly present in Clade B. Multiple isolates collected from a single patient have shown diverse genotypic and phenotypic variability, suggestive of infection via a mixed population of genotypes. We conducted phenotype microarray (PM) analysis for hundreds of different growth conditions to compare the metabolic capabilities of 21 selected isolates, including Af293 and CEA10 as reference isolates. The PM analysis showed that most phenotypic differences involved carbon and nitrogen source utilisation and high pH tolerance. Fewer variabilities and higher tolerance were observed among isolates for osmolytes, apart from a mutant isolate with low tolerance to 5.5–9% NaCl. All selected isolates showed low tolerance to 50–

200 mM sodium benzoate, 10% NaCl and 5–7% urea. The PM data were integrated with genome-wide association studies to establish genotype-to-phenotype associations and reconstruct a pathway map for the metabolism of selected substrates for further elucidation of differences between the isolates.

524V Exploring Pan-Genomes At MycoCosm *Richard D. Hayes*[1], Robin A. Ohm[2], Florian Freimoser[3], Sajeet Haridas[1], Li-Jun Ma[4], Igor V. Grigoriev[1] 1) DOE Joint Genome Institute, Lawrence Berkeley National Laboratory; 2) Department of Microbiology, Utrecht University, Ultrecht, The Netherlands; 3) Agroscope, Wädenswil, Switzerland; 4) Department of Biochemistry and Molecular Biology, University of Massachusetts, Amherst, MA, USA.

At the DOE Joint Genome Institute (JGI), the scope of sequencing now routinely looks beyond a single reference genome to collections of multiple samples of the same species or related strains. The concept of a pan-genome allows us to analyze the collective gene content and structural diversity of a population for features such as the set of orthologous genes shared by all samples, or the set of unique genes that control the diversity of strain-specific phenotypes. At JGI's web resource for fungal comparative genomics, MycoCosm (https://mycocosm.jgi.doe.gov/), we host many sets of related genomes, consisting of our own genome assembly and annotation projects and related genomes from other sources, that are amenable to this population-scale analysis. We present the results of a custom workflow combining multiple software approaches to construct protein sequence-based pan-genomes and analysis of shared synteny, the evolutionary conservation of genomic locus model order across groups of orthologous sequences, to investigate both whole-genome scale trends in protein family presence/absence differences and to resolve paralogy and thereby enrich for single-copy lineage specific genes.

525V Complete Genome Sequences and Genome-Wide Characterization of *Trichoderma* Biocontrol Agents Provide New Insights into their Evolution and Variation in Genome Organization, Sexual Development and Fungal-Plant Interactions Wan-Chen Li[1], Ting-Chan Lin[1], Chia-Ling Chen[1], Hou-Cheng Liu[1], Hisn-Nan Lin[1], Ju-Lan Chao[1], Cheng-Hsilin Hsieh[1], Hui-Fang Ni[2], Ruey-Shyang Chen[3], *Ting-Fang Wang*[1] 1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; 2) Department of Plant Protection, Chiayi Agricultural Experiment Station, Council of Agriculture, Chiayi, Taiwan; 3) Department of Biochemical Science and Technology, National Chiayi University, Chiayi, Taiwan.

Trichoderma spp. represent one of the most important fungal genera to mankind and in natural environments. The genus harbors prolific producers of wood-decaying enzymes, biocontrol agents against plant pathogens, plant-growth-promoting biofertilizers, as well as model organisms for studying fungal-plant-plant pathogen interactions. Pursuing highly accurate, contiguous, and chromosome-level reference genomes has become a primary goal of fungal research communities. Here, we report the chromosome-level genomic sequences and whole-genome annotation datasets of four strains used as biocontrol agents or biofertilizers (*T. virens* Gv29-8, *T. virens* FT-333, *T. asperellum* FT-101 and *T. atroviride* P1). Our results provide comprehensive categorization, correct positioning and evolutionary detail of both nuclear and mitochondrial genomes, including telomeres, AT-rich blocks, centromeres, transposons, mating-type loci, nuclear-encoded mitochondrial sequences, as well as many new secondary metabolic and carbohydrate-active enzyme gene clusters. We have also identified evolutionarily-conserved core genes contributing to plant-fungal interactions, as well as variations potentially linked to key behavioral traits such as sex, genome defense, secondary metabolism and mycoparasitism. The genomic resources we provide herein significantly extend our knowledge not only of this economically important fungal genus, but also fungal evolution and basic biology in general.

526V *Metarhizium robertsii* is a multifunctional insect pathogen and plant growth promoter *Huiyu Sheng*[1], Raymond St. Leger[1] 1) University of Maryland, College Park.

Some strains of *Metarhizium robertsii* (*M. robertsii*) have a dual lifestyle as insect pathogens and plant symbionts. They trade nitrogen extracted from their insect hosts for plant carbohydrates, thereby boosting plant growth as well as their own. This dualism makes *M. robertsii* a useful and tractable model to study insect-plant-fungus coevolution. We sequenced the genomes of eight *M. robertsii* strains that represent a range of contrasted abilities for colonization of insects and plants in order to relate these phenotypic differences to their genomic determinants. We found no trade-offs between pathogenicity to insects and either plant endophytic ability or physiological adaptability. The early diverged *M. robertsii* strains are weak to moderate insect pathogens, weak plant endophytes and have low physiological adaptability, whereas more recently diverged strains are more adaptable and better suited to various insect and plant environments. Ongoing work will identify and characterize the alleles that directly affect the mechanisms that underlie differences in insect pest control and plant growth promoting phenotypes. This will provide training in using genomes as predictive tools for estimating biocontrol properties, determine the genetic/phenotypic properties required for a biocontrol agent to persist and work in the field, and overall enhance efforts to optimize application strategies.

527V Genomic and transcriptomic differences between maize- and sorghum-specific *Exserohilum turcicum* isolates Pragya Adhikary[1], Pummi Singh[1], Jamann Tiffany[1], *Santiago Mideros*[1] 1) University of Illinois at Urbana-Champaign.

Exserohilum turcicum causes northern corn leaf blight in maize and sorghum leaf blight in sorghum. Isolates are host-specific, meaning that an isolate causing northern corn leaf blight cannot cause sorghum leaf blight and vice-versa. Genomic and transcriptomic studies of host-specific isolates can help identify structural variation between the genomes of host-specific isolates, genes coding for host specificity, and understand the mechanisms underlying host-specific virulence in *E. turcicum.* The objectives of our study were to i) obtain and compare genomes of two host-specific isolates to identify structural variation; and ii) obtain and compare gene expression between two host-specific isolates in order to identify genes conferring host specificity. We sequenced the genomes of maize-specific and sorghum-specific isolates with the PacBio Sequel system and de novo assembled the genomes. Both assemblies were in the range of ~ 44Mb - 45Mb with N50 of ~2.5 Mb and estimated to be 98% complete. The percentage of assembly covered by repeats was higher in the sorghum-specific isolate (29.1%) than that of the maize-specific isolate (19.6%). The estimated number of genes was 11,804 and 11,817 in the sorghum and maize-specific isolates, respectively. The comparative analysis of structural variation between the two genomes is in progress using the Funannotate pipeline. In addition, we generated transcriptomic data by sequencing RNA from maize and sorghum leaves inoculated with host-specific isolates ten days post-inoculation and from axenic cultures of each isolate. The

comparative gene expression analysis between the two host-specific isolates is in progress. Together, our study will provide insights into the mechanism of host specificity in *E. turcicum* and will further characterize the interactions of this pathogen with the grasses.

528V Asymmetrical dose-responses shape the evolutionary trade-off between antifungal resistance and nutrient use *Philippe C Després*[1,2,3,4], Angel F Cisneros[1,2,3,4], Emilie MM Alexander[1,2,3,4], Ria Sonigara[1,2,4,5], Cynthia Gagné-Thivierge[1,2,3,4,5], Alexandre K Dubé[1,2,3,4,5], Christian R Landry[1,2,3,4,5] 1) Département de Biochimie, de Microbiologie et de Bio-informatique, Faculté des Sciences et de Génie, Université Laval; 2) Institut de Biologie Intégrative et des Systèmes, Université Laval; 3) PROTEO, Le regroupement québécois de recherche sur la fonction, l'ingénierie et les applications des protéines, Université Laval; 4) Centre de Recherche sur les Données Massives, Université Laval; 5) Département de Biologie, Faculté des Sciences et de Génie, Université Laval.

Antimicrobial resistance is an emerging threat for public health. The success of resistance mutations depends on the trade-off between the benefits and costs they incur. This trade-off is largely unknown and uncharacterized for antifungals. Here, we systematically catalog the effect of all amino acid substitutions in the yeast cytosine deaminase FCY1, the target of the antifungal 5-FC. We identify over 900 missense mutations granting resistance to 5-FC, a large fraction of which appear to act through destabilisation of the protein. The relationship between 5-FC resistance and growth sustained by cytosine deamination is characterized by a sharp trade-off, such that small gains in resistance universally lead to large losses in canonical enzyme function. We show that this steep relationship can be explained by differences in the dose-response function of 5-FC and cytosine. Our results provide a powerful resource and platform for interpreting drug target variants in fungal pathogens as well as unprecedented insights into resistance-function trade-offs.

529V Quantifying the path to resistance to one of the oldest antifungal drugs *Romain Durand*[1,2,3,4], Jordan Jalbert-Ross[1,2,3,4], Alexandre Dubé[1,2,3,4], Christian Landry[1,2,3,4] 1) Département de Biochimie, Microbiologie et Bio-informatique, Faculté de Sciences et Génie, Université Laval, Québec, QC, Canada; 2) Institut de Biologie Intégrative et des Systèmes, Université Laval, Québec, QC, Canada; 3) PROTEO, Université Laval, Québec, Canada; 4) Centre de Recherche en Données Massives (CRDM), Université Laval, Québec, QC, Canada.

Pathogenic fungi display a higher mortality rate than malaria or breast cancer and are listed by the World Health Organization as needing immediate attention. Unlike bacteria, fungal cells are biologically close to animal cells, which makes the development of selective antifungals challenging. As a result, there's only 3 major classes of antifungals available. Moreover, some compounds belonging to the azoles' class are used to treat human infections as well as control crop diseases. Their massive use in the clinic and the field has inevitably led to the development of resistance-conferring mutations. Some strains of *Candida auris* are typically resistant to all available classes of antifungals, strongly limiting the ability to treat an infection. In order to increase the efficacy of current treatments, we need to better understand the mechanisms driving the emergence of resistance. In particular, we need to know which ways of evolution will lead to resistance, and the probability that a population of fungal cells will evolve in a direction or another. In this study, we propose a pipeline to characterize the evolution of resistance in yeast. Using two wild *Saccharomyces cerevisiae* strains, we performed a fluctuation assay in minimal medium to generate hundreds of mutants resistant to 5-fluorocytosine (5-FC). This pyrimidine analogue is one of the oldest antifungals. It is a prodrug, which after being imported in the cell, is then converted into the chemotherapeutic agent 5-fluorouracil, a toxic compound which ultimately causes the death of the cell. Our study confirms previous reports showing that mutations affecting the import and the conversion of 5-FC are rather rare. In contrast, around 30% of all mutants gained resistance by giving up mitochondrial function, thereby overexpressing crucial efflux pumps. Although this way of evolution comes with a fitness cost, it also confers cross-resistance to one of the most often used antifungal in both the field and the clinic: fluconazole.

530V Genome-wide Functional Analysis of WD40 Repeat-containing Proteins in *Cryptococcus neoformans* *Jin-Tae Choi*[1], Yu-Byeong Jang[1], Seong-Ryong Yu[1], Yujin Lee[1], Yong-Sun Bahn[1] 1) Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University.

Cryptococcus neoformans is one of the major human fungal pathogens which cause life-threatening meningoencephalitis in immunocompromised patients. The protein-protein interaction (PPI)-dependent cell signaling regulation in response to various environment cues and host conditions is known to be important for the pathobiological functions of *C. neoformans*. The WD40 domain is one of the most common and abundant domains related to PPIs, and yet the role of WD40 repeat-containing proteins remains elusive. In this study, we aim to systematically analyze the functions of the WD40 repeat-containing proteins in *C. neoformans*. Here we identified 132 putative WD40 repeat-containing proteins based on annotation in the *C. neoformans* var. *grubii* genome database provided by the WD40-repeat protein Structures Predictor (WDSP) and performed a BLAST search with their protein sequences to identify any corresponding orthologs in *Saccharomyces cerevisiae*, *Candida albicans*, and *Schizosaccharomyces pombe*. Thus far, we have successfully constructed 94 signature-tagged gene-deletion strains representing 47 WD40 repeat-containing proteins through homologous recombination methods. We are in the middle of examining their phenotypic traits under 30 different in vitro conditions, including growth, differentiation, stress response, antifungal resistance and virulence factor production. For the genes that could not be deleted and therefore considered to be putatively essential for the growth of *C. neoformans*, we replaced their native promoter with the copper-regulated *CTR4* promoter. Among the 47 genes, we discovered several genes that led to dramatic phenotypic changes upon gene deletion, such as *RAV1, HIRA, CTF4,* and *PWP1*. Among these, Rav1, which was known as a subunit of the regulator of ATPase of vacuoles and endosomes (RAVE) complex in *S. cerevisiae*, was related to cellular growth on various stress conditions, including temperature upshift, and the production of virulence factors in *C. neoformans*. Through further analysis of the remaining WD40 repeat-containing genes, we will uncover the roles of WD40 proteins and identify their PPI partners as well as explore how WD40 proteins affect pathobiology of *C. neoformans*, which will provide insights into development of novel antifungal agents utilizing these PPI partners.

531V Balancing positive and negative selection: metabolic consequences of antifungal resistance via constitutive Mrr1 activity in *Candida lusitaniae* *Elora Demers*[1], Amy Biermann[1], Marina Ruzic[1], Jason Stajich[2], Alix Ashare[3], Deborah Hogan[1] 1) Geisel School of Medicine at Dartmouth, Hanover, NH; 2) University of California-Riverside, Riverside, CA; 3) Dartmouth-Hitchcock Medical Center, Section of Pulmonary and Critical Care Medicine, Lebanon, NH.

The evolution of pathogens in response to selective pressures present during chronic infections, such as nutrient limitation and interac-

tion with immune cells or other microbes, can influence persistence, virulence, and the outcomes of antimicrobial therapy. Our analysis of a set of clonally derived *Clavispora (Candida) lusitaniae* isolates from a fungus-dominant lung infection in an individual with cystic fibrosis (CF), found striking genomic changes in the transcription factor encoded by *MRR1* that appeared to repeatedly arise in this chronic infection. Genetic and genomic analyses found evidence for repeated acquisition of gain-of-function mutations that conferred constitutive Mrr1 activity, resulting in increased drug resistance. In the same population, there were multiple alleles with both gain-of-function mutations and secondary suppressor mutations that either attenuated or abolished the constitutive activity suggesting the presence of counteracting selective pressures. In our recently published study, we demonstrated tradeoffs between high Mrr1 activity, which confers resistance to the antifungal fluconazole, host factors, and bacterial products through its regulation of *MDR1*, and resistance to hydrogen peroxide, a reactive oxygen species produced in the neutrophilic environment associated with this infection. Further investigation of *MDR1*-independent phenotypes however has yielded multiple links between Mrr1 activity and metabolism, such as the Mrr1-dependent regulation of methylglyoxal reductases, which detoxify a common metabolic byproduct, and multiple alcohol dehydrogenases. Analyses of the metabolic consequences of constitutive Mrr1 activity have revealed decreased in growth across a wide variety of carbon sources, including amino acids shown to be highly abundant in the CF lung environment, altered intracellular ATP and metabolite accumulation, and increased oxygen consumption. While in some cases these metabolic changes may be beneficial *in vivo*, given the dynamic environment in the CF lung, they may also contribute to the selection for mutations causing the reversion of Mrr1 to a low activity state further complicating the drug resistance profile of this population.

532V Evolution of mycorrhizal symbiosis in Inocybaceae *Faheema Khan*[1], Marisol Sanchez-Garcia[2], Hanna Johannesson[1], Martin Ryberg[1] 1) Uppsala University; 2) Swedish University of Agricultural Sciences.

Ectomycorrhiza (EcM) is one of the major types of symbiotic relationships between fungi and plants, where fungi get carbon fixed by plants and in return provide nutrients like nitrogen, phosphorus and water etc to plants. This symbiosis plays a key role in recycling of nutrients in the ecosystems by facilitating the nutrient uptake of plants. Different phylogenetic studies propose that ectomycorrhizal fungi have evolved multiple times in several lineages from saprotrophic ancestors but molecular research has only scratched the surface on understanding the genetic basis of this important symbiosis. The molecular signature of EcM lifestyle include contraction of ancestral carbohydrate degrading proteins and an expansion of mycorrhiza specific effector proteins in ectomycorrhizal genomes. Now, it is not clear if these changes are sudden at the shift to ECM or if there has been gradual change along the course of evolution. My current study, therefore, attempts to answer this important question related to EcM in the most extendedly sampled ectomycorrhizal lineage to date, Inocybaceae, and its saprotrophic sister taxa, Crepidotaceae. For this, the whole genome of 20 Inocybaceae species along with 2 Crepidotaceae species were sequenced by Illumina Hiseq. The genomes were assembled and annotated and different comparative genomic/phylogenomic analyses are being carried out. Our preliminary results show contractions in Cazyme (Carbohydrate Active Enzyme) protein families in EcM as compared to the saprotrophs as was observed previously in other EcM lineages and that most of these contractions happened at the transition point to EcM in this lineage. This study will give insights into the expansion/contraction pattern of proteins important for the establishment of this symbiotic relationship and also general genomic adaptation to symbiosis.

533V Biocontrol activity and genomic analyses of the antagonistic, yeast-like fungus *Aureobasidium pullulans* Maria Paula Rueda-Mejia[1], Lukas Nägeli[1], Stefanie Lutz[1], Richard D Hayes [2], Adithi R. Varadarajan [1], Igor V. Grigoriev[2,3], Christian H. Ahrens[1,4], *Florian Freimoser*[1] 1) Agroscope; 2) U.S. Department of Energy Joint Genome Institute (JGI); 3) Department of Plant and Microbial Biology, University of California Berkeley; 4) SIB, Swiss Institute of Bioinformatics.

Aureobasidium pullulans is an extremotolerant, cosmopolitan yeast-like fungus that successfully colonises vastly different ecological niches. The species is widely used in biotechnology and successfully applied as a commercial biocontrol agent against postharvest diseases and fireblight. However, the exact mechanisms that are responsible for its antagonistic activity against diverse plant pathogens are not known at the molecular level. Thus, it is difficult to optimise and improve the biocontrol applications of this species.
In a brod screen for antagonistic activity, *A. pullulans* was one of the most active species against a range of plant pathogenic fungi. We have therefore characterised this yeast-like fungus with respect to its biocontrol activity against plant pathogens in the phyllo- and rhizosphere and by comprehensive genomic analyses. We determined its competitiveness and antagonistic activity against other yeasts and plant pathogens, *de novo* assembled a high-quality reference genome of a strongly antagonistic *A. pullulans* strain, performed dual RNA-seq experiments, and analysed proteins secreted during the interaction with the plant pathogen *Fusarium oxysporum*.
These studies documented strong competitiveness on apples, but also the capability to colonise roots and prevent *Fusarium* infections. Based on our genome annotation, we identified potential biocontrol genes (e.g., genes encoding secreted hydrolases or being part of secondary metabolite clusters). Transcriptome and secretome analyses defined a subset of 79 *A. pullulans* genes (among the 10,925 annotated genes) that were transcriptionally upregulated or exclusively detected at the protein level during the competition with *Fusarium oxysporum*. These potential biocontrol genes comprised predicted secreted hydrolases such as glycosylases, esterases, and proteases, as well as genes encoding enzymes, which are predicted to be involved in the synthesis of secondary metabolites. This study highlights the value of a sequential approach starting with genome mining and consecutive transcriptome and secretome analyses in order to identify a limited number of potential target genes for detailed, functional analyses. In the longterm, understanding the biocontrol phenotype at the molecular level will lead to improved and more efficacious formulations and application methods of *A. pullulans* and thus lay the foundation for the future development of new plant protection products.

534V Identification of key genes of the defense response of the mushroom-forming fungus *Schizophyllum commune* against fungal and bacterial antagonists *Erik Beijen*[1], Marieke van Maanen[1], Janieke Klusener[1], Robin A. Ohm[1] 1) Utrecht University.

Mushrooms are a valuable food source for a growing world population. However, commercial production suffers from many pests and diseases, which can lead to significant crop losses. Mushroom-forming fungi have evolved defense strategies to resist attacks, such as the production of effector proteins, but regulatory mechanisms of this immune system are still unknown. Therefore, we investigated interactions between the mushroom-forming fungus *Schizophyllum commune* and multiple antagonists: the fungi *Trichoderma harzianum, Trichoderma aggressivum* and *Purpureocilium lilacinum*, and the bacterium *Serratia quinivorans*.

Differential gene expression in *S. commune* was assessed by RNA-seq in the direct interaction zone, in the area outside the direct interaction zone, and in *S. commune* growing alone. Overall, gene expression in each condition was distinct, indicating that *S. commune* responds differently to each antagonist. Moreover, there is a small but clear systemic effect, suggesting that a signal travels from the interaction zone to non-interacting parts of the colony.
The transcription factor TF31 was up-regulated during interactions with each antagonist. This GATA-zinc-finger transcription factor is the only transcription factor that shows differential expression and might therefore play an important role in general defense. A knockout strain of this gene was indeed more sensitive during interactions with *T. harzianum*.
More downstream-acting, a putative ABC (ATB-Binding Cassette) transporter gene was found to be differentially expressed. ABC transporters are common transmembrane transporters of several substrates, including antifungals. They are involved in multidrug resistance in multiple yeasts, as well as in some *Aspergilli.* However, in mushroom-forming fungi very little is known about their role in the defense against antagonists. We created a reporter strain by placing the gene encoding red fluorescent dTomato under the control of the promoter of the ABC transporter. In mono-culture, no fluorescence was observed. However, it displayed strong fluorescence when grown in co-culture with several antagonistic fungi. We hypothesize that upon detection of excessive internal levels of xenobiotics, this transporter maintains a healthy intracellular environment by pumping undesirable compounds out of the cytosol.

535V Revisiting Meiotic Mutants in *Coprinopsis cinerea* using a Genomic Approach Marilee Ramesh[1], *Abigail Burke*[1], Cameron Sammons[1] 1) Roanoke College.

The basidiomycete *Coprinopsis cinerea* is a genetic model system for studying eukaryotic processes in fungi including the relationship between meiosis and DNA repair. As part of eukaryotic evolution, genes were recruited from DNA repair processes to manage the double strand breaks that are necessary to initiate recombination events between homologous chromosomes in meiosis. This group of genes are expressed in vegetative tissue for their DNA repair role and in meiotic tissue for the meiotic role to manage the double strand DNA breaks that occur in both processes. Previous work in *C. cinerea* sought to identify genes involved in DNA repair and meiotic pathways through mutagenesis, screening and complementation. While this approach was successful in identifying some of the genes, many of the mutants were not able to be characterized using conventional methods at the time. Genomics and bioinformatics have provided new tools to revisit these mutant strains. This study revisited the twelve unidentified mutants using a comparative genomic approach. The genome of each mutant strain was sequenced and compared to the reference genomes to eliminate all identical protein predictions. Through this process, we were able to eliminate the majority of the 13,356 ORFs, creating a short list of protein predictions (5-80) that contain SNPs between the reference genome and the mutant genome; these candidates were evaluated individually for functionality. Criteria to evaluate the candidate genes for roles in DNA repair and meiosis included gene structure, gene expression, protein function based on pFam domains and BLASTx analysis. This approach enabled us to identify strong candidates for six of the twelve mutant strains, including Ku80 (non-homologous end joining), rhp42 (nucleotide excision repair), rad53 homolog, p63, and DNA Pol epsilon. Further work to extend these results examined the pathways in which these candidates' function and surveyed the genome for other key proteins to establish the presence of entire DNA repair/meiosis pathways in *C. cinerea*.

536V *Trichoderma reesei* Rad51 tolerates mismatches in hybrid meiosis with diverse genome sequences Wan-Chen Li[1], Chia-Yi Lee[2], Wei-Hsuan Lan[3], Tai-Ting Woo[1], Hou-Cheng Liu[1], Hsin-Yi Yeh[2], Hao-Yen Chang[2], Yu-Chien Chuang[1], Chiung-Yan Chen[1], Chi-Ning Chuang[1], Chia-Ling Chen[1], Yi-Ping Hsueh[1], Hung-Wen Li[3], Peter Chi[2], *Ting-Fang Wang*[1] 1) Institute of Molecular Biology, Academia Sinica; 2) Institute of Biochemical Sciences, National Taiwan University; 3) Department of Chemistry, National Taiwan University.

Sexual eukaryotes fall into two groups with respect to their RecA-like recombinases. The first group possesses Rad51 (ubiquitous) and Dmc1 (meiosis-specific), which cooperate to conduct interhomolog recombination in zygotes with high sequence heterogeneity. Interestingly, Dmc1 was lost from the second group of eukaryotic organisms. Here we used the industrial workhorse fungus *Trichoderma reesei* to address if and how Rad51-only eukaryotes carry out hybrid meiosis. We show that *T. reesei* Rad51 (*Tr*Rad51) is indispensable for interhomolog recombination during meiosis and that *Tr*Rad51, like *Saccharomyces cerevisiae* Dmc1, possesses a better mismatch tolerability than *S. cerevisiae* Rad51. Our results indicate that the ancestral *Tr*Rad51 evolved to acquire Dmc1-like properties by adopting multiple structural variations in the L1 and L2 DNA-binding loops (https://www.pnas.org/content/118/8/e2007192118)

537V Kingdom-wide analysis of fungal transcriptomes and tRNAs reveals conserved patterns of adaptive evolution *Rhondene Wint*[1,2], Asaf Salamov [2], Igor Grigoriev[2,3] 1) University of California - Merced; 2) US Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory; 3) Department of Plant and Microbial Biology, University of California Berkeley.

Codon usage in protein-coding genes is shaped by the combined, but uneven, influence of adaptive and non-adaptive evolutionary pressures. Studies in model fungi agree on codon usage bias as an adaptation for fine-tuning gene expression; however, such knowledge is lacking for most other fungi. By analyzing transcriptomic and tRNA data of over 400 species across the 6 major phyla, we show that codon usage and tRNAs have co-evolved to optimize the speed of mRNA translation. Fitting of maximum likelihood macroevolutionary models best supports adaptive codon usage as a realization of convergent evolution. Principally, the interspecies variation of most codons and tRNAs could not be fully explained by phylogenetic relatedness or drift. Yet, we found remarkable conservation of translation bias wherein the highest expressed mRNAs are enriched with codons decoded by the most abundant tRNAs, especially inosine-modified tRNAs, in contrast to the least expressed mRNAs being codon-biased for the least abundant tRNAs. To leverage the prevalence of adaptive codon usage, we developed a neural network tool, *Codon2Vec*, that predicts gene expression directly from native coding sequences, achieving a median accuracy of 83.8% ±0.05 on 300 fungal genomes. Altogether, our analyses reveal that selection on codon usage for translation efficiency is a conserved regulatory grammar for controlling fungal gene expression. Our findings have implications for better understanding the evolutionary success of fungi, as well as informing the biosynthetic manipulation of fungal genes.

538V Comparative genome analysis of *Fusarium culmorum* and *F. graminearum* reveals a different type of chromosomal organization but a common gene repertoire linked to virulence on wheat *Martin Urban*[1], Dan Smith[1], Robert King[1], Joseph Hearn-

shaw[1], Keywan Hassani-Pak[1], Kim Hammond-Kosack[1] 1) Rothamsted Research, Harpenden, UK.

The pathogenic ascomycete fungus *Fusarium culmorum* causes floral, seed, stem-base and root diseases of both cereal and non-cereal plant species. *F. culmorum* thrives in cooler conditions than *F. graminearum*. The annotated assembly of the historic strain UK99 (DON/3-ADON chemotype, isolated from a wheat crop in 1998) was obtained using PacBio sequencing. The assembly/annotation pipeline identified four core chromosomes, rich in orthologues present in *F. graminearum* and other closely related Fusaria species. The UK99 *F. culmorum* genome also has a gene sparse, transposon-rich fifth supernumerary chromosome of 1.91 MB absent from other genome sequenced Fc strains (UK97, UK98, PV1 and CS7071), but contains genes related to more distant Fusaria.
F. culmorum was previously presumed to be an asexual species but was found to possess two mating loci in isolates from three continents. Secondary metabolite gene clusters were expanded in *F. culmorum* compared to *F. graminearum*. Functional evaluation of six selected transcription factors essential for floral pathogenesis in *F. graminearum,* indicates only a subset are required for *F. culmorum* virulence on wheat spikes, stem-bases and coleoptiles.
In an additional computational approach devised to survey candidate Fc virulence genes, we built a combined knowledge gene network graph for *F. culmorum* and nine related fungal genomes including five Fusaria, *Magnaporthe oryzae* and *Zymoseptoria tritici*. The network graphs are searchable at https://knetminer.org/Fusarium_culmorum using the KnetMiner[1] gene network discovery platform. For each gene available information on gene ontology, protein domains, PHI-base[2] retrieved phenotypes and publication are provided. Protein-protein interaction networks were build using orthologous data from STRING (https://string-db.org). This newly available resource allows researchers to pinpoint inter-species differences and similarities in graphical displayed gene networks.

[1] Hassani-Pak et al (2021) Plant Biotech J, doi: 10.1111/pbi.13583
[2] Urban et al (2022) Nucleic Acids Research, PHI-base in 2022 (Database issue)

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539V Genome of the ginger pathogen *Pythium myriotylum* uncovers the most extensive arsenal of virulence-related genes amongst *Pythium* plant pathogens *Paul Daly*[1], Dongmei Zhou[1], Danyu Shen[2], Qimeng Zhang[1], Siqiao Chen[1,3], Taiqiang Xue[1], Yifan Chen[1,4], Jamie McGowan[5], David Fitzpatrick[5], Sheng Deng[1], Jingjing Li[1], Gunseli Bayram Akcapinar[6], Irina Druzhinina[3], Lihui Wei[1,4] 1) Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, Nanjing, China; 2) College of Plant Protection, Nanjing Agricultural University, Nanjing, China; 3) Fungal Genomics Group, Nanjing Agricultural University, Nanjing, China; 4) School of Environment and Safety Engineering, Jiangsu University, Zhenjiang, China; 5) Genome Evolution Laboratory, Maynooth University, Co. Kildare, Ireland; 6) Institute of Health Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey.

The *Pythium* (Peronosporales, Oomycota) genus includes devastating plant pathogens that cause widespread diseases and severe crop losses. Here we have uncovered a far greater arsenal of virulence factor-related genes in the necrotrophic *Pythium myriotylum* compared to other *Pythium* plant pathogens. The genome of a plant-virulent *P. myriotylum* strain (~70 Mb and 22,657 genes) isolated from a diseased rhizome of ginger (*Zingiber officinale*) encodes the largest repertoire of putative effectors, proteases, and plant cell wall degrading enzymes (PCWDEs) amongst the studied species. *P. myriotylum* has twice as many predicted secreted proteins compared to any other *Pythium* plant pathogen. Arrays of tandem duplications appear to be a key factor to the enrichment of the virulence factor-related genes in *P. myriotylum*. The transcriptomic analysis performed on two *P. myriotylum* isolates infecting ginger leaves showed that proteases were a major part of the upregulated genes along with PCWDEs, NEP1-like toxin (NLPs) and elicitin-like proteins. A sub-set of *P. myriotylum* NLPs were analyzed and found to have necrosis-inducing ability from agroinfiltration of tobacco (*Nicotiana benthamiana*) leaves. One of the heterologously produced infection-upregulated putative cutinases found in a tandem array showed esterase activity with preferences for longer chain-length substrates and neutral to alkaline pH levels. Our results allow the development of science-based targets for the management of the ginger soft rot disease as insights from the genome and transcriptome show that gene expansion of virulence-factor related genes play a bigger role in the plant parasitism of *Pythium* spp. than previously thought.

540V Comparative pathogen-host interaction phenotype analysis in human and plant microbial pathogens using PHI-base *Martin Urban*[1], Elzbieta Janowska-Sedja[1], Alayne Cuzick[1], James Seager[1], Kim Rutherford[2], Valerie Wood[2], Kim Hammond-Kosack[1] 1) Rothamsted Research, Harpenden, UK; 2) University of Cambridge, UK.

PHI-base, www.phi-base.org, is a gold-standard manually curated phenotype database storing molecular information on genes implicated in virulence[1]. Our Sep 2021 release (version 4.12) of the database provides information on 278 pathogens tested on 228 hosts. Information is also given on the target sites of commercial and experimental anti-infective chemistries and first host targets of pathogen effector genes. PHI-base's mission is to be a primary information source for researchers studying plant, animal, and/or human pathogens. Manually curated information from more than 4,300 peer reviewed articles are made accessible and searchable to provide relevant molecular and biological facts on pathogenicity, wild-type/mutant genes and fungicide target sites. Species neutral high-level phenotypes are used to describe the overall pathogen-host interaction outcomes using our newly developed PHI Phenotype Ontology (PHIPO) registered at the OBO Foundry. Together this allows comparative phenotype analysis across a wide spectrum of pathosystems. We recently developed a web-based community annotation tool, called PHI-Canto (canto.phi-base.org) that captures mutant phenotype data during manuscript submission by authors. PHI-base phenotype data and ontology terms are disseminated to Ensembl Genomes, NCBI, UniProtKB, FungiDB and the KnetMiner knowledge graph tool. The combined resources allow linking of phenotypes to genomes and enable computational analysis including variant analysis, RNAseq and mapping to biochemical pathways.
Here we present the construction of a predicted protein-protein interaction network resource for twelve Ascomycete fungal pathogens using PHI phenotypes in a use case study[2]. For the cereal infecting fungus *Fusarium graminearum* thirty-five putative virulence interaction proteins and siRNA targets were identified as candidate virulence genes.

[1] Urban et al., (2021) Nucleic Acids Res, doi 10.1093/nar/gkab1037; [2]Janowska-Sejda et al (2019), Front Microbiol, doi 10.3389/

fmicb.2019.02721.

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541V Full genome sequence of onion black mold causing *Aspergillus welwitschiae* reveals the presence of putative mycotoxin gene clusters *Jagath Ranasinghe*[1], Anupama Halmillawewa[2], Maciej Kaczmarek [3], Ian Singleton[4], Cristóbal Gallardo[5], Renuka Attanayake[1] 1) Department of Plant and Molecular Biology, University of Kelaniya, Sri Lanka; 2) Department of Microbiology, University of Kelaniya, Sri Lanka; 3) Crop and Soil Systems Group, SRUC, West Mains Road, Edinburgh, UK; 4) School of Life, Sport & Social Sciences, Edinburgh Napier University, UK; 5) Lehrstuhl für Bioinformatik, Institut für Informatik, Albert-Ludwigs-Universität Freiburg, Germany.

Black mold of onion is a postharvest disease causing a significant economic loss around the world. *Aspergillus niger* is the known pathogen causing the disease for many years and recently *A. welwitschiae* has been identified as a causative agent for black mold in onion. The present study was conducted to determine the post-harvest losses caused by onion black mold in Sri Lanka, to determine the pathogen species causing the disease and to obtain its full genome data in order to determine whether *A. welwitschiae* carries toxin metabolite genes. Almost all the surveyed onion vendors complained about the presence of onion black mold and the disease incidence ranged from 90-100%. Pathogen was isolated into pure culture and Calmodulin gene sequence of a selected isolate was compared to the NCBI data base confirming that the species was *A. welwitschiae*. DNA was isolated using QIAGEN fungal DNA isolation kit and library preparation and whole genome sequencing was conducted using Illumina sequencing platform. The sequenced genome of *A. welwitschiae* was assembled and annotated using the open source web-based platform, Galaxy (https://usegalaxy.eu/). The genome assembly was 38.65 Mb in size with 11,585 protein encoding genes. Based on the similarity searches, 386 putative secondary metabolite genes were identified in the genome which are also found in other *Aspergillus* species like *A. flavus, A. japonicus, A. fischeri* and *Talaromyces islandicus*. Most of the putative secondary metabolite genes found were polyketide types. It is also found that the isolate contained polyketide, tetracycline like Viridicatumtoxin synthesis gene. *In silico* analysis also revealed that the genome of *A. welwitschiae* harbors two toxin gene clusters; HC-toxin synthetase and AM-toxin synthetase. HC-toxin is a cyclic tetrapeptide and it is a virulence determinant for the plant pathogenic fungus, *Cochliobolus carbonum.* AM-toxin synthetase is a cyclic host specific toxin found in *Alternaria alternata.* Interestingly, genes coding ochratoxin and fumonicin, which were reported in other *Aspergillus* spp. were not found in the present study. Present study provides new insights into underlying metabolic pathways of *A. welwitschiae* causing onion black mold.

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542V Genome Wide Association Mapping to characterize a virulent sexual population of wheat stem rust (*Puccinia graminis* f. sp. *tritici*) from the Pacific Northwest using barley and wheat differentials *Arjun Upadhaya*[1], Sudha GC Upadhaya[1], Robert Brueggeman[1] 1) Washington State University, WSU, Pullman, WA.

Wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), is an important disease of barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*), worldwide. In North America, the Pacific-Northwest (PNW) region is a center of stem rust diversity due to sexual cycle completion in primary cereal and grass hosts and secondary sexual hosts including *Mahonia* spp. (Oregon grape) and *Barberis vulgaris* (barberry). Sexual recombination allows for virulent gene combinations that are alarming due to individuals in the PNW population with novel virulence profiles on important resistance (R) genes. For example, we recently reported on highly virulent *Pgt* isolates from this PNW population with virulence on the two major barley R-genes, *Rpg1* and *rpg4/5*, when stacked together. This was the first report of any *Pgt* isolates virulent on this combination of R-genes from collections around the world. To identify *Pgt* virulence/avirulence loci and candidate genes that interact with important barley and wheat R-genes phenotype and genotype data were generated for this PNW *Pgt* population collected from the states of Washington and Idaho consisting of 96 isolates from barley, wheat, barberry, and Oregon grape. The infection type data was generated for each isolate on five barley and ten wheat R-gene differentials that contained phenotypic variation from virulence to avirulence. The Phenotyping indicated diverse race structure and segregation of pathogen virulence/avirulence loci in the population on distinct barley and wheat R-genes indicating a diverse sexual population amenable to genome wide association studies (GWAS). Genotype data was generated by whole genome sequencing using shotgun libraries and the Illumina Novaseq 6000 platform. On average, 66 million paired-end reads (PE – 150 bp) were generated for each isolate averaging 77x coverage of the whole genome with a total of 1.2 million high quality SNPs identified. From the extensive phenotyping and genotyping data set we will report on the results of the GWAS and identification of virulence/avirulence loci and candidate genes. This research will help identify wheat stem rust effectors and fill knowledge gaps in rust effector biology and mechanisms of virulence evolution in this important cereal rust pathosystem.

543V A Pyrenophora resource to identify protein structural homologues *Paula Moolhuijzen*[1], Pao Theen See[1], Harold R. Powell[3], Gongjun Shi[2], Zhaohui Liu[2], Caroline S. Moffat[1] 1) Curtin University; 2) North Dakota State University; 3) Imperial College London.

Paula Moolhuijzen[1], Pao Theen See[1], Harold R. Powell[3], Gongjun Shi[2], Zhaohui Liu[2] and Caroline S. Moffat[1]
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Necrotrophic fungi such as *Pyrenophora tritici-repentis* can possess a large repertoire of *in silico* predicted effector proteins, characterised by very diverse sequences. The prediction of these proteins depends on machine learning which is based on sequence classifiers and the detection of a signal peptide for secretion. Although tools have improved the identification of potential candidate genes, very few can be clustered based on sequence identity into orthologous groups. It has been shown however that some effectors

with low sequence identity are conserved by protein tertiary structures and are therefore only related by a 3-dimensional structure. For example, the known *P. tritici-repentis* effectors ToxA and ToxB have only a tertiary structure in common that is characterised by two anti-parallel β-sheets linked by disulphide bonds.

To conduct a comprehensive protein tertiary structure search we engaged the whole genome of *P. tritici-repentis* available through the Protein Homology/analogy Recognition Engine (Phyre) BackPhyre application. This resource is the first necrotrophic fungal pathogen publicly available through BackPhyre for effector and other protein tertiary structure searches, providing further annotation evidence for a number of hypothetical genes. We also present how the use of protein three-dimensional structure modelling improved the identification of a number of proteins which includes effector candidates potentially involved in pathogenicity.

544V Genus-wide analysis of *Fusarium* polyketide synthases reveals broad chemical potential *Daren Brown*[1], Hye-Seon Kim[1], Amy McGovern[1], Crystal Probyn[1], Robert Proctor[1] 1) United States Department of Agriculture, ARS-USDA, Peoria, IL.

Many species of the fungus *Fusarium* are of concern to agriculture because they cause economically important diseases of cereal crops such as barley, corn, and wheat. The diseases not only reduce the yield and quality of the crops, but they can also leave crops contaminated with toxins (mycotoxins) that pose health risks to humans, pets and livestock animals. Natural products (NPs) synthesized by *Fusarium* can contribute to pathogenesis or competitiveness of the fungus in the environment and to disease in animals including cancer and neural tube defects. Polyketide synthases (PKSs) are a family of large, multi-domain enzymes that are required for synthesis of most fungal NPs. To gain insight into the NP potential of *Fusarium*, we retrieved 2974 PKS gene sequences from the genomes of 206 *Fusarium* species. Phylogenetic analysis resolved these PKSs, along with 118 previously described PKSs from other fungi, into 122 clades. Previously, we proposed that PKSs in the same clade synthesize the same polyketide, which is structurally distinct from polyketides synthesized by PKSs in other clades. Here we predict that the 122 clades potentially produce 112 structurally distinct families of polyketide-derived NPs because some NPs (e.g., zearalenone) require two PKSs for their synthesis. The clades include PKSs required for synthesis of six NPs whose production has not previously been reported in *Fusarium*, including two NPs with significant pharmaceutical interest: chaetoviridin and lovastatin. We will discuss how our results highlight the NP diversity of *Fusarium* and the potential of the genus to produce metabolites with medical and other applications.

545V Analysis of 22 *Apiospora* genome assemblies uncovers a great biosynthetic potential for secondary metabolites *Trine Sørensen*[1], Celine Petersen[1], Lavinia I. Fechete [1], Asmus T. Muurmann[1], Jens Laurids Sørensen[1], Kåre L. Nielsen[1], Teis Esben Sondergaard[1] 1) Department of Chemistry and Bioscience, Aalborg University, Denmark.

The filamentous fungal genera *Apiospora* and *Arthrinium* have undergone a period of different phylogenic affiliation during the last ten years. Recently, they were divided into two separate clades with the majority being *Apiospora* and several species changed genus from *Arthrinum* to *Apiospora*. The genus *Apiospora* is believed to have a high biosynthetic potential for secondary metabolites. Since only little work have been done on characterizing and identifying of these metabolites, there is a high probability for discovery of novel secondary metabolites within this genus. Novel secondary metabolites are of interest for the pharmaceutical, food and agricultural industry. The development of long-read sequence technology has improved genome assemblies making it possible to obtain highly contiguous genome sequences and thereby facilitates mining of secondary metabolites gene clusters. We investigated the biosynthetic potential of polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs) in 22 *Apiospora* genomes sequenced by an in-house sequencing pipeline using MinION sequencing. It was possible to obtain highly contiguous genome assemblies from all species. A total of 753 PKS genes and 286 NRPS genes was predicted in the all the genomes. Phylogenetic analysis was used to identify 97 distinct PKS clades and 30 distinct NRPS clades in the different *Apiospora* species. Furthermore, only 7 PKS clades and 5 NRPS clades was shown to be conserved across all species underscoring the diversity of secondary metabolite genes within the genus. While elucidation of which genes produce exactly which secondary metabolite remains to be resolved, the diversity of distinct gene clades increases the chance of novel compound discoveries from *Apiospora*.

546V Evolution-driven discovery of new bioactive fungal molecules *Olga Mosunova*[1], Ella Schunselaar[1], Elske Dwars[1], Jorge Navarro[1], Jacq van Neer[1], Diksha Haksar[1], Jelmer Hoeksma[2], Wouter Beenker[2], Ronnie Lubbers[2], Jeroen den Hertog[2], Jerome Colemare[1] 1) Westerdijk Fungal Biodiversity Institute; 2) Hubrecht Institute.

Among other actions, finding new antimicrobials is key to treat infections caused by multi-resistant pathogens and at the same time better manage the emergence of resistance. Around 40% of the developed therapeutics drugs approved by the US Food and Drug Administration (FDA) are derived from natural molecules produced by plants, bacteria or fungi, showing these organisms are a unique resource of bioactive molecules. Recent genomic studies have revealed great and largely unexplored biosynthetic potential of fungi to produce such molecules, and gaining access to this hidden diversity in a rational manner requires new approaches. In the present study, we focus on lichen mycobiont genomes, which can be hardly manipulated in the lab but have been used for centuries in traditional medicine and show a unique potential for the production of polyketide compounds. Using this lichen-associated fungal novelty as a starting point, we have employed a combined evolution-guided dereplication and comparative genomics strategy to identify novel polyketide biosynthetic pathways that could yield previously unreported molecules. Two biosynthetic pathways were selected for functional characterization using heterologous expression in *Aspergillus oryzae*. One pathway was linked to the production of naphthalenone compounds in fungi, and the other one yielded a molecule that exhibited antibacterial activity towards both Gram positive and Gram negative bacteria, including methicillin resistant *Staphylococcus aureus* and *Acinetobacter baumanii*. Thanks to this approach, we have generated new fundamental knowledge by linking genes to compounds, and importantly, found a new antibiotic to characterize further.

547V Nonribosomal peptide synthetase gene clusters and characteristics of NRPS-dependent siderophore synthetases in *Armillaria* and other species in the Physalacriaceae Deborah Narh Mensah[1], Brenda Wingfield[1], *Martin Coetzee*[1] 1) Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

Armillaria spp. are terrestrial ubiquitous basidiomycetes in the family Physalacriaceae, most of which are facultative necrotrophs of

plants. In fungi, secondary metabolites are often pathogenicity or virulence factors. Genes involved in these metabolites are usually contained in secondary metabolite gene clusters (SMGCs), such as nonribosomal polypeptide synthetase (NRPS) clusters. NRPSs contain domains, are either mono- or multi-modular, and produce peptides such as siderophores. Siderophores are high affinity ferric iron chelating compounds required for iron uptake under aerobic conditions. The aim of this study was to investigate NRPS-encoding genes and clusters of *Armillaria* spp. and selected species from the Physalacriaceae using a comparative genomics approach. Siderophore biosynthesis by *Armillaria* spp. was also evaluated using CAS and split-CAS assays. Our results showed that the genomes contained at least one NRPS cluster. Other clusters identified included NRPS-like, NRPS-independent siderophore synthetases, terpenes, type 1 polyketide synthetase, and some hybrid clusters. No corelation was observed between the number and types of SMGCs and reported pathogenicity of the species studied. All NRPSs containing full modules were multimodular with the domain architecture $(ATC)_3(TC)_2$. These clusters putatively encode hydroxamate siderophores based on homology with characterised NRPSs, domain architecture, and predicted substrates of A-domains of the NRPS genes. Microsynteny was observed among the NRPS clusters of the *Armillaria* spp. Genes encoding L-ornithine-N^5-monooxygenase were not identified in the NRPS clusters and atypical Stachelhaus codes were predicted for the A3 domains. Based on these results, we postulate that the siderophore biosynthesised by the NRPS clusters of *Armillaria* spp. and the other Physalacriaceae species will differ from earlier characterised siderophores. Bioassays with strains of *A. fuscipes, A. gallica, A. luteobubalina, A. mellea,* and *A. nabsnona* revealed production of mainly hydroxamate type siderophores and some catecholate type siderophores. We expect that the NRPS clusters identified in the *Armillaria* genomes will be responsible for the biosynthesis of the hydroxamate siderophores secreted by these strains. This study is the first report on siderophore biosynthesis by *Armillaria* spp. Results from this and future studies will shed light on the biology of fungal pathogens at the molecular level.

548V Transposons activate during monokaryosis in a mushroom-forming fungus *Markus Hiltunen*[1], Lorena Ament-Velásquez[2], Martin Ryberg[1], Hanna Johannesson[1] 1) Uppsala University; 2) Stockholm University.

In fungi, genetic variability can be generated during every stage of the life cycle by an array of different mechanisms, including mutations, mitotic and sexual recombination, parasexuality and transposon mobilization. Recent findings have revealed remarkable genome stability at the nucleotide level in mushroom-forming fungi, but if this pattern extends to include large-scale genotypic changes is unknown. In addition, whether genetic variability is predominantly generated during the mono- or dikaryotic life cycle stage is still an open question. Here we used a *Marasmius oreades* fairy ring that had grown as a dikaryon for an estimated 12 years in nature to look into these questions. We collected four fruiting bodies from different parts of the fairy ring and separated the two nuclear genotypes through protoplasts before sequencing these haploid genomes. We then generated nearly gapless genome assemblies, unlocking full genomic access to discover changes to the genotype of any size, in addition to any exchange between the two nuclear genotypes. During dikaryotic growth in nature, we found that no recombination or new structural variants had arisen, reinforcing the genome stability hypothesis in long-lived dikaryons. In the monokaryons, however, a considerable amount of structural variation had started to accumulate. In particular, activity of autonomous and associated non-autonomous *hAT* transposons was triggered, and translocations of up to several hundred kilobases were discovered. Furthermore, activity of the same transposon families was inferred from single-spore isolates of *M. oreades.* The finding that transposition occurred in both asexually and sexually generated monokaryons suggests that transposition is not necessarily triggered by protoplasting or meiosis, but rather that the monokaryotic condition in itself allows movement of transposable elements. Based on our results, we suggest that genetic variation in mushroom-forming fungi is mainly generated at the presumably short-lived monokaryotic stage of the life cycle in nature, and that once formed, the dikaryon is remarkably resistant to any type of genotypic change.

549V Comparative genomics highlights the importance of drug efflux transporters during evolution of mycoparasitism in *Clonostachys* subgenus *Bionectria* *Magnus Karlsson*[1], Martin Broberg[1], Mukesh Dubey[1], Mudassir Iqbal[1], Mikael Gudmundsson[1], Katarina Ihrmark[1], Hans-Josef Schroers[2], Dan Funck Jensen[1], Mikael Brandström Durling[1] 1) Swedish University of Agricultural Sciences, Uppsala, Sweden; 2) Agricultural Institute of Slovenia, Ljubljana, Slovenia.

Species in the genus *Clonostachys*, subgenus *Bionectria*, are characterized by their ecological generalist behaviour, including plant endophytism, rhizosphere competence, polyphage ability and mycoparasitism. These traits are tightly connected to the commercial exploitation of certain *Clonostachys* strains as biological control agents for the control of fungal plant diseases in agricultural crop production. Knowledge-based improvement of biocontrol efficacy relies on a thorough understanding of the evolution and mechanistic basis of the nutritional versatility of species in the subgenus *Bionectria*. Here, we determined the genome sequences of 11 *Clonostachys* strains, representing five species, and performed a comparative genomic analysis with the aim to identify gene families evolving under selection for gene gains or losses. Several gene families of ATP-binding cassette (ABC) transporters and major facilitator superfamily (MFS) transporters predicted to contribute to drug efflux evolved under selection for gene gains ($P \leq 0.05$) in the *Bionectria* subgenus lineage. More specifically, drastic increases in the paralog numbers of the drug:H+ antiporter-2 MFS and ABC-G1 pleiotropic drug resistance (PDR) transporters were associated with the *Bionectria* subgenus ancestral lineage. Reconciliation analysis of species and PDR transporter gene trees placed the gene duplication event of the ABCG5/ABCG6 paralog pair in the *Bionectria* ancestor, making them suitable for functional investigation of the ecological features driving the evolution of drug efflux transporters in *Clonostachys*. In the species *C. rosea*, both genes were induced ($P < 0.001$) by exposure to the antifungal *Fusarium* mycotoxin zearalenone and various fungicides. Sequence analyses and homology modelling predicted structural differences between ABCG5 and ABCG6 in loops contributing to substrate transport specificity. Gene deletion mutants of *abcG5* and *abcG6* identified distinct functional differences related to fungal antagonism and mycoparasitism, biocontrol and drug resistance. In conclusion, neutralization of antifungal compounds produced by other microorganisms, including defence molecules from other fungi or from plants, may facilitate the ecological generalist lifestyle and mycoparasitism in *Clonostachys* subgenus *Bionectria*.

550V Loss of *SAF1* and *RRM3* together leads to Growth Defects and Compromise in Genome Stability in *Saccharomyces cerevisiae* Meenu Sharma[1], V Verma[1], *NARENDRA BAIRWA*[1] 1) Shri Mata Vaishno Devi University.

Synthetic growth defects due to inactivation of genes indicate the role of genes in growth fitness. Saf1 contain the F-box motif which-constitutes the part of SCF-E3 ligase complex and recruits the adenine deaminase, Aah1 for ubiquitination and subsequent deg-

radation to regulate the cell cycle transition during nutrient stress in budding yeast *Saccharomyces cerevisiae.* The Pif1 helicase family member Rrm3, facilitate the replication fork movement through the difficult to replicate sites and promotes genome stability. The disruption of both the *SAF1* and *RRM3* together results in the growth defects and increase in the genome instability. The loss of both gene together with synthetic growth defects also results in compromise to in genome stability and cellular growth response to stress agents *i.e.* HU, MMS, Nocodazole, Benomyl, calcofluor white (CFW) and Sodium dodecyl sulphate. Our results show genetic interaction between *SAF1* and *RRM3* which function in parallel pathway to maintain the growth fitness, genome stability and cell wall integrity in *S.cerevisiae*. This study reports the SAF1 regulating the growth fitness in interaction with conserved Pif1 family helicase encoding *RRM3* which may have synthetic lethality application.

551V Genome comparison of 45 fungal endophytes from Rubiaceae *Kelsey Scott*[1], Humberto Castillo-Gonzalez[2], Guillermo Valero-David[1], Lauren Slattery[1], Efraín Escudero-Leyva[3], Priscila Chaverri[2,3], Jason Slot[1] 1) The Ohio State University, Columbus, OH; 2) University of Maryland, College Park, MD; 3) University of Costa Rica, San José, CR.

Rubiaceae is a diverse plant family known to produce important specialized metabolites including caffeine in *Coffea*, quinine in *Cinchona*, and dimethyltryptamine in *Psychotria*. Foliar fungal endophytes must detoxify or avoid certain defensive metabolites in plant leaves. Additionally, fungal endophytes from Rubiaceae are known for their production of diverse bioactive secondary metabolites; in some cases these fungal metabolites have been shown to contribute to plant defense and health. Our goal was to identify key genome features associated with fungal endophytism, particularly those involved in secondary metabolism. We isolated 415 diverse fungal endophytes from the leaves of 13 Rubiaceae species in Costa Rican rainforests. We selected 45 isolates from this culture library for whole-genome sequencing using Illumina technology. This selection primarily included fungi isolated from *Psychotria panamensis,* as this genus is known to produce a variety of important metabolites. For each *P. panamensis* isolate we also sequenced a closely-related "partner" isolate from a different Rubiaceae species. We also sequenced fungi known to be pathogenic on cultivated coffee. We annotated these genomes and identified metabolic gene clusters associated with the biosynthesis and degradation of secondary metabolites. Here we report our fungal genomes, which include species of *Colletotrichum, Diaporthe, Xylaria, Mycena, Trichoderma,* and *Pestalotiopsis*. These genomes exhibit differences in the number, type, and distribution of metabolic gene clusters and suggest pathways that may provide benefit in the *P. panamensis* host environment.

552T Regulation of fungal gene expression in ectomycorrhizal roots underlying heavy metal soil stress *Haihua Wang*[1], Steven Wu[2], Khalid Hameed[3], Rytas Vilgalys[3], Alan Kuo[4], Kerrie Barry[4], Igor V. Grigoriev[4,5], Hui-Ling Liao[1] 1) University of Florida; 2) National Taiwan University; 3) Duke University; 4) U.S. Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory; 5) Department of Plant and Microbial Biology, University of California Berkeley.

Plant-associated microbes can regulate heavy metal (HM) accumulation in plant tissues and reduce metal bioavailability for rhizosphere stress alleviation. Among them, ectomycorrhizal fungi (EMF) form symbiotic associations with host plants to modulate plant nutrient uptake, as well as physiological and molecular processes, including detoxification in terrestrial ecosystems. An example is zinc-tolerant Suillus luteus, which grows in association with conifers, resulting in benefits for the host plant such as protection from zinc stress. However, the molecular and regulatory mechanisms by which the root microbiome improves plant HM tolerance are largely unknown, including the functional genes manipulated by EMF. In this study, contaminated soils from Silver Hill, NC, including mine bare soil (SHBS), mine moss soil (SHM), mine pine soil (SHP), and adjacent pine forest soil (PF) were used for in-house Pinus taeda bioassay experiments. In addition, Suillus hirtellus was inoculated to study how EMF improve HM tolerance in plants. Gene expression of root microbiome was compared between P. taeda grown in different soils, and with or without S. hirtellus inoculation. Our results demonstrate that HM-contaminated soil significantly reduced plant biomass, while inoculation with S. hirtellus improved host pine growth. Comparative metatranscriptomics reveals that the root microbiome genes encoding ion-binding, transferase, and kinase activities were enriched in response to HM in contaminated soil. In addition, genes of root fungi associated with metal ion transport activity and transcription regulator activity were significantly enriched in HM soil in response to S. hirtellus inoculation, simultaneously with a decrease of genes related to ion-binding activities. Furthermore, up-regulation of fungal genes for zinc and iron binding, and copper ion transport by S. hirtellus inoculation may help reduce the HM ions in plant cells and protect the host plant from HM toxicity.

553F Pathogenic fungi in Norwegian barns - first survey of *Aspergillus fumigatus* azole resistance in Norway *Erik Magnus Nedland Henriksen*[1], Hege Divon[1], Elin Rolén[1], Lonny Margrethe Kløvfjell[1], Ellen Christensen[1], Ida Skaar[1] 1) Norwegian Veterinary Institute, Ås, Norway.

Azoles are efficient fungicides commonly used to treat and prevent fungal diseases in humans and animals. Moreover, the azole class of fungicides is widely used in agriculture to ensure healthy plants and high yield in food production, horticulture and wood industry. Unfortunately, several international studies indicate that residual azoles in the environment act as potential drivers for development of azole resistance in human pathogenic fungi, such as *Aspergillus fumigatus.*

A. fumigatus is an opportunistic human pathogen and the causal agent for aspergillosis, one of the most common fungal diseases in human medicine world wide. The increasing number of reports about azole resistance development in *A. fumigatus* is alarming. With the spread of resistance, treatments of such fungal diseases will be progressively more ineffective, with fatal consequences. This is a growing global concern.

The goal of the BARNS project was to provide preliminary data on how widespread *A. fumigatus* azole resistance is in Norwegian farms. Citizen science was used to sample *A. fumigatus* from indoor environments in animal housing and storage rooms on farms across the country. Adhesive PCR plate foiles were used as spore traps, and trapped spores were cultivated and identified both morphologically and by calmodulin sequencing as *A. fumigatus.* Pure isolates were further screened for resistance against itraconazole, voriconazole, and posaconazole using VIPCheck™ and E-tests. Resistant isolates were in addition characterized by sequencing of the *cyp51A* gene.

Out of a total of 108 isolates tested, three (2.8%) were found resistant, exhibiting cross-resistance to all three azoles. Two of the resistant isolates had documented mutations in the *cyp51A* gene, one with the TR_{34}/L98H mutation and the other with the TR_{46}/Y121F/T289A mutation. The third isolate did not have mutations in the cyp51A gene and has gained resistance by a, so far, unknown mechanism. While the percentage of resistant isolates are in line with data from other countries, a larger study is in progress to more accurately reflect the occurrence of azole-resistant *A. fumigatus* in Norway.

554W Analysis of Wood-Decay Fungal Communities Associated with Contrasting Zones of the American Wood Protection Association Decay Hazard Map *Jed Cappellazzi*[1], Amy Bishell[2], Nathan Bechle[2], Sam Glass[2], William J. Hickey[3], Gerald Presley[1], Grant Kirker[2] 1) Oregon State University, College of Forestry, Wood Science and Engineering; 2) USDA-FS Forest Products Laboratory, Madison, WI; 3) Univ of Wisconsin, Dept of Soil Sciences, Madison, WI.

Wood deterioration caused by decay fungi (predominantly basidiomycetes) can shorten the useful life span of wood and wood-based materials. Prescriptive preservative treatment is an effective way to limit the detrimental impacts decay fungi cause to wood structures, particularly in soil contact and critical use areas. A thorough understanding of the potential decay hazard of varied climates is critical for the proper selection and use of wood products. The American Wood Protection Association (AWPA) guidelines specify 3 zones of severity regarding wood decay fungal hazard, however, ecological information on the diversity and abundance of the basidiomycete communities that colonize and decay wood in ground contact is lacking. The goal of this work is to compare the wood decay fungal communities across the United States that span the current AWPA hazard map zones. To do so, fifteen southern pine field stakes (450 x 25 x 25 mm) per overstory type (pine or hardwood) were installed at 14 National Forests (N = 30) in the summer of 2016 and remained exposed for 1-year, after which DNA was extracted from the wood and adjacent soil and the fungal communities were sequenced using the Illumina MiSeq platform. Results from this research will provide important ecological data on decay fungal communities across climatically different decay zones and improve upon the historical AWPA decay hazard zone classifications.

555T Genetic determinants of azole stress in *Aspergillus fumigatus* *Shivani Ror*[1], Sanjoy Paul[1], Scott Moye-Rowley[1] 1) University of Iowa, Iowa City, IA.

Development of azole resistance in *Aspergillus fumigatus* is a growing concern as disease associated with resistant isolates has a worse outcome than that associated with azole-susceptible organisms. Adaptation to azole stress occurs by transcription activation of several genes, some of which are involved in enhancing the azole resistance. Two well-studied genes encoding products that impact azole resistance are *cyp51A* and *abcG1*. The *cyp51A* gene encodes the lanosterol a-14 demethylase enzyme that is the target of azole drugs while *abcG1* (aka *abcC*/*cdr1B*) produces an ATP-binding cassette transporter protein that is thought to efflux azole drugs from the cell. Previous studies have identified a positive regulator of transcription that binds to both the *cyp51A* and *abcG1* promoters. This factor was designated AtrR and is an important determinant of azole resistance in *A. fumigatus*. Experiments primarily in *Neurospora crassa* have shown the role of two transcription factors, ADS-4 (antifungal drug sensitive-4) and CCG-8 (clock-controlled gene), in the regulation of adaptive responses and resistance to antifungal azoles. While deletion of the analogues of these genes in *A. fumigatus* led to azole hypersensitivity, no direct comparison was possible with the relative contribution of AtrR. Here using CRISPR-mediated gene deletion we construct a series of isogenic strains to directly compare the contributions of *ads-4* (Afu1g16460), *ccg-8* (Afu5g09420) and *atrR* to azole resistance. Loss of *ccg-8* caused azole hypersensitivity in our wild-type background but this was a modest phenotype compared to isogenic *atrRΔ* cells. Strains designed to overproduce Ccg-8 or Ads-4 were not observed to have significant effects on azole resistance. Removal of *atrR* from these strains did not uncover any latent azole resistance that could be seen. Western blotting of an epitope-tagged version of Ccg-8 detected the presence of a number of high molecular weight proteins suggesting the presence of some post-translational modification on this protein. Finally, we have constructed a doxycycline-repressible (dox-off:DO) form of *atrR* to allow the rapid depletion of this factor from the cell. This DO *atrR* fusion makes doxycycline-dependent AtrR and azole resistance. Our goals are to use these genetic tools to dissect the contribution of these transcriptional circuits to azole resistance in *A. fumigatus*.

556F Categorizing Filamentation Phenotypes Across Divergent *C. albicans* Strains *in vitro* and *in vivo* *Nichole Brandquist*[1], Jill Blankenship[1] 1) University of Nebraska at Omaha.

The human pathogen *Candida albicans* causes systemic infections with a high mortality rate. *C. albicans* has diverged genetically into numerous distinct clades, five of which (I, II, III, E, and SA) are the most common cause of serious systemic infection in the United States. Previously, these were divided by geographic region, with clade 1 being associated with North America, however clade 1 strains only make up 35% of infections in the US. We utilized Multilocus Sequence Typing (MLST) to identify strain diversity throughout Nebraska. The majority of the 22 strains isolated from systemic infection from the University of Nebraska Medical Center are novel strains, with allele profiles not found in the MLST database.

It is well known that filamentation is linked to pathogenicity and virulence, and cells that are unable to undergo yeast to hyphal switching are unable to establish *in vivo* infections. Our laboratory previously found that filament inducing conditions used throughout the field are not interchangeable, therefore we tested the clinical strains across 8 filament inducing conditions and two non-inducing controls. The majority of strains exhibited defects in filamentation, most notably on solid media. Additional *in vivo* filamentation studies utilizing *Caenorhabditis elegans* provide further phenotypic characterization outside of the variable laboratory studies. To our knowledge this is the first study of its kind characterizing clinical strains from Nebraska both genetically and phenotypically.

557W RNAseq analysis identifying a core gene set of *Linnemannia elongata* involved in the chitin process *Kaile Zhang*[1], Haihua Wang[1], Khalid Hameed[2], Gregory Bonito[3], Hui-Ling Liao[1] 1) University of Florida; 2) Duke University; 3) Gregory Bonito.

Linnemannia is a genus of early-diverging Mortierellomycota previously classified as *Mortierella*, which is one of the dominant fungal groups in soils and terrestrial ecosystems. *Linnemannia* species function as saprotrophs and chitin decomposers as well as root endophytes, affecting soil C and nutrient processes and plant growth. Little is known how this *Linnemannia* molecularly responds to chitin substrate. To that end, we cultured *Linnemannia elongata* (PMI93) on the modified (1%) Malt Extract media containing 2% colloidal chitin and compared it with the same fungus cultured on media without chitin as control. Biomass from 32 hours of cultured *L.*

elongate (PMI93) was collected and its RNA was extracted using a CTAB/LiCl extraction for cDNA construction, followed by HiSeq 150PE sequencing. We detected a similar number of expressed genes from *L. elongata* culture (12,254 genes) and *L. elongata* + chitin samples (12,249 genes). However, the chitin substrate led to significant differences in the expression of *Linnemannia* genes. As compared to *L. elongata* culture, *L. elongata* with chitin culture upregulated 375 genes that were annotated into 90 gene ontology (GO) terms, with 69 GO terms belonging to molecular function. Among them, ATP binding, protein folding, protein binding, and heat shock protein binding were highly enriched compared to other GO terms. Of 375 upregulated genes, 27 genes belong to putative chitinase genes. According to the KOG category, these putative genes could be indirectly involved in cell wall/membrane/envelope biogenesis as well as carbohydrate transport and metabolism. These results suggest that chitin substrate serves as an energy resource and takes a biological role as a cell wall–supporting polymer for fungal growth and development. Metatranscriptomics of *L. elongata*- host plant (*Populus*) interaction as affected by soil chitin will also be investigated in this presentation.

558T The impact of dietary *Debaryomyces hansenii* yeast on the human gut mycobiome *Justin Tran*[1], Nabaraj Banjara[2], Heather Hallen-Adams[1] 1) University of Nebraska-Lincoln; 2) University of Holy Cross.

There is no doubt that microorganisms preside in the gastrointestinal tract. Today's research and technology has yielded an ever-increasing amount of information and studies of bacteria, specifically probiotics, prebiotics, and the microbiome. In contrast, there has been limited research performed on yeast and fungi. Studies dating over one hundred years show the prevalence of human-associated yeasts such as *Candida albicans, Candida tropicalis, C. krusei,* and *C. glabrata,* as well as foodborne yeasts such as *Saccharomyces cerevisiae,* in the human gut. Most yeasts that are present in the gut are still under research and provide some level of uncertainty whether or not their presence is beneficial or undesirable. *Debaryomyces hansenii* is commonly used as a processing yeast in many cheeses and has been shown to be relevant in the microbiota and mycobiome. Previous studies show ~50% of *D. hansenii* isolates from cheese produce killer toxin effective against strains of *C. albicans* and/or *C. tropicalis*. We have sequenced ITS2 from fecal samples from volunteers before, during, and after consumption of cheese naturally containing a confirmed killer strain of *D. hansenii*. The presence and relative abundance of *D. hansenii* and *Candida* yeasts in these samples, and the *in vitro* susceptibility of participants' mycoflora to killer toxin from this *D. hansenii* strain varies between individuals but suggests the possibility of using dietary *D. hansenii* to modulate *Candida* levels in the mycobiome.

559F The air mycobiome is decoupled from the soil mycobiome in the California San Joaquin Valley Robert Wagner[1], Lilliam Montoya[1], Cheng Gao[2], Jennifer Head[3], Justin Remais[4], *John Taylor*[1] 1) Department of Plant & Microbial Biology, University of California Berkeley, Berkeley, CA, USA. ; 2) Institute of Microbiology, Chinese Academy of Sciences, Beijing, China. ; 3) Division of Epidemiology, University of California Berkeley, Berkeley, CA, USA; 4) Division of Environmental Health Sciences, University of California Berkeley, Berkeley, CA, USA.

Dispersal drives the assembly of fungal communities, and the air is the primary dispersal medium for fungal pathogens. Preventing fungal disease in plants and animals can be greatly aided though a better understanding of the characteristics of airborne fungal spores. Here, we describe the fungal community in soil and in settled dust collected in the San Joaquin Valley of California, home to 4.2 million people and the most productive agricultural region in the United States. By means of metabarcoding of the internal transcribed spacer region of fungal rDNA, we report the first multiyear study of fungi representing the air mycobiome and the soil mycobiome in the area. We show that the air mycobiome in the San Joaquin Valley differs from the soil mycobiome, and that assemblages of fungi in settled dust from as far away as 160km are much more comparable to one another than to communities of fungi in soils sampled nearby. Our findings provide a better understanding of airborne fungi dispersal in which plant-associated species dominate. We hypothesize that persons who spend considerable time outdoors on land whose soil contains few detectable pathogens could still be exposed to airborne fungal pathogens from elsewhere. Given that the San Joaquin Valley is home to *Coccidioides*, the most medically important fungal pathogen of healthy humans, the findings presented here may be important to the prevention of this disease.

560W Metabarcoding as a tool for investigating the influence of endosymbiotic bacteria on Mucoromycota fungal host community structure in the Sonoran Desert *Nicole Reynolds*[1], Kevin Amses[2], Jessie Uehling[2], Rasheed Adeleke[3] 1) Cornell University, School of Integrative Plant Science, Ithaca, NY, USA; 2) Oregon State University, Department of Botany and Plant Pathology, Corvallis, OR, USA; 3) North-West University, School of Biological Sciences, Potchefstroom, North West Province, SA.

The Mucoromycota comprises a diverse group of filamentous fungi including mycorrhizal symbionts (Glomeromycetes, Endogonomycetes) and rhizosphere-associated and soil saprotrophs (Mortierellomycetes, Mucoromycetes). Several species within these groups may also be opportunistic human pathogens, post-harvest pathogens, or used in industrial applications for food or biofuel production. Despite the importance of these fungi, many questions remain regarding the patterns and drivers of their diversity, ecology, and distribution. To answer these questions, we are generating comprehensive Mucoromycota culture and sequence based genomic libraries. Furthermore, recent and ongoing discoveries about the endosymbiotic bacteria (EB) that many Mucoromycota species harbor have generated new questions. EB have different effects on the host fungi depending on the species, influencing asexual and sexual reproduction and metabolic functioning. To investigate the potential role of EB in the structuring of Mucoromycota communities, we are using metabarcoding to analyze soils collected from the rhizosphere of two different shrubs (*Larrea tridentata*, Zygophyllaceae and *Ambrosia dumosa*, Asteraceae) in the Sonoran Desert (California). Using both bacterial (16S) and fungal (28S) primers, we are investigating the co-occurrence of potential EB species and fungal hosts. Sequences are generated on the Illumina MiSeq platform, and bioinformatic analyses performed using the AMPtk pipeline with customized reference databases. One essential aspect to further understanding EB and their functional effects on their host fungi is quantifying how technical experimental biases such as primer and sequencing bias influence our interpretation of community-based sequence data. To evaluate the effect of methodological biases, we generated a biological mock community including genomic DNAs from a diversity of Mucoromycota fungi (with or without EB) and are processing it alongside the environmental samples. Our results show not only the utility of metabarcoding for understanding communities of Mucoromycota and their putative EB, but also the importance of accounting for methodological biases that can impact the results. Additionally, we explore the effects of biotic filtering influenced by host plant and dispersal filtering based on geographic distance. This work is the first step in a larger project to compare two biomes (deserts, xeric shrublands and Mediterranean scrub) across two disjunct geographic

areas (California, USA and Western Cape, SA).

561T Impact of fungal pigment from *Chlorociboria* spp. on community composition and decay *Ray Van Court*[1], Jed Cappellazzi [1], Gerald Presley[1] 1) Oregon State University.

Chlorociboria spp. are soft-rot ascomycetes commonly found on decayed wood notable for their production of a bright blue-green pigment called xylindein. Several human uses for this pigment have been developed ranging from artistic creations to semiconductors, however the biological function is not known. *Chlorociboria* spp. are slow growing, and one possibility is that xylindein functions as part of the antimicrobial defense apparatus for this fungus. To test this hypothesis, an ecological experiment is being carried out to measure the impacts of xylindein on fungal community structure. Stakes including Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), big leaf maple (*Acer macrophyllum* Pursch), alder (*Alnus rubra* Bong.), sugar maple (*Acer saccharum* Marshall), and cherry (*Prunus serotina* Ehrh.) with dimensions of 1/8"x 1.5"x 10" either untreated or treated with a xylindein-containing dichloromethane solution will be set out near five downed trees showing pigmentation associated with *Chlorociboria* spp. for 6 or 12 months prior to harvesting for DNA extraction and metagenomic analysis. In addition, decaying wood in the vicinity and other Oregon locations will be collected and included in the analysis to profile the native fungal community. Analysis will identify any differences in community structure resulting from pigment presence. Community parameters including species richness, dissimilarity and presence of known xenobiotic-tolerant fungi will be measured to infer whether xylindein modulates interspecific interactions. To relate community composition to wood decay, sample deterioration will be assessed and a controlled decay test following AWPA standard E10-16 will be carried out. Pigmented and non-pigmented blocks of species used for stake testing will tested against standard decay fungi, in addition to select fungi isolated from non-pigmented samples. Results of decay testing will validate effects of xylindein seen in community analysis.

562F Fungi adaptation to actinide contamination : accumulation of europium in the filamentous fungus *Podospora anserina* *Eva Cabet*[1], Yang Pei[3], Claire Le Naour[3], Gaelle Creff[2], Aurélie Jeanson[2], Christophe Den Auwer[2], Gwenaël Ruprich-Robert[1], Florence Chapeland-Leclerc[1], Melody Maloubier[3] 1) Université de Paris, UMR CNRS 8236, LIED Laboratoire Interdisciplinaire des Energies de Demain, Equipe B2C Biologie et Biotechnologie des Champignons, 75205 Paris Cedex 13, France; 2) Université Côte d'Azur/ CNRS, ICN, 06108 Nice, France; 3) Université Paris-Saclay, CNRS/IN2P3-IJCLab 91405 Orsay, France, .

Natural and anthropogenic radionuclides are present in the different environmental compartments (lithosphere, hydrosphere, atmosphere and biosphere). Among these radionuclides, actinides remain a major concern of modern nuclearized societies. The complexity of their chemistry, especially the light actinides (from U to Am), leads to a non-well-known behavior in the environment. Civil and military nuclear related activities have led to the releases of actinides into the environment. The mobility of these radioelements depends on the chemical composition of aquifers and soils and on the presence of microorganisms or organisms like fungi (including mushrooms) that are good metal accumulators.
Within the biosphere, fungi play a major role in the ecosystem due to their ability to absorb nutrients from substrates, but also to biodegrade them. Because of their wide geographic dispersion, their abundance, their heavy metals accumulation ability (e.g. cadmium and arsenic), their great variety and their aptitude for growing even under extreme conditions, they can be good bioindicators of environmental pollution. Until now, most of the rare studies have focused on the determination of actinide concentrations in different fungal species without explaining the molecular mechanisms involved in their accumulation and their impact on the fungal development (mycelium growth and reproduction) and on the trophic level. Then, the speciation of actinides in fungi remains unknown. Moreover, many components of fungi are likely to interact with actinides, like polysaccharides, proteins, minerals, amino acids, fibers (chitin) and antioxidants that can favor or inhibit the transfer of radionuclide into fungi.
Then, based on radiochemical and biochemical complementarity approaches, the aim of this project is to study the speciation of actinides at different oxidation levels during the different stages of development of the fungus in order to understand the transfer and accumulation mechanisms. The study will focus here on the accumulation of europium, an analog of trivalent actinides, in the filamentous ascomycete *Podospora anserina*, this fungus having the particularity of being easily cultivated under laboratory conditions and of growing rapidly (1 week for the appearance of the sporophore).
We will present here some promising results about the accumulation, the localization and the speciation of europium in *P. anserina*, allowing us to better understand the biochemical processes of transfer and accumulation of actinides in fungi.

563W Septins in the unconventional cell divisions of the extremophilic black fungus *Knufia petricola*. *Grace Hamilton*[1], Amy Gladfelter[1] 1) University of North Carolina.

Black fungi are polyextremotolerant species that grow in diverse harsh environments. They interact with multifarious other microbes in biofilms, produce many secondary metabolites, and often undergo unconventional modes of cell division. Thus, they are of interest to fields ranging from ecology to astrobiology.
I am characterizing the unconventional cell divisions of the microcolonial black fungus *Knufia petricola* at a cellular and biochemical level. *Knufia* cells can divide by two distinct modes, which resemble either budding or furrowing. When they bud, *Knufia* produce approximately linear chains of spherical cells, with occasional branching to produce dendritic colonies. How do cells "decide" between alternative possible modes of division? How do cells that bud "decide" between linear and perpendicular budding? How is polarity specified in nearly spherical cells? We hypothesize that septins are essential for all these processes, because chemical disruption of *Knufia* septins (using Forchlorfenuron) results in aberrant cell and colony morphology.
Using the CRISPR-based genetic toolkit developed for *Knufia* by the Gorbushina Lab, I am generating novel strains to define the roles of septins and other familiar elements of the cytoskeleton in the unconventional cell divisions of this species. Through a combination of *in vivo* fluorescence microscopy and *in vitro* biochemical reconstitution, I am characterizing the functions of *Knufia* septins. *Knufia* possess homologs of all four canonical septins, as well as a filamentous fungal-specific septin. By comparing these proteins to canonical septins in model budding and filamentous fungi, I hope to build an understanding of how the septin cytoskeleton has evolved to support varied cell shapes, modes of division, and extremophile lifestyles.

564T Small-spored *Alternaria* species associated with potato leaf spot across the US for nearly two decades *Ipsita Mal-*

lik[1], Neil Gudmestad[1], Binod Pandey[1], Fereshteh Shahoveisi[1], Sarah Budde-Rodriguez[2], Ohud Alam[1], Julie Pasche[1] 1) North Dakota State University; 2) BioSafe Systems.

Recent literature reported that brown leaf spot of potato in the Pacific Northwest US is caused by several small-spored *Alternaria* spp (SSA). Our laboratory has collected putative *A. alternata sensu lato* isolates from several potato producing states in the US for almost 20 years. An NCBI BLAST nucleotide match from *OPA1-3* gene sequences from a sub-set of two hundred isolates identified three species of SSA as *A. alternata sensu stricto*, *A. tenuissima* and *A. arborescens*. These three species are similar morphologically, displaying overlapping and variable characteristics, and have indistinguishable disease symptomatology. A phylogenetic characterization of sixty-nine SSA isolates were performed using four gene sequences, *OPA1-3*, *Alt a1*, *ITS* and *TEF*. The phylogenetic analysis based on *ITS*, and *TEF* revealed no diversity among the SSA isolates. The *Alt a1* analysis classified the SSA isolates into two major groups, with no distinction between *A. alternata sensu stricto* and *A. tenuissima*. The phylogenetic analysis based on *OPA1-3* gene sequences classified the SSA isolates in three distinct groups as *A. alternata sensu stricto*, *A. tenuissima* and *A. arborescens*. A multiplex real-time PCR was developed based on SNPs identified in the *OPA1-3* gene to distinguish isolates of *A. alternata* (E=100%, R^2 value=0.97), *A. tenuissima* (E=104%, R^2 value=0.98) and *A. arborescens* (E=102%, R^2 value=0.98). Multiplex real-time PCR differentiation was 99% successful when compared to sequences from the *OPA* gene, moreover closely-related phytopathogenic fungi were not amplified. The multiplex real-time PCR performed on three-hundred and four SSA isolates indicate that all three species were found in association with brown leaf spot of potato in the US and Canada. In both populations, *A. alternata* represented greater than 60% of all SSA isolates recovered. Isolates of all three species were found as early as 2000 in the US; however, the frequency of *A. alternata* isolates has decreased recently. Among US isolates collected in 2000, 2003, 2011, 2013 and 2014, the frequency of *A. alternata* ranged from 75% to 93%. In 2017, 2018 and 2019 that decreased to 47% to 61%. It is unclear if the shift in SSA species associated with brown leaf spot will affect disease severity or management but differences in sensitivity to foliar fungicides is being investigated. The use of the multiplex PCR assays will facilitate accurate characterization of the potato brown leaf spot pathogen complex in the future.

565F ***Fusarium*** **in Nebraska Corn** *Yuchu Ma*[1], Heather Hallen-Adams[1] 1) University of Nebraska-Lincoln.

Members of the *Fusarium sambucinum* (especially *F. graminearum*) and *Fusarium fujikuroi* species complexes are among the most common and economically important pathogens infecting corn in Nebraska. These fungi can produce trichothecene and fumonisin mycotoxins, respectively, causing harm to human and animal health. A total of 61 whole plant corn samples have been randomly collected from 21 countries in Nebraska. Two samples each for ear, stalk and root from each plant were placed on Fusarium Selective Media with pentachloronitrobenzene for 10 days incubation and isolates with *Fusarium* colony characteristics were transferred on to potato dextrose agar and incubated for 10-14 days. DNA was extracted and evaluated by PCR amplification reaction of the eukaryotic translation elongation factor 1 α (EF-1α), followed by Sanger sequencing and blastn. Besides *Fusarium graminearum*, we have found multiple species of both the *Fusarium sambucinum* and *Fusarium fujikuroi* species complexes and *Fusarium oxysporum*, as well as some additional species. This data is being compared with our previous characterization of *Fusarium* species on Nebraska wheat, especially in samples from areas where both corn and wheat are grown. Additionally, assays for deoxynivalenol and fumonisin B1 are being conducted.

566W Genetic diversity of *Fusarium oxysporum* f. sp. *vasinfectum* California race 4 isolates and Alabama field isolates *Miranda Otero*[1], Ambika Pokhrel[1], Seungyeon Seo[1], Laura Wendell[1], Jeffrey J. Coleman[1] 1) Auburn University, Auburn, Alabama.

Fusarium oxysporum f. sp. *vasinfectum*, the causal agent of Fusarium wilt on cotton, can lead to leaf chlorosis, wilting, darkening of the vascular tissue, and plant death. Multiple genotypes of this soil fungal pathogen have existed in the United States for over one hundred years. However, a more virulent genotype (race 4) was initially found in California in 2001. Race 4 was previously restricted to India and can cause disease in the absence of nematodes which is a main management strategy for Fusarium wilt. In 2017, race 4 was identified in Texas and New Mexico and could potentially spread to the remaining cotton belt. Pulse-field gel electrophoresis and multi-locus sequencing were used to evaluate genetic diversity among California race 4 isolates and Alabama field isolates. Among California race 4 isolates, we observed a variation in the number and of size of small chromosomes which could be associated with host-specific virulence. Two housekeeping genes (*translation elongation factor 1 alpha* and *DNA-directed RNA polymerase II core subunit*) of 130 field isolates collected in Alabama from 2014 and 2016 were used to construct a phylogenetic tree. As in previous surveys, most Alabama isolates grouped with races 1, 2, and 6, and there were no isolates that grouped with races 3 and 5. Four isolates grouped with races 4 and 7 but were determined not to be California race 4 isolates due to the absence of the *Tf01* transposon insertion in the *PHO* gene. Unexpectedly, 20 different haplotype groups were recovered. Cotton virulence assays will be conducted to assess pathogenicity of representatives from the 20 haplotype groups and to compare virulence among California race 4 isolates.

567T Genetic diversity and pathogenicity of *Botryosphaeriaceae* and *Diaporthaceae* causing defects of hazelnut nuts from Italy. *Muhammad Waqas*[1,2], Vladimiro Guarnaccia[1,2], Davide Spadaro[1,2] 1) Centre of Competence for the Innovation in the Agro-environmental Sector-AGROINNOVA, University of Turin, Grugliasco, TO, Italy; 2) Department of Agricultural, Forest and Food Sciences, University of Torino, Grugliasco, TO, Italy.

Hazelnut (*Corylus avellana*) is considered an important nut crop worldwide and a rich source of vitamins, minerals, and plant proteins. Italy is the second-largest hazelnut producing country (110,000 t/year) after Turkey, on a surface of 81,000 ha. Major constraints for hazelnut production are members of *Botryosphaeriaceae* family and genus *Diaporthe* which are responsible for several defects (internal discoloration, necrosis, blemishes) in hazelnut kernel, and can reduce the hazelnut quality and yield by altering its kernel. In order to investigate the phenomenon of rotten hazelnuts and to identify the responsible agents, a survey was initiated during 2020. A total of 383 samples having the symptoms of black rot (incidence: 32%), mouldy rot (incidence: 41%) and necrosis (incidence: 27%) were collected from Piedmont, northern Italy. Fungal genomic DNA was extracted, and multi-locus phylogeny was performed based on combined partial genomic region ITS and the partial gene *tef-1α*. ITS and *tef-1α* sequences were obtained after PCR amplification using the primers ITS1/ITS4 and EF1-728F/EF1-986R, sequencing and phylogenetic analysis for species identification. Pathogenicity tests were performed on ripening hazelnuts 'Tonda Gentile del Piemonte'. Three nuts per isolate, and per three replicates were surface disinfected with 1% NaClO. A piece of shell (5 mm diameter) from nuts was removed with a sterile cork borer and inoculated with mycelium plug cut

from 7 days old PDA colony. Research results revealed that isolates represent 6 species of *Diaporthe* (*D. eres*, *D. rudis*, *D. novem*, *D. oncostoma*, *D. ravennica*, *D. foeniculina*) and 3 species of *Botryosphaeriaceae* (*Botryosphaeria dothidea*, *Diplodia seriata* and *Neofusicoccum parvum*). Overall incidence of *Diaporthe* spp., *B. dothidea*, *D. seriata* and *N. parvum* were 39%, 20%, 15% and 5%, respectively. Pathogenicity results revealed that all these species are pathogenic to the tested cultivar. All the hazelnut kernels showed abundant development of pycnidia with different disease index. Additionally, isolates from *B. dothidea*, *D. seriata* and *N. parvum* produced black lesions with softening pulp. *D. eres* was more virulent compared with the other *Diaporthe* spp. *N. parvum* was the most pathogenic species among *Botryosphaeriaceae.* The present study improves our understanding of the species associated with hazelnut defects and provides useful information for effective management of the nut disease.

568F The Systematics of North American *Rhizopogon* Using Modern Molecular Techniques *Thelmalyn Montenegro*[1], Emeline Pano[1], Alija Mujic[1] 1) California State University, Fresno, Fresno.

Rhizopogon is a genus of truffle-forming fungi that forms mutualistic relationships with Pinaceae trees, the family of pine trees, which are critical to the healthy function of coniferous forests. These mutualistic relationships are termed ectomycorrhizae (ECM), and they are important because fungi protect plant roots from pathogens, directly exchange nutrients with plants, and facilitate environmental nutrient cycling. *Rhizopogon* species possess reduced morphology, or loss of distinguishing morphological features over evolutionary time, compared with other fungi, and traditional identification methods based upon morphology have failed to accurately describe the true species diversity of the genus. The purpose of this study is to investigate the diversity of *Rhizopogon* species across North America, with a particular focus on the Pacific Northwest geographic region. The results of this research have implications for future systematic studies of many fungal genera and provide valuable information for federal land-use managers where sensitive or rare species of *Rhizopogon* are found. Previous work has used only morphological characters to assess evolutionary relationships within *Rhizopogon* and generated many species-level classifications which may be a misestimation of true species diversity in the genus. Previous molecular phylogenetic analysis of the genus established 5 subgeneric levels in genus *Rhizopogon*, and this work seeks to refine these taxonomic delimitations and expand sampling of type specimens to further clarify true species diversity. Accomplishing these tasks are the primary goals of this study. This work achieves a many-fold increase in holotype sequence data compared with previous studies by using modern enzyme technologies and refined DNA extraction protocols. Here we infer multigene phylogenies incorporating *Rhizopogon* holotype sequence data, using maximum likelihood and Bayesian analyses, and significantly revise species hypotheses and systematic relationships of North American *Rhizopogon* species.

569W Genomic diversification of the specialized parasite of the fungus-growing ant symbiosis *Kirsten Gotting*[1], Daniel May[1], Jeffrey Sosa-Calvo[2], Lily Khadempour[3], Charlotte Francoeur[1], Margaret Thairu[1], Shelby Sandstrom[1], Caitlin Carlson[1], Marc Chevrette[1], Monica Pupo[4], Tim Bugni[1], Ted Schultz[2], J. Spencer Johnston[5], Cameron Currie[1] 1) University of Wisconsin-Madison, Madison, Wisconsin; 2) Smithsonian Institution, Washington, DC; 3) Rutgers University, Newark, New Jersey; 4) University of São Paulo, Ribeirão Preto, Brazil; 5) Texas A&M University, College Station, Texas.

Fungi shape the diversity of life. Characterizing the evolution of fungi is critical to understanding symbiotic associations across kingdoms. In this study, we investigate the genomic and metabolomic diversity of the genus *Escovopsis*, a specialized parasite of fungus-growing ant gardens. Based on 25 high-quality draft genomes, we show that *Escovopsis* forms a monophyletic group arising from a mycoparasitic fungal ancestor 61.82 million years ago (Mya). Across the evolutionary history of fungus-growing ants, the dates of origin of most clades of *Escovopsis* correspond to the dates of origin of the fungus-growing ants whose gardens they parasitize. We reveal that genome reduction is a consistent feature across the genus *Escovopsis*, largely occurring in coding regions, specifically in the form of gene loss and reductions in copy numbers of genes. All functional gene categories had reduced copy numbers, but antimicrobial resistance and pathogenic virulence genes maintained functional diversity. Biosynthetic gene clusters contribute to differences among *Escovopsis* spp., and a similar diversity is also present in metabolomes of sister taxa in the Hypocreaceae. Taken together, our results indicate that *Escovopsis* spp. evolved unique genomic repertoires to specialize on the fungus-growing ant-microbe symbiosis. This genomic evolution represents an example of a eukaryotic genus evolving a reduced genomic toolkit while maintaining ancient host associations.

570T Interrogating the poplar fungal microbiome interactions using meta-transcriptomics and constructed communities *Jake Nash*[1], Keaton Tremble[3], Brian Looney[1], Corbin Bryan[1], Khalid Hameed[1], Yi-Hong Ke[1], Melissa Cregger[2], Nicholas Dove[2], Christopher Schadt[2], Rytas Vilgalys[2] 1) Duke University, Durham, NC; 2) Oak Ridge National Laboratory, Oak Ridge, TN; 3) The University of Utah, Salt Lake City, UT.

Poplar trees (genus *Populus*) are host to a diverse root fungal microbiome including ectomycorrhizal, arbuscular mycorrhizal, and endophytic fungi. These fungi perform services for the plant host including growth promotion, nutrient acquisition, protection from pathogens, and conferral of abiotic stress tolerance. Meta-transcriptomics can provide large amounts of data on the function and taxonomic composition of the poplar root fungal microbiome. We developed an RNA-seq method using a synthetic spike-in standard curve that allows for the calculation of absolute abundances of fungal transcripts on poplar roots. We implemented a bioinformatics workflow that provides taxonomic and functional annotations of assembled fungal contigs from meta-transcriptomic data. These methods were applied to an ecosystem-scale time-series field experiment to document taxonomic and functional shifts of the poplar fungal microbiome in response to a historic drought in the semi-arid American West during the summer of 2021. We identified transcripts from a previously isolated dark septate endophyte in the genus *Hyaloscypha* as a highly active root colonizer across our field sites. Dark septate endophytes are a functionally diverse group of root associates that have been described as either mutualists, commensalists, or latent pathogens. We conducted further work to understand the characteristics of the *Populus-Hyaloscypha* association. *In* vitro inoculations with this fungus demonstrated compatibility with both *Pinus* and *Populus*, and suggested that it engages in antagonistic interactions with arbuscular mycorrhizal fungi during plant host colonization. We were also able to establish simplified constructed communities with this fungus and three common ectomycorrhizal fungi, ranging in diversity from one to four species. These constructed communities will allow us to identify interactions between fungi during root colonization and evaluate the effects of fungal diversity on plant performance and nutrient uptake. Future work will also 1) dissect the molecular mechanisms of the antagonistic interaction with arbuscular mycorrhizal fungi, 2)

evaluate the ability of this fungus to confer drought tolerance to *Populus*, and 3) identify common and unique symbiosis-induced genes when colonizing different plant hosts.

571V Examining the legacy of moderate drought on the wheat seed mycobiome *Lindsey Becker*[1], Marc Cubeta[1] 1) Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC.

Winter wheat (*Triticum aestivum*) is a staple crop grown throughout the world. In 2021, wheat growers in the United States experienced extreme climatic weather events that reduced harvest and increased grain prices. In North Carolina (NC), wheat growers often experience moderate drought during flowering and ripening stages prior to harvest. Our research examines the influence of moderate drought stress imposed post-flowering on wheat physiology and seed fungal communities. We hypothesize that moderate drought stress and reduced plant water availability imposed over a single generation will have a greater impact on beta than alpha diversity of the wheat seed mycobiome. To test this hypothesis, untreated Generation 0 (G0) seeds were harvested and sourced from three winter wheat cultivars (Catawba, Shirley, and USG-3640) grown in a single production field in Raleigh, NC. Wheat seeds were vernalized and 8 week-old seedlings of each cultivar were grown in 5 cm diameter x 36 cm deep custom PVC pots in a growth chamber at the NC State University Phytotron facility. Moderate drought was imposed on a subset of plants for 1 week after 90% flowering by using a gravimetric method to reduce plant water availability to 45%. Leaf chlorophyll content was measured in wilted plants and compared to well-watered control plants as a proxy for photosynthetic activity. G1 wheat seeds were harvested from individual plants following ripening. ITS sequence-based analysis of G0 and G1 fungal communities in wheat seeds was conducted with Illumina Miseq to determine alpha and beta diversity for each wheat cultivar. A comparative fungal community analysis will be presented to examine differences between water regimen, cultivar, and generation. Our research will provide a better understanding of the impact of moderate drought stress on the wheat seed mycobiome. By identifying key fungal taxa associated with drought legacy over generations, we can investigate and harness plant-fungal interactions to mitigate crop losses due to late season drought in wheat.

572V Dissecting Ascochyta blight disease of Field pea using genomics and population genetics approaches *Yvonne Ogaji*[1,3], Robert Lee[4], Garry Rosewarne[2], Tim Sawbridge[1,3], Ben Cocks[1,3], Hans Daetwyler[1,3], Sukhjiwan Kaur[1] 1) Agriculture Victoria, AgriBio, Centre for AgriBioscience, 5 Ring Road, Bundoora, Victoria 3083, Australia; 2) Agriculture Victoria, AgriBio, Grains Innovation Park, 110 Natimuk Road, Horsham, Victoria 3400, Australia; 3) School of Applied Systems Biology, La Trobe University, Bundoora, Victoria 3086, Australia; 4) Centre for Crop and Disease Management, School of Molecular and Life Sciences, Curtin University, Western .

Ascochyta blight (AB) is the second most important disease of field pea able to cause yield losses of up to 70%. AB is known to be caused by up to seven fungal pathogens of which three are most relevant in Australia including *P. pinodes*, *P. pinodella* and *P. koolunga*. AB complex complicates disease management and poses a research challenge. Limited genomic resources have hampered breeding progress for the development of resistant cultivars. The aim of our study was to develop genomic resources including whole-genome reference assemblies from the AB complex, perform comparative pan-genome analysis to explicate species similarities and differences, understand population genetics using resequencing from 110 isolates and elucidate host-pathogen interaction mechanism. We report the first high-quality fully annotated near chromosome-level nuclear genome as well as mitochondrial genome assemblies from 18 isolates of the Australian AB complex, using Oxford Nanopore long-read sequencing technology. Our results showed that the nuclear genome size for *P. koolunga* (~58Mb) is larger followed by *P. pinodella* (~37Mb) and *P. pinodes* (~36Mb). We confirmed the mating type of *P. pinodella* (heterothallic) and *P. pinodes* (homothallic) and identified the *P. koolunga* mating type as being heterothallic. Homology and orthologous gene clusters between *P. pinodes* and *P. pinodella* were greater compared to *P. koolunga* indicating closer similarity between the former two species. CAZyme repertoire showed enrichment for enzymes specific for degradation of cell wall components enabling colonization and pathogenicity. Secondary metabolite analysis highlighted differences in biosynthetic gene repertoire that could elicit different host responses. Phylogenetic analysis revealed clear separation of the AB species with *P. pinodella* having a common *P. pinodes* ancestor. Genetic diversity showed *P. pinodes* and *P. pinodella* exhibits significant interspecific divergence from *P. koolunga* both having an Fst of 0.8 to *P. koolunga* and 0.3 between themselves. Host-pathogen interactions were studied using a time-course study (RNAseq) to understand differential gene expression from both the host and pathogen. Preliminary results for the host showed gene enrichment for several heat shock proteins and protein kinases. Different genes were elicited in the host at different time points indicating primary and secondary fungal defense response targets. Analysis for pathogens is still underway and will include identifying DEGs at the different time points between susceptible and resistant cultivars. Results from this study provide the much-needed genetic resources, characterization of the AB species, and explain the complex interactions between pathogen and host. This will drive detailed research in key areas like epidemiology, introgression, and genomic selection for the development of durable resistant cultivars.

573V Genome diversity in *F. musae* isolated from banana and human host. *Luca Degradi*[1], Valeria Tava[1], Andrea Kunova[1], Cristina Pizzatti[1], Marco Saracchi[1], Paolo Cortesi[1], Matias Pasquali[1] 1) University of Milan.

Fusarium musae is a postharvest pathogen in banana causing crown rot and it is also the cause of infection in immunocompromised patients as well as a cause of keratitis and onychomycosis.
The genome of a *F. musae* strain causing skin infection in a patient was obtained by a combination of short illumina reads and long nanopore reads. The complete genome includes 14 chromosomes. The genome size is approximately 1.5 Mb larger when compared with the two existing genomes from banana. Two supplementary chromosomes were found to be present in the clinical strain. Interestingly, the two extra mini-chromosomes contain 117 and 47 genes. Forty percent of the genes of the unique mini-chromosomes were present only in the human strain. Among those, over 50% of genes are putative effectors involved in interaction with the host. There is evidence of horizontal gene transfer from other distant fungal species. Further investigations are warranted to demonstrate, as shown for other *Fusarium* species, that the acquisition of supplementary chromosomes may confer plasticity in host adaptation.

574V Interspecies interactions between a mycoparasite and its prey are mediated by a conserved cell-cell communication mechanism. *Natascha Stomberg*[1], Antonio Serrano[1], André Fleißner[1] 1) Institut fuer Genetik.

Cell-cell communication and fusion are important mechanisms for the colony development of filamentous fungi. An established mod-

el organism to study cell-cell-fusion is *Neurospora crassa*. Germlings of this fungus grow towards to each other, establish physical contact, and fuse into a supracellular network. These cell-cell interactions employ an unusual signaling mechanism, in which the two fusion partners alternate between signal sending and receiving in a dialog-like manner. In this process the MAP kinase MAK-2 and the SO protein are dynamically recruited in an antiphase manner to the growing cell tips of the interacting germlings. Homologs of MAK-2 (BMP1) and SO (BcPro40) were also detected in the grey mold *B. cinerea*, where they exhibit comparable subcellular dynamics in fusing spore germlings. In addition, interspecies interactions occur between *N. crassa* and *B. cinerea* germlings, which also involve the described dynamic membrane recruitment of BMP1 (*B. cinerea*) and MAK-2 (*N. crassa*). Together, these observations suggest that the "cell dialog" signaling mechanism is conserved in distantly related fungi. However, interspecies cell-cell fusion was never detected between these two species. The observed interspecies interactions prompted us to hypothesize that the cell dialog mechanism might also contribute to the interaction of mycoparasitic fungi and their prey. To test this idea, we investigated the interaction of the mycoparasite *T. atroviride* with *N. crassa*. Inter- and intraspecies interactions occur in the same frequency in mixed spore populations of the two fungi. Fluorescence microscopy during co-cultivation showed that MAK-2-GFP oscillation and localization in *N. crassa* is comparable in intra- and interspecies interactions. SO-GFP is also dynamically recruited, but was mostly mislocalised. In rare cases, cytoplasmic exchange appeared to occur between germlings of the two different species. This observation could reinforce the hypothesis that anastomosis might enable horizontal gene transfer. In our future studies we will characterize the molecular mechanisms and the biological role of these interspecies interactions.

575V Measuring the mutagenic properties of antifungal drugs within *Aspergillus fumigatus* *Michael Bottery*[1], Chris Knight[1], Michael Bromley[1] 1) University of Manchester.

Antifungal resistance in the opportunistic pathogen *Aspergillus fumigatus* is becoming increasingly common, creating a barrier to the successful treatment of life-threating fungal infections. It is becoming increasingly evident that the environmental use of antifungals, such as azoles, is creating a strong selective pressure for the evolution of antifungal resistance in the environment. In addition, resistance evolves *de novo* during the prolonged treatment of chronic aspergillosis. In order to predict the rate and trajectory of resistance evolution within *A. fumigatus*, an understanding of both the spontaneous mutation rate and the mutagenic effect of antifungal compounds are essential but are currently unknown. Here, we develop a bespoke *in vitro* fluctuation assay to measure spontaneous mutation rates of *A. fumigatus*, allowing the study of how an organism's genotype and environmental context affects mutation rate. We first validate our method through the identification of mismatch repair (MMR) system genes of *A. fumigatus*, essential to maintain genomic integrity and improve fidelity during DNA replication. We show that the loss of function of the MMR system genes significantly increases the probability of spontaneous mutation, and thus the probability of antifungal resistance arising within a population. We also demonstrate how sub-inhibitory concentrations of some antifungal compounds induce elevated mutation rates in *A. fumigatus*, again increasing the probability of resistance mutations to arise. These finding suggest that mutator phenotypes together with the sub-inhibitory selection by antifungal drugs may play an important role in the rapid evolution of resistance both within the environment and clinic.

576V The maize mycobiome and implication on mycotoxin contamination in relation to climatic patterns *Bwalya Kataiti*[1,2], Bas J. Zwaan[1], Anne van Diepeningen[1], Pierre Schoenmakers [1], Paul Kachapulula[3], Henry Njapau[2], Sijmen E. Schoustra[3] 1) Wageningen University and Research, Wageningen, Netherlands; 2) National Institute for Scientific and Industrial Research, Lusaka, Zambia; 3) University of Zambia, Lusaka, Zambia.

Maize is often contaminated with an array of fungi at field. The implication is mycotoxin contamination. We investigated the fungal microbiome contaminating maize in the field in relation to climatic patterns and aflatoxin (AF) and fumonisin (FB) contamination thereof. The study was done over two seasons and two contrasting agroecological zones (AEZs) in Zambia. AEZ1 is comparatively drier and hotter than AEZ3 which is wetter and milder.
Maize samples were collected from 40 fields per season, spread across eight selected districts over the AEZs. AF and FB were analysed by High Pressure Liquid Chromatography. For mycobiome study, isolated DNA from maize kernels surface wash was analysed by amplicon sequencing targeting the fungal nuclear ribosomal internal transcribed spacer 1 region. Influence of AEZ and season on AF and FB was analysed by Kruskal Wallis non-parametric approach. Sequencing output data was processed using the Divisive Amplicon Denoising Algorithm 2 pipeline. Taxonomy was assigned to output sequences using the UNITE taxonomic fungal database. Principle coordinate and component analyses, non-metric multidimensional scaling and Phyloseq were used to study species abundances and relations with environmental variables.
Results revealed 87 fungal genera present on the maize mycobiome whose composition was climatic pattern related. Over the two seasons, *Fusarium* and *Sarocladium* had the highest relative abundance of 44% and 37%. PCA loading factors revealed *Fusarium* presence to be antagonistic with *Sarocladium*. *Aspergillus* had no clear antagonism or mutualism with *Fusarium*.
Fusarium was abundant throughout seasons and AEZs without any correlated environmental variables. Further, FB was detected throughout AEZs and seasons. Overall, FB contamination in season 1 was higher than season 2. *Aspergillus* was a low abundance genus across seasons and AEZs (0 – 10%). Its ingress into maize was higher in the hotter and drier zone during season 1 which had a dry spell. Overall, its abundance correlated with AF contamination. This resulted in high AF levels in maize in the hot drier zone in the first season with the dry spell. No AF was detected in second season and AEZs. Further, the AEZ with dry spell had higher levels of *Aspergillus, Penicillium, Meyerozyma, Ustilago* and *Kodamaea*.
Overall the maize mycobiome was diverse. The implication on the crop is AF contamination due to *Aspergillus* ingress into maize, depending on climatic conditions. Further observed implication is perpetual FB contamination due to *Fusarium* irrespective of climatic conditions.

577V Fusarium spp. associated with wheat nodes and grain in representative sites across the western Canadian Prairies *Mohamed Hafez*[1], Ryan Gourlie[1], Melissa Telfer[1], Kelly Turkington[2], Brian Beres[1], Reem Aboukhaddour[1] 1) Agriculture and Agri-food Canada, Cereal Pathology, Lethbridge, Alberta, Canada; 2) Agriculture and Agri-Food Canada, Lacombe Research and Development Center, Lacombe, Alberta, Canada.

Fusarium head blight (FHB) and Fusarium crown and root rot (FCRR) are two major wheat diseases worldwide. Populations of FHB

and FCRR pathogens are highly dynamic, and shifts in these populations in different regions is reported. In this study, we characterized the major Fusarium spp. and other mycobiota associated with wheat node and grain samples that were collected from four different sites in western Canada. In total, 994 fungal isolates were recovered and based on culture and molecular diagnostic methods, three genera constituted over 90% of all fungal isolates, namely Alternaria (39.6%), Fusarium (27.8%), and Parastagonospora (23.9%). A qPCR test was developed to quantify the most frequently isolated Fusarium spp. (F. avenaceum, F. culmorum, F. graminearum and F. poae). The qPCR results showed that F. graminearum was not detected frequently in samples collected from all tested locations. Whereas, F. poae was the most abundant species in grain samples in all locations. However, in node samples, F. culmorum (in Beaverlodge and Scott) and F. avenaceum (in Lacombe and Lethbridge) were the most abundant species. Trichothecene genotyping showed that the 3ADON is the most dominant trichothecene genotype (68%), followed by type-A trichothecenes (29.5%), while the 15ADON trichothecene genotype was least dominant (2.5%) and the NIV genotype was not detected. Moreover, a total of 129 TEF1α sequences from nine Fusarium spp. were compared at haplotype level to evaluate genetic variability and haplotype distribution. F. avenaceum and F. poae exhibited higher diversity as reflected by higher number of haplotypes present in these two species compared to the rest.

578V ***Pyrenophora tritici-repentis*** **in Japan: first report on race structure and a novel** ***ToxA*** **haplotype** *Mohamed Hafez*[1], Kaori Nakajima[2], Reem Aboukhaddour[1] 1) Agriculture and Agri-food Canada, Cereal Pathology, Lethbridge, Alberta, Canada; 2) Mie Prefectural Agricultural Research Institute, Matsusaka, Japan.

Tan spot, caused by *Pyrenophora tritici-repentis* (*Ptr*), is a destructive foliar wheat disease worldwide. The fungus was described first as a pathogen on wheat in Japan in 1920s, but since then there is no reports on the race structure or the dominant effectors secreted by Ptr in Japan. In this study, ten single-spore isolates of Ptr were collected from bread wheat and four locations within the Mie prefecture in Japan. These isolates were evaluated for virulence on four differential wheat genotypes, and tested for the presence/absence of the effector genes, *ToxA*, *ToxB* and its homolog *toxb* in multiplex PCR assays. Eight isolates were designated as race 2 (ToxA-producers), and two isolates were classified as race 1 (ToxA and ToxC-producers), based on their virulence patterns. The necrosis inducing *ToxA* gene was present in all tested isolates, whereas *ToxB*, the chlorosis inducing gene, and its homolog *toxb* were totally absent. Sequence analysis of *ToxA* amplicons from these isolates indicated the presence of a novel *ToxA* haplotype (denoted PtrH2). A comparative sequence analysis and re-sequencing of *ToxA* from reference Ptr isolates showed that all the previously published Ptr ToxA haplotypes (3 haplotypes) were identical. This is the first report correctly confirming the presence of an additional new ToxA haplotype in Ptr, which has two non-synonymous mutations at the DNA level that alter the amino acid sequence of the ToxA effector. The overall results highlight the value of exploring variability in Ptr and its *ToxA* haplotypes around the world to gain a better understanding of the evolution of this important fungus.

579V **An updated checklist of wood decay fungi in the Maltese Islands.** *Marco Iannaccone*[1], Joseph Buhagiar[1] 1) University of Malta.

The most abundant organic compound on Earth is lignocellulose and consists of three major components: cellulose, hemicellulose, and lignin. White-rot fungi are able to degrade lignin efficiently through lignin-modifying enzymes, in contrast to brown-rot fungi which can predominantly degrade cellulose, hemicellulose, through cellulase degrading enzymes. Wood-decaying fungi are very important study subjects for their potential biotechnological applications. They can also create structural damage to stored wood, wooden structures and to trees, especially older ones with very low level of fitness.
Studies of wood decay fungal diversity in the Maltese Islands are limited to incomplete records described by handful of Authors. The aim of the present project is to provide a comprehensive description and updated checklist of confirmed records reported in past years of wood decay fungal diversity in the Maltese Islands. Several surveys have been carried out during the rainy season along the wooded areas of the Maltese Islands as well as in historical gardens. A total of 10 species of wood decay fungi were recorded on 9 different host/substrate including a new record for the Maltese Islands.

580V **The novel 15-keto NX-2 and 15-keto NX-3** ***Fusarium*** **trichothecenes: pathway, phytotoxicity, and pathogenicity** *Imane Laraba*[1], Susan P. McCormick[2], Martha M. Vaughan[2], Robert H. Proctor[2], Kerry O'Donnell[2] 1) ORISE fellow, USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Research Unit. 1815 N. University, Peoria, IL. 61604, USA; 2) USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Research Unit. 1815 N. University, Peoria, IL. 61604, USA.

Five strains from South Africa representing two novel, undescribed species within the *F. sambucinum* species complex (FSAMSC 15 and FSAMSC 16) produce two novel type A trichothecenes, 15-keto NX-2 and 15-keto NX-3. The latter two toxins are similar to NX-2 and its deacetylated form NX-3 in *F. graminearum*, but they differ in possessing a keto or aldehyde group, respectively, at the C-15 position on the trichothecene ring. A new variant of the trichothecene biosynthetic *TRI1* gene product, a cytochrome P450 monooxygenase, was found to catalyze C-7 but not C-8 hydroxylation. Loss of C-8 hydroxylation activity in FSAMSC 15 and FSAMSC 16 most likely arose independently of loss of C-8 hydroxylation activity in *TRI1* in *F. graminearum* NX-producing strains, which is the only other species known to synthesize NX toxins. Investigations are ongoing to elucidate the genetic basis underlying the formation of the keto group at C-15. The fusaria producing 15-keto NX-2 and 15-keto NX-3 were aggressive towards wheat cv. Apogee, causing severe head blight symptoms. They were also more aggressive to cvs. Alsen and Norm wheat heads than NX-producing *F. graminearum*. *In planta*, these strains also synthesized NX-3, 15-keto NX-3, and culmorin. The 15-keto NX-2 and 15-keto NX-3 trichothecenes were also toxic to wheat roots, but they appear to be less toxic than NX-2 and NX-3. However, 15-keto NX-3 was more toxic towards wheat roots when present with NX-3 and culmorin, suggesting a synergetic phytotoxic effect between these toxins.

581V **Genetic diversity of banana infecting** ***Fusarium*** **spp. strains in Cuba** *Einar Martinez de la Parte*[1,2], David Torres Sanchez[1,3], Anouk van Westerhoven[1,3], Luis Pérez-Vicente[2], Edgar A. Chavarro Carrero[1,4], Michael F. Seidl[3], Harold J. G. Meijer[1], Gert H. J. Kema[1] 1) Wageningen University and Research; 2) Instituto de Investigaciones de Sanidad Vegetal (INISAV), Cuba; 3) Theoretical Biology and Bioinformatics Group, Department of Biology, Utrecht University, The Netherlands. ; 4) Cluster of Excellence on Plant Sciences (CEPLAS), Institute for Plant Sciences, University of Cologne, Germany..

Fusarium wilt of banana, is one of the major constraints on global banana production. Recently, its causal agent, *F. oxysporum* f sp. *cubense*, was re-classified and divided over eleven distinct species; including *F. odoratissimum,* also known as Tropical Race 4 (TR4) which is highly aggressive on Cavendish bananas but also poses a significant threat to many other regionally important banana varieties, such as those in Cuba. Since, it is unknown which *Fusarium* species are present in Cuba, we sampled symptomatic banana plants across the country at geographically and environmentally different locations and obtained a collection of 166 *Fusarium* isolates. We used DArT genotyping-by-sequencing technology to explore genetic diversity across this suite of isolates and compared it with the diversity in a global panel of *Fusarium* strains. Our analysis revealed that the Cuban *Fusarium* strains infecting bananas belong to the species: *F. purpurascens*, *F. tardicrescens* and *F. tardichlamydosporum* and to races 1(R1) and 2(R2). The sampling did not reveal any TR4 strain in Cuba. The distribution of the three species throughout the country was associated with the cultivated banana varieties in each region. Oxford Nanopore DNA sequencing of a representative isolate of each species resulted in (near) chromosome-level genome assemblies of 50.6, 51.2 and 52.6Mbp with 14, 16 and 18 contigs, respectively. Whole-genome comparisons revealed that R2 isolates of *F. tardicrescens* and *F. tardichlamydosporum* from Cuba, contain two accessory chromosome that could not be identified in the genome assemblies of *F. purpurascens* (R1) nor in TR4, suggesting that these extra chromosomes are specific for R2 isolates. Thus, we here provide the first report on the genomic diversity and genomic structure of banana infecting *Fusarium* species across Cuba, which will facilitate the identification of effector candidates that are crucial for disease development.

582V ***Fusarium musae*** **diversity from a mitochondrial comparative perspective** *Valeria Tava*[1], Degradi Luca[1], Kunova Andrea[1], Pizzatti Cristina[1], Cortesi Paolo[1], Saracchi Marco[1], Vande Velde Greetje[2], Pasquali Matias[1] 1) University of Milan, Department of Food, Environmental and Nutritional Sciences, Milan, Italy; 2) KU Leuven, Department of Imaging and Pathology, Biomedical MRI unit/ MoSAIC, Leuven, Belgium.

Fusarium musae is a pathogenic species, previously misclassified as *F. verticillioides*, described in 2011 and belonging to the *Fusarium fujikuroi* species complex. It has the ability to infect taxonomically distant hosts: banana fruits mostly in Central and South America as well as human patients in Europe and USA. We studied a worldwide collection of 19 *F. musae* strains isolated from banana and human hosts in central America, northern America and Europe. We thereby verified the ability of the different strains to cause infection on banana fruits and *Galleria mellonella* as "human proxy". All strains were able to cause comparable levels of infection in both hosts. Sequencing and comparative studies of the mitochondrial genomes in *F. musae* led to the identification of a specific endonuclease polymorphic site that allows the distinction from *F. verticillioides* and that differentiates *F. musae* geographic subgroups. By analysing the distribution of the endonuclease polymorphism in *F. musae* we could potentially trace the geographic origin of the clinical infections in Europe and US. We did not find any correlation between the mitochondrial subgroups and their pathogenic activity in the two hosts.

583V Tandem-approach of direct-infusion HRMS and LC-QTOF-MS for the evaluation of food safety and useful secondary metabolites in *Aspergillus oryzae* *Sharon Marie Bahena-Garrido*[1], Ryota Saito[1], Yuko Komatsu[1], Ken Oda[1], and Kazuhiro Iwashita[1] 1) National Research Institute of Brewing, Higashi-Hiroshima, Japan.

Aspergillus oryzae has a plenty number of secondary metabolite gene clusters (SMGCs) of unknown functions and its investigation on genome and secondary metabolite (SM) production particularly on mycotoxins is still limited. There is also a wide array of *A. oryzae* species used in the brewing industry, therefore it is necessary to evaluate the safety of the entire *A. oryzae* which is closely related to *Aspergillus flavus*-notorious for its aflatoxin production, as well as to explore the potential wealth of useful SMs among *A. oryzae* species. In detail, there were 13 *A. oryzae* strains selected based on our previous phylogenetic tree and these strains along with *A. flavus* NRRL3357 were grown in various culture conditions, including rice-*koji a*nd soy sauce-*koji.* The SMs from the extracted fractions were analyzed by adopting a tandem-approach of direct-infusion high-resolution mass spectrometry (DI-HRMS) based metabolomics for efficient, high-throughput screening of metabolites and liquid chromatography quadrupole time-of-flight mass spectrometry LC-QTOF-MS (MS/MS) for further metabolite validation.

In the first approach, DI-HRMS analysis focused on 21 mycotoxins regulated by Joint FAO/WHO Expert Committee on Food Additives (JECFA). Aflatoxin B2 putatively detected in soy sauce-*koji* condition, aflatoxin G2 in corn and citrinin, ergot alkaloids among others detected in different conditions were further validated by LC-QTOF-MS (MS/MS). Results revealed no significant traces of 21 mycotoxins found in all 13 *A. oryzae* strains grown in various conditions. In the second approach, DI-HRMS analysis detected putative SMs which were further subjected to multivariate analysis to determine the SM production pattern resulting from diverse responses among the species. Distinct SM pattern was observed among the strains particularly in *A. oryzae* RIB40, RIB128, RIB915, RIB1172 grown in rice-*koji* and in RIB301, RIB915, RIB1108 grown in soy sauce-*koji* conditions possibly contributed by varying putative production of useful known and nonelucidated SMs. Furthermore, it was observed that *A. flavus* was clearly separated among the *A. oryzae* when grown in corn, YES and CYA suggesting the production of aflatoxins as well as other metabolites likely induced by plant material and nutrient-rich culture media under laboratory conditions.

Taken together, the efficient tandem-approach of metabolomic analysis in various growth conditions provides a plethora of candidate metabolites such as possible novel biomarkers useful for rapid discrimination between *A. oryzae* and the aflatoxigenic *A. flavus* as well as the interesting SM candidates produced by the dependable *A. oryzae* for promising pharmaceutical and other bio-industrial uses.

584V Can the quality of ITS regions in genome assemblies be trusted? *Barbara Robbertse*[1] 1) National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD.

The internal transcribed spacer (ITS) region of the nuclear ribosomal cistron is the primary barcode marker for Fungi and together with any taxon-specific secondary barcodes, serve to help identify species. The ITS marker is widely used in biodiversity studies, which depend on accurate reference databases for identification. The RefSeq ITS database at NCBI curates ITS PCR amplicons from type material specimens, submitted to GenBank. Since sequences from type material provide the most unambiguous link to species names it is generally recommended that taxonomists publicly deposit ITS sequences in addition to sequences from multiple other markers when describing new species. A recent trend in new species descriptions is that only genome assemblies are included. In addition, the

growing number of genome assemblies from type material of previous descriptions serves as a valuable source for trusted reference sequences. ITS sequences could be parsed from such genome assemblies and used as additional references to link environmental and genome data, but can they be trusted?
It was noted previously that the ITS region is absent in a number of genome assemblies of Trichoderma and Colletotrichum species, and when present, had quality issues and identity discrepancies. This has also since been observed for other genera with multiple genomes. We report additional information including sources of sequence variation and comparisons with PCR amplicons.

585V Selection controls genetic diversity among nuclei populating strains of arbuscular mycorrhizal fungi *David Manyara*[1], Marisol Sánchez-García [1,2], Markus Hiltunen[4], Mercè Montoliu-Nerin [1,3], George Cheng[1], James D. Bever[5], Hanna Johansson[4], Anna Rosling[1] 1) Department of Ecology and Genetics, Evolutionary Biology, Uppsala University, Uppsala, Sweden. ; 2) Uppsala Biocentre, Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden.; 3) Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden.; 4) Department of Organismal Biology, Systematics Biology, Uppsala University, Uppsala, Sweden. ; 5) Department of Ecology and Evolutionary Biology, and Kansas Biological Survey, University of Kansas, Lawrence, Kansas, USA..

Arbuscular mycorrhizal (AM) fungi form obligate symbioses with the roots of the majority of land plants, and are found in all terrestrial ecosystems. The degree of intra-organismal genetic variation in AM fungi has been a long-standing topic of debate due to difficulties in the axenic cultivation and generation of high-quality genome assemblies from most species of AM fungi. Furthermore, how the fungus survives long-term without a single nuclear stage is puzzling, and there are hypotheses on selection at the nuclear level functions to purge deleterious mutations.

In this study, we aimed to characterize intra-organismal genetic variation by analyzing nuclei that propagate within a strain. We examined 24 nuclei extracted from spores of three strains in the AM fungi genus *Claroideoglomus* that have recently been sequenced by our group. We analyzed shared variance across species, and assessed signatures of selection among nuclei in the strains.

In line with earlier studies in the model AM fungal genus *Rhizophagus*, we confirmed nuclei to be haploid and we identified one mating type allele in each of the three strains, indicating that the strains were homokaryotic. We also observed an overall low genetic variation within the strains and a low number of fixed differences between the species. Additionally, we observed shared variance across the species, and propose that it represents variance that predates speciation and is maintained in strains as a result of balancing selection or large population size. Strain-specific variance is mostly by rare, presumably young variants that appear to be under strong purifying selection.

Taken together, these results align with our conceptual understanding that the strains function as populations of asexually reproducing nuclei individuals. New deleterious mutations are purged by purifying selection while some mutations that rise to higher frequency can be maintained across speciation events likely as a result of a large populations size. Further, we infer that there is selection acting on different levels within the individual, and on nuclei within the nuclei population of a strain.

586V 1000 lichen MAGs: a reference-free census of lichen symbionts *Gulnara Tagirdzhanova*[1], Paul Saary[2], Robert D. Finn[2], Toby Spribille[1] 1) University of Alberta; 2) European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI).

About one fifth of described fungal species are involved in lichen symbioses. The genomics of these fungi remains understudied, in part owing to the fact that lichen fungi can be resistant to attempts to culture them. In recent years, metagenomics-based culture-independent studies have begun to fill this gap. One strength of metagenomics is that it allows unbiased exploration of lichen symbiosis. While culture or amplicon-based studies might miss certain lineages due to their unculturability or primer bias, metagenomic data can give insight into any and all organisms present in the sample. However, the potential of lichen metagenomics has yet to be fully realized.

Here we present a first systematic overview of lichen metagenomics. We analyzed a total of 437 lichen metagenomes, including nearly every publicly available lichen metagenome and 24 metagenomes generated de-novo. From these metagenomes, we extracted 1000 metagenome-assembled genomes (MAGs), including 294 fungal MAGs, which will be deposited in open access for research use. Using the metagenomes, we analyzed: 1) diversity and abundance of symbionts in every analyzed lichen symbiosis, and 2) co-occurrence patterns of the symbionts, including fungal, algal, and bacterial lineages. We also explored how depth of sequencing (i.e. the amount of raw sequencing data) determines success in detection of organisms and MAG recovery.

587W *Investigating heterologous expression in N. crassa* *James Mierendorf*[1], Thomas Hammond[1] 1) Illinois State University.

The CRISPR-associated Cas9 enzyme is commonly used to genetically modify organisms. However, our lab has recently demonstrated that *Neurospora crassa* is recalcitrant to heterologous expression of a *cas9*-containing transgene. To determine if there is a genetic component to this phenomenon, we have developed a genetic screen for heterologous expression positive (*hep*) mutants. The genetic screen uses a strain carrying two DNA constructs consisting of *cas9* DNA fused to the 5' ends of the *leu-1* and *his-3* metabolic genes. Mutations that allow for expression of the transgenic DNA correlate with increased growth rate on medium lacking leucine and histidine. Here, we present details of our genetic screen as well as results from a preliminary analysis of a candidate *hep* mutant.

588T Complex and critical roles for the AtrR transcription factor in control of *cyp51A* expression in *Aspergillus fumigatus* *Sanjoy Paul*[1], Paul E. Verweij[2], Willem J.G. Melchers[2], W. Scott Moye-Rowley[1] 1) The University of Iowa; 2) Radboud University Medical Centre, 6500 HB Nijmegen, The Netherlands.

Aspergillus fumigatus is the major filamentous fungal pathogens in humans, causing invasive aspergillosis in immunocompromised patients that can often be fatal. Unfortunately, there is a limited repertoire of antifungal drugs available to treat this fungus. The most popular and only oral antifungal drug used to treat invasive aspergillosis is the azole class of drugs that target an important step in the fungal specific ergosterol pathway. However, persistent and prolonged use of azoles, not only in chronic aspergillosis treatment but also

in agriculture as fungicides, have resulted in the appearance of azole-resistant isolates at an alarming rate. Arguably the most common azole-resistant mutations found are associated with the gene that encodes the enzymatic target of azole drugs, referred to as *cyp51A*. Azole-resistant alleles of cyp51A often have the unusual promoter containing a duplication of a 34 bp region in the promoter that leads to enhanced gene transcription. This 34 bp duplication is called TR34 and contains binding sites for the SrbA and AtrR transcription factors that play an important role in regulating expression of cyp51A. In this study, we provide genetic evidence that the presence of the atrR gene is essential for clinical strains containing the TR34 or its related TR46 (46 bp repeat) versions of the *cyp51A* genes to confer enhanced azole resistance. Using site-directed mutagenesis, we demonstrate that both the binding site for SrbA (sterol response element: SRE) and the AtrR binding site (AtrR response element: ATRE) are required for normal expression of the *cyp51A* gene. In the context of the TR34 promoter, loss of either the SRE or ATRE from the distal 34 bp repeat (further 5' from the transcription start site), caused loss of expression of *cyp51A* and decreased voriconazole resistance. Surprisingly, loss of these same binding sites from the proximal 34 bp repeat led to increased *cyp51A* expression and voriconazole resistance. A similar increase in gene expression and drug resistance was also observed at the TR46 promoter upon alteration of either SRE or ATRE elements in the 46 bp repeat proximal to the cyp51A gene. These data dissect the molecular inputs to *cyp51A* transcriptional regulation and reveal a complicated function of the promoter of this gene that is critical in azole resistance.

589F Duplication, Redundancy, and Divergence: roles of the *Aspergillus nidulans* paralogous transcription factors LeuR and LeuB in leucine biosynthesis, nitrogen assimilation, and transcriptome regulation *Joel T. Steyer*[1], Damien J. Downes[1], Cameron C. Hunter[1], Richard B. Todd[1] 1) Kansas State University, Manhattan, KS.

Numerous fungal species, including *Aspergillus, Candida,* and *Saccharomyces*, can synthesize branched chain amino acids (BCAAs) while animals cannot. Proper regulation of BCAA metabolism is important for protein synthesis, growth, secondary metabolite production and virulence. In *Aspergillus nidulans,* the Zn(II)2Cys6 transcription factor LeuB is modulated from repressor to activator by the biosynthetic intermediate α-isopropyl malate (α-IPM) to regulate the leucine biosynthesis pathway. LeuB also regulates expression of *gdhA*, which encodes the key nitrogen assimilation enzyme NADP-glutamate dehydrogenase, NADP-GDH. We have identified a paralog of LeuB named LeuR and examined the intersection of LeuB and LeuR in leucine biosynthesis and *gdhA* regulation. Phylogenetic analysis shows that while LeuB is conserved in Ascomycetes, LeuR is conserved only within Eurotiomycetes. The *leuB*Δ mutant is a leaky leucine auxotroph. We deleted *leuR* and found the *leuR*Δ mutant to be a prototroph. However, the *leuB*Δ *leuR*Δ double mutant is a tight leucine auxotroph, indicating a role for LeuR in regulating leucine biosynthesis. Using a *gdhA-lacZ* translational fusion reporter gene and exogenous leucine, we show that LeuR also regulates *gdhA* expression. By artificially altering the levels of α-IPM through loss of function mutants, we are working to determine if α-IPM also modulates LeuR activity. Previously, we used a series of promoter deletions in *gdhA-lacZ* to identify two sites of action for LeuB in the *gdhA* promoter. We have now used these promoter deletions to compare the wild type strain to *leuB*Δ, *leuR*Δ, and *leuB*Δ *leuR*Δ mutants and identified the site of action for LeuR. Additionally, we performed RNA-Seq with the wild type, *leuB*Δ, *leuR*Δ, and *leuB*Δ *leuR*Δ mutants to determine the genome-wide direct and indirect targets and overall physiological roles of LeuB and LeuR in *A. nidulans*. Our experiments show that the transcription factors LeuB and LeuR overlap in regulating nitrogen assimilation and leucine biosynthesis. However, LeuB and LeuR diverge in the total number of genes regulated and play distinct roles in the regulation of metabolic pathways for iron metabolism, metabolism of other amino acids, and ribosomal RNA biogenesis and processing.

590W Analysis of *defective in silencing* (*dis*) mutants of *Fusarium graminearum* to understand the formation and maintenance of facultative heterochromatin Allyson Erlendson[1], John Ridenour[1], Mareike Möller[1], Lanelle Connolly[1], Xiao Lan Chang[1], Brett Pierce[1], Corinne Fargo[1], Brian Josephson[1], Zackary Bango[1], *Michael Freitag*[1] 1) Oregon State University, Corvallis, OR.

In most fungi, Polycomb Group (PcG) proteins generate facultative heterochromatin by histone H3 lysine 27 trimethylation (H3K27me3). Members of the conserved Polycomb Repressive Complex 2 (PRC2) include the H3K27 methyltransferase, Kmt6, the allosteric binding partner, Eed, and a putative specificity subunit, Suz12. Deletion of *kmt6*, *eed*, or *suz12* leads to complete loss of H3K27me3, accompanied by developmental defects and novel or increased expression of ~25% of all genes. While most genes silenced by PcG have unknown functions, many are predicted to be involved in development and differentiation, secondary metabolism, and pathogenicity. To uncover suppressors of H3K27me3 silencing, we used a forward genetic selection to isolate dozens of primary mutants, which we call *defective in silencing* (*dis*) or *drug response attenuated* (*dat*), based on their transcriptional profiles as determined by RNA-seq. These mutants fall into morphological groups with distinct phenotypes and global gene expression patterns. By bulk segregant analysis followed by high-throughput sequencing (BSA-seq), we identified the underlying mutation in more than a dozen mutants. Here we will report on how deletion of *dis2* (aka "BP1"; Tang *et al.* 2021 NAR 49: 18) affects gene silencing by altering both distribution and global enrichment of chromatin with H3K27me3.

591T A reverse genetics approach to identify genes affecting H3K27 methylation levels in *Fusarium graminearum* *Elizabeth Milford*[1], John Ridenour[1], Mareike Möller[1], Michael Freitag[1] 1) Oregon State University, Corvallis, OR.

Histone H3 lysine 27 trimethylation (H3K27me3) is a conserved chromatin modification involved in reversible gene silencing through formation of facultative heterochromatin. H3K27me3 is established by Polycomb Repressive Complex 2 (PRC2), a protein complex comprised of the lysine methyltransferase, Kmt6, an allosteric activator, Eed, and a putative targeting protein, Suz12. Although facultative heterochromatin is essential for proper development in many eukaryotes, the mechanisms by which H3K27me3 is distributed and maintained are not well understood. The filamentous fungus, *Fusarium graminearum*, is an exceptional model to answer unresolved questions surrounding PRC2-mediated H3K27me3. More than 30% of the *F. graminearum* genome is enriched with H3K27me3, and loss of any PRC2 subunit abolishes H3K27me3 resulting in global activation of transcription and dramatic developmental defects. In this study, we sought to identify additional genes involved in the regulation of facultative heterochromatin in *F. graminearum*. We identified twenty candidate genes, including genes encoding proteins known to affect H3K27me3 in other organisms (e.g., a putative homolog of PRC2 Accessory Subunit, PAS), known to be important for histone modifications that may interact with H3K27me3 (e.g., a histone acetyltransferase, Gcn5), or predicted to have chromatin modification activity (e.g., a novel SET-domain protein). Targeted deletion of these candidate genes revealed a variety of phenotypes, including defects in morphology, asexual reproduction, and secondary me-

tabolism that are comparable to *kmt6* deletion strains. We are using ChIP-seq and additional genetic data to assess how these genes impact global distribution of H3K27me3 and H3K4me2, a histone modification correlated with active transcription. Additional work will be devoted to determine how proteins with promising ChIP-seq data are involved in the distribution or maintenance of H3K27me3 in *F. graminearum*.

592F Methylation of H4 controls gene expression in facultative heterochromatin *Mareike Moeller*[1], Devin Wright[1], Michael Freitag[1] 1) Department of Biochemistry and Biophysics, Oregon State University, Corvallis.

Facultative heterochromatin controls the development and differentiation in many eukaryotes. In metazoans, plants, and many filamentous fungi, facultative heterochromatin is characterized by transcriptional repression and enrichment with histones that are trimethylated at histone H3 (H3K27me3). While loss of H3K27me3 results in de-repression of transcriptional silencing in many species, additional up- and downstream layers of regulation are necessary to control transcription in these regions. Here, we investigated the effects of histone marks on histone H4 in the plant pathogen *Zymoseptoria tritici*. Deletion of the methyltransferase responsible for H4 methylation resulted in global gene activation, especially in facultative heterochromatin, and to a much greater extent than the loss of H3K27me3 we had previously observed. This gene activation is accompanied by chromatin reorganization affecting H3K27me3 distribution, H3K4me2 levels, and a complete loss of ASH1-mediated H3K36me in facultative heterochromatin regions. Strains with specific mutations in the single H4 gene of *Z. tritici* resemble these chromatin changes, underlining the importance of H4 methylation for overall chromatin structure. The mutants we obtained are more sensitive to genotoxic stressors and show a greatly increased rate of accessory chromosome loss. Using epifluorescence microscopy and immunoprecipitation, we are disentangling the interactions of three different histone methyltransferase complexes *in vivo*. Taken together, our results provide insights into a novel, and unsuspected, mechanism controlling the assembly and maintenance of facultative heterochromatin.

593W Development of genetics and molecular tools to study DNA N^6-adenine methylation in early-diverging fungi *Carlos Lax*[1], José Francisco Martínez-Hernández[1], José Antonio Pérez-Ruiz[1], Maria Isabel Navarro-Mendoza[2], Carlos Pérez-Arques[2], Eusebio Navarro[1], Teresa E. Pawlowska[3], Francisco Esteban Nicolás-Molina[1], Victoriano Garre[1] 1) Departamento de Genética y Microbiología, Facultad de Biología, Universidad de Murcia, 30100 Murcia, Spain; 2) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27710, USA; 3) School of Integrative Plant Science, Plant Pathology & Plant-Microbe Biology, Cornell University, Ithaca, New York, USA.

The study of biologically relevant processes in early-diverging fungi such as the role of epigenetics in gene expression regulation and chromatin structure has been hampered by the scarcity of genetically tractable models that allow a deep characterization of these complex and intricate mechanisms. We have developed a methodology based on the use of a plasmid-free CRISPR/Cas9 system coupled with microhomology repair templates that has proven to be highly efficient in producing targeted and stable gene disruption in the fungus *Rhizopus microsporus* (Lax et al., 2021), which belongs to the Mucorales order. This fungus is an attractive model of study because it is one of the most frequent causal agents of mucormycosis and interacts both antagonistically and mutualistically with bacteria. Interestingly, as in bacteria and unlike most eukaryotes, early-diverging fungi rely on the use of adenine methylation (6mA) as the predominant DNA epigenetic modification, a characteristic shared with microalgae and ciliates. In these organisms, it has been proposed that a methylation complex (MTA1c), formed by two DNA methyltransferases (MTA1 and MTA9) belonging to the MTA-70 superfamily and two Myb-like DNA binding proteins (P1 and P2), is directly involved in 6mA deposition. The interaction between these proteins in *R. microsporus* has been confirmed with a yeast two-hybrid assay. Taking advantage of the new procedures that we have established in *R. microsporus*, we have knocked out the three components, *mta1*, *mta9*, and *p1* (p2 is absent), of this complex. Deletion of any of these genes was lethal as only heterokaryotic strains could be obtained, suggesting an essential role of MTA1c in *R. microsporus*. To study the role of the MTA1c in virulence, we have established the conditions for virulence assays in mice using *R. microsporus*. This assay revealed uracil biosynthesis is essential for virulence as uracil auxotrophs showed a drastic reduction in virulence that was reverted by complementation with the wild-type allele of the mutated gene. Strikingly, heterokaryotic mutants for the *mta1* gene showed an increased virulence compared to the wild-type strain, suggesting that the MTA1c positively regulates genes involved in saprophytic growth, but negatively regulates those required for virulence. The combination of the new tools available along with the further characterization of the regulation of 6mA will be remarkably useful to understand its implications in the symbiotic interactions with bacteria and concerning the pathogenicity of *R. microsporus*.

Lax, C., Navarro-Mendoza, M.I., Pérez-Arques, C., Navarro, E., Nicolás, F.E., Garre, V., 2021. Stable and reproducible homologous recombination enables CRISPR-based engineering in the fungus Rhizopus microsporus. Cell Reports Methods 100124. https://doi.org/10.1016/j.crmeth.2021.100124

594T Systematic deletions of histone methyltransferase and demethylase genes reveal their role in RIP and sexual development. *Pierre Grognet*[1], Mengyuan Li[1], Fabienne Malagnac[1] 1) I2BC, Universite Paris-Saclay, CNRS, Gif-sur-Yvette.

The model fungus *Podospora anserina* is widely used to study a great variety of processes. Its life cycle is short and relies on sexual reproduction only. In addition to the well-described stages of the sexual development, a key event is the occurrence of a silencing phenomenon called RIP (Repeat-Induced Point mutation) after fecundation, during the dikaryotic stage, prior to karyogamy. First described in *N. crassa*, RIP can target sequences that occur at least in two copies and introduces CpA to TpA mutations in repeated sequences and lead to DNA methylation and heterochromatin formation on such sequences regardless of their coding capacity and relative position in the genome. Beside RIP, epigenetic regulation has been shown to be crucial for proper development in many organisms including fungi. We focused our study on histone lysine methylation, a post-translationnal modification that is deposited by enzyme containing a SET domain (histone methyltransferase, KMT) and removed by enzyme containing a Jmj domain (histone demethylase, KDM). Searching the genome of *P. anserina* for putative protein with SET domain and JmJ domain, we were able to predict 32 and 12 genes respectively.
Thanks to the ease of gene knockout in *P. anserina*, we generated a collection of KO strains for KMT and KDM genes. One of the first candidates, namely *PaKmt6*, have been shown to be involved in all stage of sexual development including RIP.

Here we show that among the other genes we inactivated, several of them are required for normal development and more interestingly, we found a new gene involved in RIP.
In addition, an experimental evolution approach using the PaKmt6 mutant showed that after only a few generations, genome instability may appear.

595F Loss of EZH2-like or SU(VAR)3-9-like proteins causes simultaneous perturbations in H3K27 and H3K9 tri-methylation and associated developmental defects in the fungus *Podospora anserina* Florian CARLIER[1, 2], Mengyuan LI[1], Laetitia MAROC[4], Robert DEBUCHY[1], Charbel SOUAID[1, 3], Daan NOORDERMEER[1], Pierre GROGNET[1], *Fabienne MALAGNAC*[1] 1) University Paris Saclay, I2BC; 2) Pasteur Institute; 3) INSERM, Aix-Marseille University; 4) University Paris Saclay, IDEEV.

Selective gene silencing is key to development. We used the fungus *Podospora anserina*, a valuable alternative to higher eukaryote models, to question the biological relevance and functional interplay of two distinct chromatin conformations: the H3K9me3-enriched heterochromatin and the that H3K27me3-enriched heterochromatin.
We established genome-wide patterns of H3K27me3 and H3K9me3 modifications, and found these marks mutually exclusive within gene-rich regions but not within repeats. We generated the corresponding histone methyltransferase null mutants and showed an interdependence of H3K9me3 and H3K27me3 marks. Indeed, removal of the PaKmt6 EZH2-like enzyme resulted not only in loss of H3K27me3 but also in significant H3K9me3 reduction. Similarly, removal of PaKmt1 SU(VAR)3–9-like enzyme caused loss of H3K9me3 and substantial decrease of H3K27me3. Removal of the H3K9me binding protein PaHP1 provided further support to the notion that each type of heterochromatin requires the presence of the other. Altogether, these results suggest an intriguing evolutionary fluidity in the repressive histone deposition machinery, which challenges canonical definitions of constitutive and facultative heterochromatin.
We also established that *P. anserina* developmental programs require H3K27me3-mediated silencing, which supports a conserved function of the Polycomb Repressive Complex 2 (PRC2) in fungal development.

596W The histone variant H2A.Z in *Fusarium graminearum*, its genomic location and its environment *Aurelie Etier*[1], Fabien Dumetz[1], Nadia Ponts[1] 1) MycSA.

Fusarium graminearum is the predominant causal agent of Fusarium Head Blight (FHB) of small grain cereals in most areas worldwide [1]. This disease causes severe economic losses due to a reducing yield and contaminating grains with harmful mycotoxins, such as the deoxynivalenol (DON) [2]. To prevent these devastating effects on the grain production we have to accumulate knowledge about ways and mechanisms of infection, and ways of mycotoxins production. In the last past decade, several studies highlighted the major role of chromatin structure to regulate secondary metabolites [3]. Our recent work highlighted the crucial function of the histone variant H2A.Z in several *F. graminearum* developmental processes, such as growth, sexual and asexual reproduction, stress response and in the DON's production [4]. In addition, the observation of compensatory mutations in H2A.Z deleted mutants may suggest major gene network reorganisations following the loss of H2A.Z [4]. These results permitted us to emphasis the essential role of H2A.Z in *F. graminearum,* but do not to define the precise function of this variant. The realization of a H2A.Z ChIP-seq combined with a RNA-seq has been performed to localize it and to identify its function on gene expression regulation in the I349WT and OE strains. In parallel, considering the already proven crosstalk between this histone variant and H3K4me3 and H3K27me3 to respectively activate[5] or repress[6] gene expression in other organisms, a ChIP-seq for these mark has been performed. This study would allow us to better define the function of this histone variant in *F. graminearum*.

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[4] Z. Chen, et al., 2020, doi: 10.1371/journal.pgen.1009125.
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[6] Y. Wang *et al.*, 2018, doi: 10.1186/s12915-018-0568-6.

597T The ATP-dependent chromatin remodeling factor, Isw1, governs development in *Fusarium graminearum*, partially through regulation of facultative heterochromatin *John Ridenour*[1], Mareike Möller[1], Michael Freitag[1] 1) Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR .

Facultative heterochromatin is a transcriptionally repressed chromatin state characterized by histone H3 lysine 27 trimethylation (H3K27me3). Although facultative heterochromatin is essential for proper development in many eukaryotes, the mechanisms by which it is established and maintained are poorly understood. ATP-dependent chromatin remodeling factors regulate chromatin structure through nucleosome sliding, disassembly, and exchange. Recent evidence suggests that homologs of the conserved chromatin remodeling factor, ISWI, affect H3K27me3 distribution in fungi. In this study, we examine how H3K27me3 distribution changes during development of *Fusarium graminearum* and how its single ISWI homolog, Isw1, contributes to the generation and maintenance of facultative heterochromatin. To this end, we used quantitative ChIP-seq to assess changes in the distribution of H3K27me3 and H3K4me2, a modification correlated with active transcription, and RNA-seq to assess changes in gene expression over a developmental time series. Our results revealed quantitative, stage-specific changes in H3K27me3 and H3K4me2 distribution and changes in gene expression. Deletion of *isw1* had dramatic impacts on development, including defects in morphology, asexual reproduction, and secondary metabolism, and concomitant changes in global gene expression. We observed regional changes in H3K27me3 distribution in the *isw1* deletion strain, but numerous genes localized within facultative heterochromatin were derepressed without apparent loss of H3K27me3, indicating that the presence of H3K27me3 is not necessarily sufficient for repression. We are using nucleosome positioning data as well as additional phenotypic and genetic data derived from strains deficient in proteins that interact with Isw1 (Acf, Hfp1, and Hfp2) or are required for H3K27me3 (Kmt6) to elucidate underlying mechanisms. Together, our results provide novel insights into dynamic regulation of facultative heterochromatin and identify a central role for ISWI homologs in fungal development.

598F Gene expression divergence correlates with histone modifications in the fungal plant pathogen *Verticillium dahl-*

iae *David E Torres Sanchez*[1,2], Bart PHJ Thomma[3], Michael F Seidl[2] 1) Wageningen University and Research, The Netherlands; 2) Theoretical Biology & Bioinformatics Group, Department of Biology, Utrecht University, The Netherlands; 3) Institute for Plant Sciences, Cluster of Excellence on Plant Sciences (CEPLAS), University of Cologne, Germany.

Gene expression levels are often evolutionary conserved between related species or strains of the same species. However, within and between species phenotypic diversity is at least partially explained by the divergence in gene expression, which can be caused by changes in regulatory sequences as well as in chromatin landscapes. This diversity enables adaptation to changing environments, which is particularly relevant for plant pathogens that engage in 'arms races' with their hosts. *Verticillium dahliae* is a soil-borne fungal plant pathogen that causes wilt diseases on hundreds of plant hosts. The *V. dahliae* genome contains genomic regions that are hypervariable between strains and are characterized by a distinct chromatin landscape. Furthermore, chromatin changes are associated with gene expression variation during different *in vitro* conditions, but it remains unknown how gene expression is conserved between genetically distinct *V. dahliae* strains. Here, we used *in vitro* transcriptome data to study the conservation of expression levels between two *V. dahliae* strains. We observed that gene expression levels *in vitro* are highly conserved, and that expression as well as nucleotide conservation levels at protein-coding genes are positively correlated. Nevertheless, 18% of the genes in both strains showed variable expression levels, and 6% of the genes are highly expressed in one strain while absent in the other. To start explaining to which extent gene expression differences are caused by divergence of regulatory sequences or the chromatin landscape, we used sequence conservation as well as ChIP-seq data of seven activating and repressing histone modifications that localize at gene bodies as well as at promoters. We show that specific histone modifications (H3K9ac, H4K16ac, H3K4ac, and H3K4me3) are differentially enriched in conserved promoters in variably expressed genes. In contrast, these histone modifications are conserved at genes for which gene expression patterns are conserved between the two *V. dahliae* strains. Collectively, our results indicate that gene expression levels in *V. dahliae* are largely conserved yet gene expression divergence is associated with differences in chromatin landscape rather than regulatory sequence divergence.

599W A prion accelerates proliferation at the expense of lifespan *David Garcia*[1,2], Edgar Campbell[2], Christopher Jakobson[2], Mitsuhiro Tsuchiya[3], Ethan Shaw[1], Acadia DiNardo[1], Matt Kaeberlein[3], Daniel Jarosz[2] 1) University of Oregon, Institute of Molecular Biology; 2) Stanford University, Department of Chemical and Systems Biology; 3) University of Washington, Department of Pathology.

In fluctuating environments, switching between different growth strategies, such as those affecting cell size and proliferation, can be advantageous to an organism. Trade-offs arise, however. Mechanisms that aberrantly increase cell size or proliferation—such as mutations or chemicals that interfere with growth regulatory pathways—can also shorten lifespan. Here we report a natural example of how the interplay between growth and lifespan can be epigenetically controlled. We find that a highly conserved RNA-modifying enzyme, the pseudouridine synthase Pus4/TruB, can act as a prion, endowing yeast with greater proliferation rates at the cost of a shortened lifespan. Cells harboring the prion grow larger and exhibit altered protein synthesis. This epigenetic state, [*BIG*$^{+}$] (*b*etter *i*n *g*rowth), allows cells to heritably yet reversibly alter their translational program, leading to the differential synthesis of dozens of proteins, including many that regulate proliferation and aging. Our data reveal a new role for prion-based control of an RNA-modifying enzyme in driving heritable epigenetic states that transform cell growth and survival.

600T RNAi and heterochromatin independently control gene expression and transposable elements in Mucorales *María Isabel Navarro-Mendoza*[1], Carlos Pérez-Arques[1], Joseph Heitman[1] 1) Department of Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, NC, USA.

The RNA interference machinery silences expression and prevents movement of transposable elements in fungal species with active RNAi systems. The early-diverging fungus *Mucor lusitanicus* has an intricate RNAi system with two interacting pathways that control transposable elements and endogenous gene expression: one involves a canonical or Dicer-dependent pathway and the other an alternative Dicer-independent pathway. The pericentric regions flanking *Mucor* mosaic centromeres harbor numerous Mucoromycotina-specific L1-like retrotransposable elements known as Grem-LINE1s that are actively silenced by the canonical RNAi machinery. Inactivation of key components of the alternative RNAi pathway was found to increase silencing of these retrotransposons. We conducted ChIP-seq experiments to determine if this increase in post-transcriptional gene silencing was correlated with enhanced heterochromatin formation. Our results identified the presence of di- and trimethylated H3K9 (H3K9me2 and -me3) at Grem-LINE1 and other transposable elements genome-wide, often correlating with abundant antisense small RNAs. But surprisingly, H3K9me2 and -me3 levels were not significantly altered in mutants lacking key components of either the canonical (Dicer2, Ago1) or alternative RNAi pathways (Rdrp1, Rdrp3, R3B2), indicating that RNAi is dispensable for heterochromatin maintenance at transposable elements. RNAi also controls endogenous gene expression in the Mucorales. We identified protein-coding loci that harbor high levels of small RNAs displaying canonical siRNA features. In contrast to transposable elements, H3K9me2 or -me3 was not present in these genomic locations. The absence of heterochromatin marks at genes actively silenced by RNAi indicates that RNAi does not in and of itself recruit the machinery that forms heterochromatin. Taken together, our results suggest that RNAi and heterochromatin formation are independent genome defense and regulatory mechanisms in the Mucorales, contributing to a shift in paradigm from the co-transcriptional gene silencing observed in fission yeasts to models in which heterochromatin and RNAi operate independently in early-diverging fungi. This may also be the case in other ascomycetes and basidiomycetes such as *Neurospora crassa* and *Cryptococcus neoformans*, and the phylogenetic position of *Mucor* suggests that independence of function of RNAi and heterochromatin may represent an ancestral state in the fungal kingdom.

601F Regulation and product identification of FmPKS8, a so far cryptic PKS in *F. mangiferae* *Anna Atanasoff-Kardjalieff*[1], Bernhard Seidl[2], Katharina Steinert[3], Svetlana Kalinina[3], Hans-Ulrich Humpf[3], Rainer Schuhmacher[2], Lena Studt[1] 1) University of Natural Resources and Life Sciences, Vienna, Department of Applied Genetics and Cell Biology, Institute of Microbial Genetics, Tulln an der Donau, Austria; 2) University of Natural Resources and Life Sciences, Vienna, Department of Agrobiotechnology (IFA-Tulln), Institute of Bioanalytics and Agro-Metabolomics, Tulln an der Donau, Austria; 3) Westfälische Wilhelms-Universität, Institute of Food Chemistry, Münster, Germany.

The genus *Fusarium* comprises a species-rich group of mycotoxigenic plant pathogens, known to cause a wide range of various plant

diseases in agri- and horticulture, being detrimental for the northern- and southern hemisphere. The mango plant pathogen *Fusarium mangiferae* is the causal agent of the mango malformation disease and a so far not well-characterized member of the *Fusarium fujikuroi* species complex (FFSC). Environmental cues, such as growth and plant infection, trigger the production of low molecular weight substances or secondary metabolites (SMs), which contaminate food- and feed sources.

Biosynthesis of these SMs underlies a complex regulatory network modifying the chromatin structure to ensure the targeted expression of SM-related genes. Alterations in the chromatin structure through the deposition of posttranslational modifications are key in the regulation of fungal SM-gene expression. We have previously shown that the methyltransferase FmKmt1 involved in tri-methylation of histone 3 lysine 9 (H3K9me3) - a hallmark of heterochromatin formation - is required for fusapyrone biosynthesis mediated by the PKS40 gene cluster in *F. mangiferae* [1].

Here we show that FmKmt1 is involved in the expression of additional PKS-related genes. Biosynthesis of the so far cryptic FmPKS8 is under control of FmKmt1 and independent of the H3K9 acetylation status. Next to this, we delineated cluster borders *via* molecular and chemical means. By the functional characterization of single cluster genes, we unraveled a four-gene cluster to be involved in the biosynthesis of the final FmPKS8 product. Intriguingly, while FmPKS8 lacks an intrinsic enoyl reductase (ER) domain, our results suggest that the ER function is taken over by a tailoring enzyme located within the cluster. Next to this, we show that biosynthesis is induced under nitrogen limiting conditions but repressed under nitrogen-sufficient conditions. While the PKS8 gene cluster is abrogated in the closely related *Fusarium fujikuroi*, comparative genomics suggest that the PKS8 gene cluster is conserved also in *Fusarium verticillioides* and *Fusarium oxysporum*. In line with this, *F. verticillioides* produced the same PKS8-related compounds as determined by molecular and chemical analyses.

[1] A. K. Atanasoff-Kardjalieff et al., *Frontiers in Fungal Biology*, vol. 1, p. 671796, 2021, doi: 10.3389/ffunb.2021.671796.

602W Heterochromatin marks perturb transcriptional robustness and underpin dispensability of genes across evolutionary timescales in fungi *Sabina Tralamazza*[1,2], Leen Abraham[1], Claudia Reyes[1], Benedito Correa[2], Daniel Croll[1] 1) Laboratory of Evolutionary Genetics, Institute of Biology, University of Neuchatel, Neuchatel, Switzerland ; 2) Department of Microbiology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil.

Epigenetic modifications are key regulators of gene expression and underpin genome integrity. Yet, how epigenetic changes affect the evolution and transcriptional robustness of genes remains largely unknown. Here, we show how repressive histone marks underpin the trajectory of genes across evolutionary timescales. To determine transcriptional robustness and protein sequence evolution, we focused on two parallel systems of major crop pathogens, the *Fusarium graminearum* species complex (FGSC) and *Zymoseptoria tritici*. We performed transcriptomic and methylome (H3K27me3, H3K4me2) profiling of by including multiple closely related species. Furthermore, we used a collection of a thousand isolates of *Z. tritici* spanning the world-wide distribution to define precise boundaries of epigenetic marks and regions of high variation in gene content. Expanding to closely related species, we found that gene expression variation is negatively correlated with gene conservation confirming that highly conserved genes show higher expression robustness. Interestingly, highly conserved genes with repressive histone marks show no clear association between protein conservation and transcriptional robustness compared to unmarked genes. Importantly, we show that genes marked by H3K27me3 result in low phenotypic response during host infection. Highly conserved marked genes show enrichment in environmental stress related functions, carry hallmarks of fast evolving genes, result in low phenotypic response during host infection and, hence, do not follow the housekeeping gene archetype. Hence, histone modifications provide a key association between protein evolvability and gene essentiality across evolutionary timescales.

603T A new role in G2-M control revealed by a reciprocal translocation involving the *snxA* shuttling mRNA-binding protein and a GYF-domain protein in *Aspergillus nidulans* *Steven James*[1], Jonathan Palmer[2], Nancy Keller[3], Sarah Lea Anglin[4] 1) Gettysburg College, Gettysburg PA; 2) IFF Health and Biosciences, Palo Alto, CA; 3) University of Wisconsin - Madison, Madison, WI; 4) Millsaps College, Jackson MS.

Aspergillus nidulans snxA, an ortholog of *Saccharomyces cerevisiae* Hrb1/Gbp2 messenger RNA shuttle proteins, is – in contrast to budding yeast – involved in cell cycle regulation, in which *snxA*1 and *snxA*2 mutations as well as a *snxA* deletion suppress mutations in regulators of the CDK1 mitotic induction pathway (James *et al.* 2014. doi.org/10.1534/genetics.114.167445). *snxA* mutations confer cold-sensitivity, and *snxA*1/*A*2 mRNA and protein expression are strongly repressed at permissive temperature. Genetic studies demonstrated linkage of *snxA*1/*A*2 cold-sensitivity to markers on two chromosomes, suggesting a chromosomal rearrangement. Whole-genome sequencing revealed a chromosome I – II reciprocal translocation with breakpoints in the *snxA* first intron and in the fourth exon of a GYF-domain gene that we have named *gyfA*. Surprisingly, a complete deletion of *gyfA* and a reconstructed *gyfA* translocation allele suppressed the heat-sensitivity of CDK1 pathway mutants in a *snxA*$^{+}$ background, demonstrating that the reciprocal translocation simultaneously disrupted two unrelated genes, *snxA* and *gyfA*, each of which act through the CDK1-CyclinB axis to restrain the G2-M transition, and for the first time identifying a role in G2-M regulation for a GYF-domain protein.
The translocation breakpoint in the *snxA* first intron eliminated an 11-exon transcript and allowed only weak expression of a wild-type 9-exon transcript. To better understand the basis for *snxA*1/*A*2 reduced expression, we generated suppressors of *snxA*/1/*A*2 cold-sensitivity, and discovered that loss of *setB*^{Set2}histone H3 lysine36 (H3K36) methyltransferase rescued the mutants by restoring full transcriptional proficiency. These phenotypes were largely mirrored in a non-methylatable histone H3K36L mutant, indicating that methylation of H3K36 acts to restrain 9-exon *snxA* expression. These observations are in line with known *SET2* functions in preventing excessive and cryptic transcription of active genes, and suggests that restoration of *snxA*1/*A*2 expression may occur by reactivating a strong transcription startsite(s) (TSS) within intron 2, proximal to the 9-exon start codon, or by activating more distal TSS(s) mapped to intron 1.

604F Data-driven modelling captures dynamics of the circadian clock of *Neurospora crassa* Amit Singh[1], Congxin Li[2], Axel Diernfellner[1], Thomas Höfer[2], *Michael Brunner*[1] 1) Heidelberg University Biochemistry Center, Heidelberg, Germany; 2) Theoretical

Systems Biology, German Cancer Research Center, Heidelberg, Germany.

Eukaryotic circadian clocks synchronize physiology and metabolism with the environment via recurrent stimuli (zeitgebers) and are based on self-sustaining feedback loops. Many components and network interactions of biological clocks are known, their quantitative contribution to clock function is only partially understood. Data-driven mathematical clock models are limited by the lack of sufficient data. We present a model of the circadian clock of *Neurospora crassa* that describes free-running an light-dark entrained oscillations based on high-resolution time courses of luciferase reporters. These data allowed estimating parameters governing circadian phase, period length and amplitude, and the light response of gene expression. Our model suggests that functional maturation of the core clock protein Frequency (FRQ) causes a delay in negative feedback that is critical for circadian rhythms.

605W FREQUENCY Phosphosite Mutations Perturb Temperature Compensation of the *Neurospora* Circadian Clock *Elizabeth-Lauren Stevenson*[1], Christina M. Kelliher[1], Jennifer J. Loros[2], Jay C. Dunlap[1] 1) Department of Molecular & Systems Biology, Geisel School of Medicine at Dartmouth, Hanover, NH; 2) Department of Biochemistry & Cell Biology, Geisel School of Medicine at Dartmouth, Hanover, NH.

The molecular circadian clock in animals and fungi consists of a transcription-translation feedback loop that is highly regulated post-translationally throughout the circadian day by phosphorylation events. In the classic clock model *Neurospora crassa,* the positive arm of the clock, a heterodimeric complex of transcription factors (White Collar-1 and White Collar-2)*,* activates the transcription of the negative arm of the clock, Frequency (FRQ), which complexes with FRQ-interacting RNA Helicase (FRH) and Casein Kinase I to inactivate the positive arm via phosphorylation, thereby inhibiting their own transcription. Several key features define circadian rhythms, including the capacity to be entrained by external cues, the ability to continue oscillating in the absence of such cues, and incredibly, the maintenance of a consistent period across temperatures (temperature compensation/TC).
We have previously demonstrated that a classical *N. crassa* period mutant with overcompensated TC (in which period lengthens with temperature), *prd-3,* is a Casein Kinase II (CKII) hypomorph. This led to a model in which CKII phosphorylates FRQ preferentially at higher temperatures, which compensates for the increased levels of FRQ present at such temperatures. Given that FRQ phosphorylation may contribute to the TC mechanism, we further probed which phosphosites on FRQ are important for TC by determining the circadian period of strains bearing serine to alanine mutations on FRQ at 20°C, 25°C, and 30°C, using a transcriptional reporter in which the *frq* promoter drives luciferase expression. We found that no disruption of a single phosphosite on FRQ alone can perturb TC, but that when several key sites are mutated together, the strains are abnormally compensated against temperature. Both under-compensation and over-compensation TC phenotypes were observed, depending on which phosphosites were mutated. Our results further support a role for FRQ phosphorylation in TC and begin to define the precise mechanism that underlies how circadian period is temperature compensated.

606T Novel NuA4 subunits reveal a crucial role of dynamic expression of the negative arm of the circadian clock *Bin Wang*[1], Xiaoying Zhou[1], Arminja Kettenbach[2], Hugh Mitchell[3], Jennifer Loros[1, 2], Jay Dunlap[1] 1) Department of Molecular and Systems Biology, Geisel School of Medicine at Dartmouth, Hanover, NH 03755, USA; 2) Department of Biochemistry and Cell Biology, Geisel School of Medicine at Dartmouth, Hanover, NH 03755, USA; 3) Biological Sciences Divisions, Pacific Northwest National Laboratory, Richland, WA, 99352, USA.

Post-translational modifications (PTMs) on histones have been found to play diverse functions in regulating chromatin events and gene expression. The operation of circadian clocks heavily relies on finely tuned and timely expression of the proteins comprising core oscillators. However, most studies of PTMs' effects on circadian clocks have been conducted using static systems in which circadian clocks are rendered arrhythmic due to the essential role of PTMs on gene expression. In the Neurospora circadian system, the White Collar Complex (WCC), a heterodimeric transcription factor formed from White Collar-1 (WC-1) and White Collar-2 (WC-2), serves the function of the BMAL1/CLOCK heterodimer in mammals, driving expression of circadian negative arm component(s), a principal one in Neurospora encoded by the gene frequency (frq). FRQ interacts with FRH (FRQ-interacting helicase) and CK-1 forming a stable complex that represses its own expression by inhibiting WCC. In this study, a genetic screen identified a gene, designated as eaf-8, that encodes a novel conserved subunit of the NuA4 histone acetylation complex. Loss of eaf-8 reduces H4 acetylation and RNA polymerase (Pol) II occupancy at frq and other known circadian genes, and leads to a long circadian period, delayed phase, and defective overt circadian output at some temperatures. In addition to strongly associating with the NuA4 histone acetyltransferase complex, EAF-8 is also found complexed with the transcription elongation regulator BYE-1. Expression of eaf-8, bye-1, histone hH2Az, and several NuA4 subunits is controlled by the circadian clock, indicating that the molecular clock both regulates the basic chromatin status and is regulated by changes in chromatin. Taken together, our data identify a new type of the NuA4 complex including EAF-8 and BYE-1 which, along with conventional NuA4 subunits, is required for timely and dynamic frq expression and thereby a normal and persistent circadian rhythm.

607F *carP*, a long non-coding RNA with broad effects on *Fusarium fujikuroi* transcriptome. *Javier Pardo-Medina*[1], Gabriel Gutiérrez[1], M. Carmen Limón[1], Javier Avalos[1] 1) Universidad de Sevilla.

The synthesis of neurosporaxanthin in *Fusarium* is a well-studied model for the regulation of carotenogenesis in filamentous fungi. Most of the genes in this pathway are organized in a co-regulated cluster. Their transcription is strongly stimulated by light and it›s repressed by the CarS protein, a putative ubiquitin ligase, whose mutation gives rise to a carotenoid overproducing phenotype. A potentially non-coding 1.2 kb transcript, which was denominated *carP*, was detected by RNA-seq in the region upstream the *carS* gene. Genomic alterations around the transcript led to the deregulation of the carotenoids production; and the targeted deletion of this gene, not previously annotated, causes an albino phenotype due to a sharp drop in the mRNA levels of the structural genes of carotenogenesis. A combination of bioinformatic analysis of this sequence together with the phenotypes of the *carP* mutants in *F. fujikuroi* and *F. oxysporum* ruled out its coding capacity. The reintroduction of the wild-type carP sequence in the mutant of *F. fujikuroi* showed that only reintegration in its native locus allows the recovery of the ability to produce carotenoids. Transcriptomic studies in *F. fujikuroi* found that *carP* not only disturbs carotenogenesis genes but also other photoinducible ones. Many of these changes appear to be due to the cascade effect caused by changes in the level of transcription of *carS*, but the data suggest that *carP* could have other functions as an independent regulatory element. In conclusion, our data show that *carP* participates as a regulatory lncRNA in the production of

carotenoids in *Fusarium*, possibly through the control of CarS by a mechanism yet to be determined.

608W Circadian Clock-Controlled Translation of Specific *Neurospora crassa* mRNAs Requires Rhythmic eIF2α Activity and P-bodies *Kathrina Castillo*[1,2], Cheng Wu[2], Zhaolan Ding[1,2], Matthew Sachs[2], Deborah Bell-Pedersen[1,2] 1) Center for Biological Clocks Research, Texas A&M University; 2) Department of Biology, Texas A&M University.

At least half of proteins that accumulate with a circadian rhythm in *Neurospora crassa* are produced from mRNAs whose levels are not clock-controlled, indicating a prominent role for clock regulation of post-transcriptional processes. Phosphorylation of at least 30% of available *N. crassa* eIF2α, a conserved translation initiation factor, is clock-controlled, peaking during the subjective day. To determine the impact of rhythmic eIF2α phosphorylation on rhythmic translation, we carried out temporal ribosome profiling and RNA-seq in WT, clock mutant Δ*frq*, eIF2α kinase mutant Δ*cpc-3*, and constitutively active *cpc-3*^{c} cells. We discovered that ~14% of *N. crassa* mRNAs are rhythmically translated in WT cells, and translation rhythms for ~30% of these mRNAs were dependent on the clock and CPC-3. FunCat term analysis revealed that these cTICs primarily function either in anticipation of, or in direct response to various stresses experienced by the cells on a daily basis. Most circadian translation initiation-controlled genes (cTICs) are expressed from non-rhythmic mRNAs, and contain a cytoplasmic P-body localization motif present in their 5' leader sequence. Deletion of the P-body component SNR-1, and deletion of the P-body motif in the 5' leader of one of these mRNAs *zip-1*, significantly altered rhythmic translation of *zip-1* mRNA. Furthermore, the deletion of P-body components SNR-1 and SNR-7 led to reduced linear growth rates in constant conditions, to a level comparable to cells with abolished rhythmic eIF2α phosphorylation. Together, these results revealed a mechanism by which the circadian clock regulates rhythmic translation of specific mRNAs, through rhythmic eIF2α activity and P-body metabolism.

609T A platform for functional analysis for *Candida albicans* strain variation *Yinhe Mao*[1], Max Cravener[1], Norma Solis[2], Scott Filler[2], Aaron Mitchell[1] 1) Department of Microbiology, University of Georgia, Athens, GA; 2) Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, CA.

Candida albicans is a major human fungal pathogen. The clinical isolate SC5314 and its derivatives have been well studied, and have revealed molecular mechanisms of cellular processes and pathogen-host interaction. However, most studies have not accessed the extensive genetic variation among *C. albicans* isolates and its functional consequences. Different clinical isolates of *C. albicans* display striking differences in phenotypes, genotypes, and regulatory network structure. Mutations can affect both ORFs and non-coding sequences. What are the determinants of this extensive diversity? We created a new platform to investigate strain variation based on genotype-phenotype relationships. An SC5314 genomic DNA library was produced in lambda phage, which maintains 20 kb inserts stably. We have introduced the library into other clinical isolates by two different approaches. The first approach uses CRISPR-Cas9 techniques to integrate the library at the *MDR1* locus. We created about 2600 homozygous transformant strains in a weakly filamentous strain, P75010, and they were screened for the ability to form hyphae and damage host cells. The second approach is to generate autonomously replicating clones capable of autonomous maintenance instead of integrating into the genome, based on the strategy of Bijlani et al., mSphere. 2019 Mar 6;4(2):e00103-19. Lambda phage libraries were added with selection marker and origins of replication at one end and terminal telomere repeats at both ends. The autonomously replicating mutants showed a high copy number of genes, providing a powerful new tool to investigate pathway components and identify strain variation. Together, the low copy complementation library and the high copy overexpression library provide exciting tools to explore *C. albicans* strain diversity.

610F *In vivo* analysis of hyphal morphogenesis in *C. albicans.* *Rohan Wakade*[1], Melanie Wellington[1], Damian Krysan[1] 1) University of Iowa.

The yeast-hyphal transition, triggered by numerous environmental stimuli, has been associated with the pathogenicity of the human fungal pathogen *Candida albicans*. *In vitro*, the set of transcription factors (TFs) responsible for the dimorphic switch is extensively characterized. However, the transcriptional requirements for filamentation vary with the specific environments raising the possibility that distinct TFs networks may mediate filamentation in the mammalian host. To examine the TFs network that regulates filamentation *in vivo*, we developed an *in vivo* imaging system and screened for a TFs deletion library [1]. To do this, the dermal layer of the DBA/2N mouse ear was inoculated with fluorescently labelled *C. albicans* and the morphology of reference vs TF mutant strain was assessed after 24 hours in the ears of mice using confocal microscopy. In contrast to the number of TFs critical for filamentation (40 TFs) *in vitro* [1], our *in vivo* data identified only five TFs (*EFG1, BRG1, ROB1, TEC1,* and *RIM101)* that are required for filamentation. Although heterozygous mutants of *EFG1, TEC1,* and *RIM101* had no filamentation defect, we observed a critical genetic interaction between *EFG1-TEC1* as well as *RIM101-TEC1* indicating *TEC1* could act as a critical co-regulator for *in vivo* filamentation. In agreement with our hypothesis, we have identified a key set of TFs that specifically regulates morphogenesis *in vivo*. Ongoing studies will focus on identifying the mechanistic basis that contributes and governs the morphogenesis of *C. albicans* within the host.

1. Homann, O.R., et al., *A phenotypic profile of the Candida albicans regulatory network.* PLoS Genet, 2009. **5**(12): p. e1000783.

611W The Ess1 prolyl isomerase and its target, the CTD of RNA polymerase II, in cold-adapted fungi. *Steven Hanes*[1], Ryan Palumbo[1], Nathan McKean[1], Erinn Leatherman[1], Kevin Namitiz[1,5], Laurie Connell[2], Aaron Wolfe[3], Kelsey Moody[3], Cene Gostinčar[4], Nina Gunde-Cimerman[4], Alaji Bah[1] 1) SUNY-Upstate Medical University; 2) School of Marine Sciences, University of Maine, Orono, ME USA; 3) Ichor Therapeutics, Lafayette, NY, USA; 4) Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia; 5) Current address... Penn State University.

Most of the world's biological diversity is adapted to living in cold, often below freezing temperatures (-1°C to 4°C). We are interested in understanding the mechanisms of transcription by RNA polymerase II (RNAPII) in the cold. Toward this end, we cloned the Ess1 gene from fungal species that survive in the extreme environments of the polar regions. Ess1 is an essential prolyl isomerase that regulates transcription by inducing conformational changes in the carboxy-terminal domain (CTD) of the large subunit of RNAPII. Ess1 enzymes from cold-adapted fungi (Arctic and Antarctic) were active in the model organism *Saccharomyces cerevisiae*, and sequence substitutions may provide clues as to how they function in the cold. More striking was that the CTDs from cold-adapted fungi are highly-di-

vergent from the near consensus repeat sequence ($YSPTSPS_{26}$) found in *S. cerevisiae* and other model organisms. This divergence profoundly affected the ability of the CTD, which is intrinsically-disordered, to undergo liquid-liquid phase separation (LLPS) *in vitro* and to localize and function *in vivo*. We propose that one mechanism for cold-adaptation (and other environmental tolerance) is altered LLPS behavior via sequence divergence within the intrinsically disordered regions of otherwise globular proteins. Indeed, phylogenetic analyses revealed that most fungal species that live outside the laboratory carry sequence-divergent CTDs. Our findings lay the groundwork for future detailed studies on a new and highly-tractable model for evolutionary cold-adaptation of a globular enzyme (Ess1/Pin1), as well as for uncovering the link between evolutionary selection for sequence divergence in intrinsically disordered regions, LLPS properties and the CTD Code.

612T Regulatory conservation of *EFG1* indirect target genes by *WOR3* *Max Cravener*[1], Eunsoo Do[1], Carol Woolford[2], Gemma May[2], Frederick Lanni[2], Charles McManus[2], Aaron Mitchell[1] 1) University of Georgia, Athens, GA; 2) Carnegie Mellon University, Pittsburgh, PA.

Candida albicans clinical isolates occupy a wide spectrum of genetic and phenotypic diversity. In particular, the ability of this fungus to switch back and forth from yeast form growth to hyphal form and the regulatory variation governing this switch among a panel of clinical isolate strains is the primary focus of this work. Hyphal induction is caused by several different factors and is necessary for a variety of reasons including the formation of a biofilm and evasion of the immune system. One of the core regulators of hyphal induction is the transcription factor, *EFG1,* which is known to positively regulate hyphal formation and hyphal-associated genes across a variety of inducing conditions. Recent work has also shown that while *EFG1* has expansive phenotypic control in one strain across many conditions, when five strains are considered, there are many genes whose relationship to *EFG1* is strain-specific and others which are conserved. Here we used a transient CRISPR-Cas9 based approach to delete *EFG1* in 17 clinical isolate strain backgrounds. Homozygous *efg1ΔΔ* strains were then shown to have a universal defect in both filamentation and biofilm formation. In addition, RNA-seq analysis revealed that there is a conserved set of 115 genes which have a conserved response to deletion of *EFG1.* Interestingly, the core *EFG1* response genes are not all direct targets of *EFG1*. Unpublished ChIP-seq data shows that 52 *EFG1* core response genes are indirect targets of *EFG1*. Among the *EFG1* direct target core genes activated by *EFG1* are transcription factors *BRG1, TYE7, UME6, WOR3, RFX2, LYS143,* and *orf19.6888*. Each of these genes was then overexpressed in three clinical isolate *efg1ΔΔ* backgrounds by substituting the highly expressed *TDH3* promoter at the native promoter locus. Overexpression of *BRG1, UME6,* and *WOR3* was sufficient to drive at least a partial rescue of the filamentation, biofilm formation, and gene expression defects caused by the deletion of *EFG1*. These data show that the conserved direct targets of *EFG1* are responsible in part for the strong conservation of the indirect core target genes as well as the universal phenotypic defect.

613F Characterization of a *Candida albicans* transcription factor family *Amelia White*[1], Aaron Mitchell[1] 1) University of Georgia.

The *C. albicans ADR1, TRY4,* and *orf19.5026* genes encode zinc cluster transcription factors with very similar DNA-binding domains. All three genes are also under control of the Mig1/2 repressors, which mediate glucose repression (Lagree et al. 2020). For these reasons, we view the three gene products as a transcription factor family, and we hypothesize that they may have shared functions. Adr1 is the ortholog of an *S. cerevisiae* activator of genes required for ethanol, glycerol, and fatty acid utilization (Young et al. 2003), but prior studies in *C. albicans* indicate that Adr1 function has diverged (Ramirez and Lorenz 2009). Our shared function hypothesis predicts that a triple deletion mutant may show prominent phenotypic defects.
We constructed mutant strains in the *C. albicans* SN152 background using a transient CRISPR-Cas9 transformation protocol (Min et al. 2016). Growth of the triple mutant versus wild type on diverse carbon sources was assayed by utilizing Biolog 96 well carbon source microarray plates. The wild type was able to use TCA cycle intermediates as carbon sources, while the triple mutant was not. In addition, an *adr1*ΔΔ *try4*ΔΔ *orf19.5026*ΔΔ mutant has a severe filamentation defect when assayed in YPD at 37 □C compared to the wild type.
The above results indicate that this transcription factor family is likely involved in both carbon source regulation and filamentation control. One model is that *ADR1* family members control carbon transport in and out of the mitochondria. The connection between these metabolic functions and filamentation remains to be established.

614W The effect of Hxk1 and Hxk2 on open chromatin regions and gene expression in *Candida albicans* *Stefanie Wijnants*[1], Dimitrios Sofras[1], Sabrina Jenull[2], Clarissa Nobile[3,4], Karl Kuchler[2], Patrick Van Dijck[1] 1) Laboratory of Molecular Cell Biology, Department of Biology, Institute of Botany and Microbiology, KU Leuven, Leuven, Belgium; 2) Max Perutz Labs Vienna, Center for Medical Biochemistry, Medical University of Vienna, Vienna, Austria; 3) Department of Molecular and Cell Biology, School of Natural Sciences, University of California, Merced, California, USA; 4) Health Sciences Research Institute, University of California, Merced, California, USA.

Candida albicans is an opportunistic human fungal pathogen that relies upon different virulence traits, including morphogenesis, invasion, biofilm formation, and nutrient acquisition from host sources as well as metabolic adaptations during host invasion. Different proteins of *C. albicans* that function in metabolism also have additional roles. Hxk1 and Hxk2 seem to have such a double function, a catalytic one and a regulatory one. Hxk1 phosphorylates GlcNAc which is necessary for different purposes in the cell. Furthermore, this protein enters the nucleus and influences the expression of Hxk2 and Glk1/4. Hxk1 also seems to be a regulator for morphogenesis via interacting with Sir2, an important chromatin remodulator. Hxk2 is important for both glucose and fructose phosphorylation and enters the nucleus in the presence of glucose. An *hxk2* mutant shows an increased transport of glucose into the cells which can be explained by the fact that Hxk2 has a negative effect on hexose transporter genes (*HGT12*, *HGT7*, *HXT10* and *HGT8*). Due to these findings, we hypothesized that Hxk1 and Hxk2 would influence different processes in the cell by regulation of open chromatin regions and gene expression. Therefore, we performed a ATACSeq and RNASeq experiment. These experiments got us valuable information about which genes and more specific which pathways are regulated by Hxk1 and Hxk2. However, we still had no idea if this regulation happened in a direct or indirect way. To elucidate this question, we performed a CUT&RUN experiment. Our major findings will be highlighted at the Fungal Genetics Meeting.

615T Dss1 and Cap60 are required for capsule formation, stress response, and virulence in *Cryptococcus neoformans* *Olumuyiwa Igbalajobi*[1], Mélissa Caza [1], James Kronstad[1] 1) University of British Columbia, Canada.

Cryptococcus neoformans is responsible for an estimated 300,000 cases of cryptococcal meningoencephalitis and over 15% of all AIDS-associated deaths per annum. The capsule polysaccharide is critical for virulence and a target for a potential anticryptococcal therapeutic strategy because the loss of capsule attenuates virulence in mice. Unfortunately, our understanding of the mechanisms of capsule formation is incomplete. We recently discovered that the proteasome inhibitor, Bortezomib, inhibits capsule production in *C. neoformans* suggesting crosstalk between capsule production and the ubiquitin-proteasome system (UPS). The UPS has been explored as a therapeutic target for cancer, malaria, and tuberculosis. To understand the interplay between the ubiquitin-proteasome system and capsule formation in *C. neoformans*, we created a loss of function mutation of the proteasomal subunit, *DSS1*. Similar to the loss of capsule formation in mutants lacking Cap60, Cap64, or Cas 35, the deletion of *DSS1* attenuated capsule production at 37°C. We found that the ability to grow at elevated temperature depended more on Dss1 and partially on the capsule proteins (Cap60, Cap64, and Cas35). A lack of Dss1 and one of the capsule proteins, Cap60, Cap64, or Cas35 resulted in sensitivity to cell wall perturbing agents, endoplasmic reticulum stress, and agents that provoke oxidative, osmotic, or nitrosative stress. Furthermore, Dss1 is required for the upregulation of genes involved in capsule production. Interestingly, the deletion of *CAP60* resulted in a reduced transcript level of *DSS1*. The *dss1 and cap60* mutants also exhibited attenuated virulence in an intranasal murine model of cryptococcosis. Our results suggest overlapping roles of Dss1 and capsule proteins in capsule formation, stress response, and virulence in *C. neoformans*. This work provides novel opportunities to develop proteasome inhibitors as potential antifungal agents

616F Analysis of pre-filamentous *Candida albicans* cells identifies differing requirements for filamentation in *in vitro* models and defines the pre-filamentation transcriptome Patricia Harte-Maxwell[1], Amanda Brookhouser-Sisney[1], Sebastian Espinoza[1], *Jill Blankenship*[1] 1) University of Nebraska at Omaha.

The ability of cells to switch between yeast and hyphal forms of growth is strongly correlated to the ability of the human pathogen *Candida albicans* to cause serious system infections in hosts. Our previous studies illustrated that *in vitro* models of filamentation were not interchangeable and that physical cues for filamentation drive distinct programs of filamentation in *C. albicans* with differing genetic requirements and transcriptomes. In this work, we have turned our attention to a timepoint prior to the emergence of filaments to determine when the transcriptional differences between solid and liquid filamentation begin to arise. We hypothesized that cells would behave similarly in the pre-filamentation phase and that transcriptional differences would begin to arise between cells grown in liquid versus solid inducing media as hyphae began to form and probe their environment. In contrast to our hypothesis, filamentation profiles were distinct in liquid and solid media prior to germ tube emergence. In addition, the core transcriptomic profiles of cells at this early timepoint suggest the cells in all inducing conditions remain in an extended post-diauxic phase following induction. We explored whether post-diauxic phase was a pre-requisite for filamentation and found that it *was*, but only for cells induced in most liquid filamentation conditions, further illustrating differences between these induction models. Contrasting the transcriptomes of pre-filamentous cells and cells 3 hours post-induction, we have identified genes that are differentially regulated throughout early stages of filamentation development, pre-filamentation to 3 hours post-induction, and have found that time post-induction is a stronger driver of differential gene regulation than filamentation model. The latter suggests that the transcriptome of filamentous cells evolves as the filamentous cells develop and suggests that analysis of core filamentation transcriptomes will provide insight into processes that are regulated at specific stages of filamentous development. This work provides insight into pre-filamentous cells and has implications for drug development targeting filamentous cells.

617W The HMG Domain-Containing Transcription Factors Hgr1 and Hgr2 are Putative Dormancy Factors of *Cryptococcus* Spores *Megan McKeon*[1], Christina Hull[1,2] 1) Department of Biomolecular Chemistry, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI; 2) Medical Microbiology and Immunology, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI.

Spores are a dormant, stress-resistant cell type used by fungi to spread to new environments. To survive, these cells must remain dormant until they encounter favorable conditions for germination, an essential differentiation process in which dormant spores transition into vegetatively growing cells. Despite the importance of spore germination in the life cycles of the majority of fungi, the molecular networks governing this fundamental process remain relatively poorly understood. To determine the molecular mechanisms controlling germination, we carried out a time course transcriptomic analysis of *Cryptococcus* spores, assessing 11 time points over the 10 hour transition from spores to yeast. We discovered that the most dynamic differences in transcript levels occurred between dormant spores (time 0) and 20 minutes after initiation of germination. During this short time span, 2078 transcript levels increased and 863 transcript levels decreased, suggesting that spores are primed to respond quickly to both synthesize new transcripts and degrade existing spore transcripts upon initiation of germination. Through an analysis of known transcription factors in *Cryptococcus* we hypothesized that two high mobility group (HMG) domain-containing proteins would be regulators of germination initiation. To test this hypothesis, we evaluated strains with deletions of *HGR1* and *HGR2* for phenotypes during germination. Using a high-resolution, quantitative germination assay, we discovered that spores produced by both *hgr1Δ* and *hgr2Δ* strains germinate at a faster rate than wild type spores. This finding suggests that Hgr1 and Hgr2 play a regulatory role during germination in which they modulate the rate at which germination initiation occurs. Our working model is that Hgr1 and Hgr2 act as dormancy factors to maintain the ungerminated spore state by repressing transcriptional responses to germination signals. Future experiments will identify environmental signals that control Hgr1 and Hgr2, directly regulated targets of Hgr1 and Hgr2, and define the transcriptional regulatory network that coordinates spore germination and subsequent vegetative growth.

618T Natural variation by collaboration between antagonistic morphotype master regulators *Eunsoo Do*[1], Max Craven-er[1], Manning Huang[2], Gemma May[2], C. Joel McManus[2], Aaron Mitchell[1] 1) University of Georgia, Athens, GA, USA; 2) Carnegie Mellon University, Pittsburgh, PA, USA.

Genetic variation is prevalent within all species. One manifestation of this variation is the diversification of the circuits regulating biofilm/hyphal formation, which is pervasive among *Candida albicans* clinical isolates (Huang et al., PLOS Pathog. 2019 May

22;15(5):e1007787). To understand the architecture of biofilm/hyphal regulatory networks among *C. albicans* clinical isolates, we used both RNA-seq and ChIP-seq analysis to elucidate diversified regulatory networks of the master biofilm regulator Efg1 among a panel of *C. albicans* isolates. Our analysis revealed that, while gene expression dependence on Efg1 is highly variable, almost all Efg1 binding sites were uniformly detected across five isolates. Hence, we focused on differentially expressed transcription factors (TFs) that share target genes with Efg1 in P75010, which is the most weakly filamentous strain tested. Increased gene dosage by using ectopic expression of Efg1 partner TFs revealed that *BRG1*, *TEC1* and *WOR1* affect the Efg1 regulatory network and also enhance biofilm formation in P75010. Strikingly, substitution of negatively charged or aromatic amino acids in prion-like domain (PrLD) of Wor1, as defined by Frazer et al., Nat Microbiol. 2020 Nov;5(11):1374-1389, abolished the Wor1 effect on Efg1 target genes. Therefore, we conclude that Efg1 and Wor1 have a collaborative interaction in biofilm/hyphal regulation, in addition to the antagonistic interaction in cell morphotype determination that is well described (Noble et al., Nat Rev Microbiol. 2017 Feb;15(2):96-108). Together, our results demonstrate that collaboration between the master regulators for different morphotypes contribute to regulatory variation within the *C. albicans* isolates.

619F Natural variation in *Candida albicans* glycolytic regulator activities *Minju Kim*[1], Aaron Mitchell[1] 1) University of Georgia, Athens, GA.

The opportunistic human fungal pathogen *Candida albicans* assimilates diverse carbon sources to thrive in a wide range of host environments. *C. albicans* Gal4 and Tye7 are known as transcriptional activators of glycolytic genes. Tye7 also has roles in the virulence traits, filamentation, and biofilm formation; Gal4 is not known to have such functions. In the model yeast *Saccharomyces cerevisiae,* Gal4 is a positive regulator of galactose genes, but studies in *C. albicans* have suggested that this role has been lost. Our studies seek to exploit natural variation to understand gene function and to exploit gene function to understand natural variation. We have begun to analyze Tye7- and Gal4-related phenotypes in five diverse clinical isolates of *C. albicans.* We have discovered evidence that the balance of activities of Tye7 and Gal4 may vary among clinical isolates. Also, in contrast to published studies with the type strain, we have found that Gal4 governs phenotypes that include filamentation and galactose utilization. We propose to use gene expression analysis and target gene manipulation to elucidate the basis for strain differences in the Tye7 and Gal4 regulatory portfolios. The research will lead us to understand mechanisms of *C. albicans* natural variation and, potentially, determinants of infection outcome differences.

620W Reaching across the aisle: Sculpting of *C. albicans* biofilm/hyphal gene expression network through collaboration among antagonistic cell type regulators Eunsoo Do[1], Max Cravener[1], Manning Huang[2], Gemma May[2], Joel McManus[2], *Aaron Mitchell*[1] 1) University of Georgia; 2) Carnegie Mellon University.

The most well characterized cell morphology transitions in *Candida albicans* are the yeast-hyphal switch, which is critical for biofilm formation, and the white-opaque switch, which is critical for mating. These switches depend upon the master regulators Efg1 (yeast-hyphal) and Wor1 (white-opaque), and occur under distinct environmental conditions. High levels of Efg1 or Wor1 can repress one another's expression and thus antagonize one another's function. Our studies of natural variation among *C. albicans* clinical isolates have revealed two collaborations that stand in stark contrast to the prior understanding of Efg1-Wor1 antagonism.
First, we find that small differences in *WOR1* expression levels, observed among clinical isolates during the yeast-hyphal switch, change the effect of Efg1 on its direct target genes. For example, Efg1 activates *HYR1* when Wor1 levels are slightly elevated, yet represses *HYR1* when Wor1 levels are slightly diminished. These gene expression effects are manifested biologically: an engineered increase in Wor1 levels enhances biofilm formation in a strain that normally expresses low Wor1 levels. Frazer et al. (2020) showed that Efg1 and Wor1 form a complex condensate in vitro that depends upon the prion-like domain of Wor1. We find that mutations in this domain of Wor1 abolish its enhancement of biofilm formation. We propose that Wor1 sculpting of Efg1 activity depends upon the prion-like domain interaction in transcription factor condensates.
A second unexpected collaboration occurs between Efg1 and Wor3, an activator of *WOR1* expression. We find that 30% of the core genes under Efg1 control in 17 different clinical isolates are indirect targets of Efg1 – not bound in ChIP assays – and instead are activated by Wor3, which is in turn activated directly by Efg1. The biological significance of this relationship is underscored by observations that increased *WOR3* expression can promote biofilm formation in the absence of Efg1, and that in some strains the loss of *WOR3* causes a biofilm defect.
Our findings show that the impact of white-opaque regulators extends to the pathogenic process of biofilm formation through productive relationships with Efg1. Efg1-Wor1 collaboration likely occurs through protein complex formation; Efg1-Wor3 collaboration likely occurs through a regulatory circuit relay.

621T The role of *C. neoformans* casein kinase Yck2 in translational reprogramming during host temperature adaptation *Amanda Bloom*[1], John Panepinto[1] 1) SUNY University at Buffalo, Buffalo, NY.

Cryptococcus neoformans is a basidiomycete that causes significant mortality in people that are immunocompromised. Our laboratory has a longstanding interest in understanding post-transcriptional and translational mechanisms that allow this environmental fungus to adapt to stresses encountered in the host, including temperature stress. Our previous studies have linked translational reprogramming to cell wall remodeling following a shift to host temperature. Here we present evidence that the casein kinase homolog, Yck2, may play a role in regulating these processes. Using cell staining and flow cytometry we show that the *yck2*Δ mutant fails to mask the cell wall β-1,3-glucan, and is recognized by the host Dectin-1 receptor following 37°C stress. Additionally, removal of abundant ribosomal protein mRNAs from the translating pool via enhanced degradation is defective in the *yck2*Δ mutant, and the mutant displays sensitivity to translational inhibitors. Our data suggests that signaling via Yck2 may mediate stress-induced changes at the ribosome that govern host temperature adaptation. We are currently investigating the role of Yck2 in shaping the translational landscape, and the possible cross-talk between Yck2 and other signaling pathways involved in the response to host temperature stress.

622F Gcn2 compensates for the absence of Hog1 in *Cryptococcus neoformans* *David Goich*[1], Amanda Bloom[1], John Panepinto[1] 1) University at Buffalo, Buffalo, NY.

Cryptococcus neoformans is an environmental fungus that causes severe opportunistic infections in immunocompromised individuals, particularly people living with HIV/AIDS. Upon entry into the host, *C. neoformans* rapidly alters its proteome in response to a variety of stressors, including elevated temperature, reactive oxygen species (ROS) in phagocytes, nutrient deprivation, and more. Our previous work demonstrated that these processes are dependent in part on the p38 MAPK, Hog1, which couples the translational response to stress-sensing signal transduction. Our preliminary data suggests that Gcn2, the *C. neoformans* eIF2α kinase, is hyperactive in *hog1*Δ strains. We generated a *hog1gcn2*ΔΔ strain to investigate whether translational defects in *hog1*Δ are a result of, or masked by, hyperactivation of Gcn2. Using polysome profiling and Northern blots, we investigated whether loss of both kinases resulted in synergistic defects in global translation or mRNA repression during thermal stress. We also investigated whether loss of both kinases resulted in synergistic growth defects under thermal and oxidative stress, as measured by spot plate analysis and kinetic growth curves. We found that *hog1gcn2*ΔΔ exhibited synergistic growth defects under both stressors, and that this was associated with defects in both global translation and repression of ribosomal transcripts not seen in either single knockout. Our data suggest that Hog1 and Gcn2 may play compensatory roles when the other gene is knocked out. Ongoing investigations will determine whether the translational defects and stress sensitivity of *hog1gcn2*ΔΔ is associated with attenuated virulence.

623W Translational suppression by ribonuclear protein (RNP) granules: a mechanism for post-transcriptional regulation of *Candida albicans* filamentation *Melissa Tosiano*[1], Gemma May[1], Frederick Lanni[1], C. Joel McManus[1] 1) Carnegie Mellon University.

While *Candida albicans* lives as a commensal organism in healthy humans, it can cause candidiasis in immunocompromised individuals. Like many fungi, *C. albicans* is dimorphic; undergoing filamentation in response to heat stress and starvation. This transformation accompanies the shift from a harmless commensal yeast to a virulent hyphal pathogen. Many fungi, including the pathogen *C. neoformans*, utilize post-transcriptional regulation to rapidly reprogram the translatome, upregulating translation of stress response genes and silencing housekeeping genes, suggesting a role for post-transcriptional regulation in *C. albicans* filamentation. While transcriptional regulation of stress-induced filamentation in *C. albicans* is well established, post-transcriptional mechanisms remain relatively unexplored.

Localization of mRNA transcripts to ribonuclear protein granules (RNPs) such as stress granules (SGs) and P-bodies has been associated with translation repression. *C. albicans* forms SGs and P-bodies during filamentation[1,2]. In diverse yeast species, heat shock induces DEAD-box helicase Ded1p condensation, selectively repressing housekeeping mRNAs in SGs[3]. Ded1p contains an N-terminal intrinsically disordered region (IDR) that appears to control condensation temperature during heat-shock[3]. Prior work demonstrated that *C. albicans* forms P-bodies under stress, and P-body component Edc3p is required for filamentation[2]. However, Ded1p granule formation has not been previously investigated in this species. We fluorescently tagged canonical SG (*PAB1*) and P-body (*EDC3*) markers as well as *DED1* in *C. albicans* to examine their condensation and potential co-localization during filamentation. In the future we will test the hypothesis that the expanded IDR of *C. albicans* influences filamentation and compare the association of transcripts with RNP condensates to translational repression during filamentation.

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2. Jung, J. H. & Kim, J. Accumulation of P-bodies in Candida albicans under different stress and filamentous growth conditions. *Fungal Genet. Biol.* **48**, 1116–1123 (2011).
3. Iserman, C. et al. Condensation of Ded1p Promotes a Translational Switch from Housekeeping to Stress Protein Production. *Cell* **181**, 818-831.e19 (2020).

624T Functional characterization of basic helix-loop-helix transcription factor family in *Cryptococcus neoformans* *Mona Pokharel*[1], Paulina Konarzewska[1], Yina Wang[1], Chaoyang Xue[1] 1) Public Health Research Institute, New Jersey Medical School, Rutgers University, Newark, NJ.

Sensing extracellular nutrient signals is critical for fungal pathogens to adapt to their hostile host environment and cause disease. Our previous studies demonstrated that myo-inositol contributes to the pathogenesis of fungal pathogen *Cryptococcus neoformans.* This yeast pathogen has developed a complex system to acquire and utilize extracellular inositol for its development and virulence. Mutants defective in inositol uptake and metabolism showed attenuated virulence. However, how *C. neoformans* regulates and coordinates the complex inositol function remains unknown. The transcription factors involved in such regulation have not been identified. In *Saccharomyces cerevisiae*, two basic helix-loop-helix (bHLH) transcription factors, Ino2 and Ino4, form a heterodimer and play a key role in regulating inositol function. In *C. neoformans,* there are nine putative bHLH transcription factors, but none of them share sequence homology with Ino2 or Ino4 beyond the bHLH domain. From screening assays, it is unclear whether any of these transcription factors are involved in phospholipid synthesis regulation as none of the single mutants showed strong phenotype related to inositol function. To test the hypothesis that there may be functional redundancy between certain bHLH proteins in *C. neoformans,* we generated double and triple deletion mutants for some of these bHLH-containing genes, and detected some additive effect based on phenotypic analysis. Interestingly, we found *HLH6 as* essential gene for fungal cell viability. Attempts to disrupt *HLH6* were unsuccessful. We hypothesized that Hlh6 is involved in regulation of phospholipid metabolism in *C. neoformans*. To study its function, we generated a strain in which the expression of *HLH6* is under the control of a copper regulated Ctr4 promoter and investigated this mutant phenotype. Transcriptional control of *HLH6* in induced and suppressed media conditions significantly changed the relative expression of selected genes involved in inositol, a precursor for phospholipid metabolism. Our functional analysis of bHLH transcription factors in *C. neoformans* provides key insight into understanding their role in complex pathway of inositol metabolism regulation.

625F Truncation of *MAT1-2-7* gene leads to changes in the pheromone response pathway of *Huntiella omanensis* *Brenda Wingfield*[1], Andrea Wilson[1], Michael Wingfield[1] 1) University of Pretoria.

Novel fungal mating-type (*MAT*) genes are being described almost as rapidly as fungal genomes are being sequenced. These genes

are often described as *MAT*genes due only to their placement within the defined *MAT*locus and unfortunately, their characterization remains somewhat lacking. This is generally because these genes are being described in non-model fungi for which tools for functional genomics are not yet available. In this study we functionally characterized the novel *MAT1-2-7*gene, which was originally described from the *MAT1-2*idiomorph of the heterothallic *Huntiella omanensis*(Ceratocystidaceae). Using a protein-based CRISPR-Cas9 protocol, the *H. omanensis MAT1-2-7*gene was truncated with a dDNA harboring an in-frame stop codon as well as a gene for hygromycin resistance. Two identical mutants were isolated and screened for their ability to undergo sexual reproduction in the presence of a suitable MAT1-1 partner. Comparative RNA-seq experiments were also performed to identify changes in overall gene expression patterns associated with *MAT1-2-7*disruption during vegetative growth and under conditions of mating. We showed that neither of the mutants was able to complete the sexual cycle, instead producing only immature proto-ascomata when co-cultured with an individual of the opposite mating type. The truncation of *MAT1-2-7*led to significant expression changes in approximately 2 000 genes, under both vegetative and mated conditions in both mutant isolates. In particular, genes associated with the pheromone response pathway, including the **a**-factor pheromone, the **α**-factor receptor, and *STE12*, the terminal transcription factor of this response pathway, were differentially expressed in the two mutant isolates compared to the wild type. Collectively, the results strongly suggest that *MAT1-2-7*is an essential mating-type gene, which encodes a transcription factor. This regulates the expression of genes involved in pheromone production, recognition and response, and subsequently, the ability to complete the sexual cycle. Future work should focus on the characterization of this gene in species with different sexual systems, including *H. moniliformis*, a unisexual species in the same genus, and other species in the Ceratocystidaceae that undergo unidirectional mating type switching.

626W Reconstructing transcriptional networks governing fungal fruiting body development *Xiao-Bin Liu*[1], Máté Virágh[1], Árpád Csernetics[1], Zhihao Hou[1], Viktória Bense[1], Manish Pareek[1], Hongli Wu[1], Zsolt Merényi[1], Botond Hegedüs[1], László Nagy[1] 1) Institute of Biochemistry, Biological Research Center, Szeged, Hungary.

Reconstructing transcriptional networks governing fungal fruiting body development
Xiao-Bin Liu1, Máté Virágh1, Árpád Csernetics1, Zhihao Hou1, Viktória Bense1, Manish Pareek1, Hongli Wu1, Zsolt Merényi1, Botond Hegedüs1, László G. Nagy1*
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Mushroom-forming fungi produce fascinating fruiting bodies which represent the most complex structures made by fungi. They play essential roles in Earth's ecosystem and also form the basis of a large industry in many countries. Thus, studying fruiting body formation has long been a research focus of mycologists. Like other complex multicellular lineages, mushroom-forming fungi make extensive use of transcription factors (TFs) during fruiting body formation. Fruiting body development starts with the reorganization of hyphal branching patterns, which gives rise to primary and secondary hyphal knots. We hypothesized that hyphal knot up-regulated TFs are key to fruiting body initiation and development. Based on a large-scale genome comparison (137 Basidiomycota species) and re-analysis of developmental transcriptome data for eight Agaricales species, we identified 40 TFs which are upregulated in hyphal knot staged and are widely conserved among Agaricomycetes. These include genes that evolved within the Agaricomycetes, but also others that are orthologous to well-known morphogenetic TFs in filamentous fungi. We knocked out 15 TF genes using CRISPR/Cas9 in the Coprinopsis cinerea AmutBmut #326 strain and performed functional characterization. We observed that the phenotypes of the mutant strains varied from the complete failure of hyphal knot formation to ones that could produce hyphal knots but were arrested at primordium 1/2 stages, among others. In the future, we will combine the RNA-seq analyses of the TF knockout strains vs. WT strain, and the DNA binding sites of the TFs determined based on DAP-seq data (collaborated with Joint Genome Institute, JGI) to infer the evolution of the transcriptional network involved in fruiting body development in mushroom-forming fungi.
Keywords: Agaricomycetes, gene editing, gene regulatory network, transcriptome, fruiting body development

627T Investigating the regulatory networks governing morphology and virulence in *Histoplasma capsulatum* *Nebat Ali*[1], Mark Voorhies[1], Anita Sil[1] 1) UCSF.

The ability to sense and effectively adapt to survive within mammalian hosts is a hallmark of clinically relevant microbial pathogens. This phenomenon is exemplified in thermally dimorphic fungi such as *Histoplasma capsulatum,* where sensing of elevated elevated host body temperature (37°C) triggers a dramatic shift in cell state that enables the pathogen to persist and disseminate within the host. In the environment, *H.c* grows in the soil as a filamentous mold that can be aerosolized and inhaled into the lungs of mammals, where host temperature triggers yeast phase growth and the expression of virulence genes. Prior studies in our lab identified an interdependent network of transcription factors (TFs) Required for yeast growth (Ryp 1,2,3,4) at 37°C. Ryp1-4 globally reprogram the transcriptome to establish yeast-phase growth while simultaneously inhibiting pathways that promote filamentation. Disruption of any of the individual Ryps through insertional mutagenesis or RNAi results in filament-locked strains that fail to induce the correct transcriptional program at 37°C. The complex network that impacts the Ryps and helps establish the two temperature states remains unknown. Furthermore, the screens used to identify Ryp1-4 were not saturated, so there are likely to be additional TFs involved in directing this response. Using a newly developed CRISPR/Cas9-based system, I will take a targeted genetic approach to identify additional TFs that may integrate with or act independently of the Ryp network to mediate thermal dimorphism. Through the use of bioinformatics, we have identified approximately 280 putative TFs in the genome of our working lab strain of *Hc,* HcG217B. Close to 10% of these TFs are yeast-phase specific, are directly regulated by at least one of the Ryp proteins, and/or physically associate with Ryp2/3. One of the TFs identified in this subset is the highly conserved and fungal specific Heat-Shock TF Skn7. In a pilot experiment, I generated Skn7 deletion mutants and observed that similar to the *ryp* mutants, *skn7Δ* strains grow constitutively as filaments. These preliminary results highlight the likelihood of additional regulators being critical in mediating this temperature-dependent developmental response. Disrupting additional candidate TFs and screening for those that affect morphology will further inform us on the dynamics of these regulatory circuits and provide valuable insight on potential mechanisms to combat *Hc* virulence and pathogenicity.

628F Transcriptional profiling of the dbcAMP response of the human pathogen Histoplasma capsulatum identifies the WOPR-family transcription factor PAC2 as a regulator of cell morphology. *Dror Assa*[1], Mark Voorhies[1], Anita Sil[1] 1) Univ. of California,

San Francisco, Dept. of Microbiology and Immunology.

Histoplasma capsulatum (*Hc*), a human pathogen that causes disease in immunocompetent individuals, is a thermally dimorphic fungus. *Hc* grows in the soil as spore-producing mold under ambient temperatures. After inhalation into the host, the fungus transitions into its parasitic budding-yeast form in response to mammalian body temperature (37°C). Similarly, yeast cells that are shifted from 37°C to room temperature halt yeast-phase growth and instead grow as hyphae (filaments). Previous studies have demonstrated that intracellular levels of cAMP rise upon transition to room temperature growth, and addition of the cAMP analog dbcAMP is able to cause filamentation even at 37°C. Here we report that the signaling mucin Msb2, which is known to be required for room temperature filamentation, is also necessary for dbcAMP-induced filamentation. To interrogate the molecular pathways that drive this filamentation response, we performed transcriptional profiling of wild-type and *msb2* mutant *Hc* in the presence of dbcAMP. We identified a subset of transcripts that are induced in the wild-type strain but fail to be induced in the *msb2* mutant. These Msb2-dependent genes include the transcription factor *PAC2*, which is a member of the WOPR family of transcriptional regulators that governs developmental decisions in many fungi. Using CRISPR-Cas9-mediated disruption, we generated a mutant that does not express functional *PAC2*. The *pac2* mutant is unable to form hyphae in response to dbcAMP, indicating that this transcription factor is required for dbcAMP-induced filamentation. Interestingly, the *pac2* mutant is able to form hyphae at room temperature, indicating that *PAC2* is dispensable for temperature-induced filamentation. Taken together, these findings highlight that there are overlapping and distinct regulators that control filamentation in response to diverse stimuli.

629W The transcription factor Roc1 is a regulator of cellulose degradation in the wood-decaying mushroom *Schizophyllum commune* *Peter Jan Vonk*[1], Ioana M. Marian[1], Ivan D. Valdes[1], Kerrie Barry[1], Benedict Bostock[1], Akiko Carver[2], Chris Daum[2], Harry Lerner[1], Anna Lipzen[2], Hongjae Park[2,3], Margo B.P. Schuller[1], Martin Tegelaar[1], Andrew Tritt[2], Jeremy Schmutz[2,4], Jane Grimwood[2,4], Luis G. Lugones[1], In-Geol Choi[2,3], Han A.B. Wösten[1], Igor V. Grigoriev[2,5], Robin A. Ohm[1,2] 1) Microbiology, Utrecht University, Utrecht, The Netherlands; 2) U.S. department of Energy Joint Genome Institute, Berkeley, CA, USA; 3) Department of Biotechnology, College of Life Sciences and Biotechnology and Graduate School, Korea University, Seoul, South Korea; 4) HudsonAlpha Institute for Biotechnology, Huntsville, AL, USA; 5) Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA.

Wood-decaying fungi of the class Agaricomycetes (phylum Basidiomycota) are saprotrophs that break down lignocellulose and play an important role in the nutrient recycling. They secrete a wide range of extracellular plant cell wall degrading enzymes that break down cellulose, hemicellulose and lignin, the main building blocks of plant biomass. Although the production of these enzymes is regulated mainly at the transcriptional level, no activating regulators have been identified in any wood-decaying fungus in the class Agaricomycetes. We studied the regulation of cellulase expression in the wood-decaying fungus *Schizophyllum commune*. Comparative genomics and transcriptomics on two wild isolates revealed a Zn2Cys6-type transcription factor gene (*roc1*) that was highly up-regulated during growth on cellulose, when compared to glucose. It is only conserved in the class Agaricomycetes. A *roc1* knockout strain showed an inability to grow on medium with cellulose as sole carbon source, and growth on cellobiose and xylan (other components of wood) was inhibited. Growth on non-wood-related carbon sources was not inhibited. Cellulase activity was reduced in the growth medium of the *Δroc1* strain. ChIP-Seq identified 1474 binding sites of the Roc1 transcription factor. Promoters of genes involved in lignocellulose degradation were enriched with these binding sites, especially those of LPMO (lytic polysaccharide monooxygenase) CAZymes, indicating that Roc1 directly regulates these genes. A GC-rich motif was identified as the binding site of Roc1, which was confirmed by a functional promoter analysis. Together, Roc1 is a key regulator of cellulose degradation and the first identified in wood decaying fungi in the phylum Basidiomycota.

630T Genome wide insights into signal integration by the G-protein pathway for regulation of carbon- and secondary metabolism *Miriam Schalamun*[1], Wolfgang Hinterdobler[1], Tiziano Benocci[1], Nicole Wanko[1], Johann Schinnerl[3], Monika Schmoll[1,2] 1) Austrian Institute of Technology GmbH, Department Health and Bioresources, Konrad Lorenz Strasse 24, 3430 Tulln, Austria; 2) University of Vienna, Department of Microbiology and Ecosystem Science, Division of Terrestrial Ecosystem Research, Djerassiplatz 1, 1030 Vienna, Austria; 3) Department of Botany and Biodiversity Research, University of Vienna, Vienna, Austria.

Nutrient sensing is of utmost importance for gene regulation in fungi, with the heterotrimeric G-protein pathway as prototypical transmission machinery. Previously we could show an interrelationship between light response and cellulase gene regulation in the filamentous fungus *Trichoderma reesei*. Recently we found that also secondary metabolism is regulated in a light dependent manner in *T. reesei*. In both cases, G-protein coupled receptors (GPCRs) exemplified these connections: the glucose sensors CSG1 and CSG2, which are responsible for posttranscriptional regulation of cellulase expression as well as GPR8, a GPCR associated with the sorbicillin cluster. To gain further insight into the balance between carbon- and secondary metabolism along with its dependence on light, we performed functional, transcriptome analyses and network analysis. We used deletion mutants Δ*gna1*, Δ*gna2* and Δ*gna3,* Δ*gnb1* and Δ*gng1* as well as strains expressing constitutively activated versions of the G-alpha proteins, GNA1QL, GNA2QL and GNA3QL in *T. reesei* QM6a. We found characteristic alterations in biomass formation, enzyme production and growth as well as an influence on sexual and asexual development. Cellulase gene transcription was differentially regulated between the investigated G-protein mutant strains with important differences between light and darkness. Analysis of secondary metabolite production revealed an impact on regulation of several compounds hence substantiating the link between cellulase regulation and secondary metabolism.
We conclude that the G-protein pathway integrates signals relevant for carbon- and secondary metabolism to optimally balance enzyme biosynthesis and growth with metabolite production.

631F Genetic and epigenetic variants underpinning within-species transcriptional polymorphism in a major fungal pathogen *Leen Abraham*[1], Ursula Oggenfuss[1], Daniel Croll[1] 1) Laboratory of Evolutionary Genetics, Institute of Biology, University of Neuchâtel, Neuchâtel.

In agricultural ecosystems, outbreaks of diseases are frequent and pose a significant threat to food security. A successful fungal pathogen undergoes a complex and well-timed sequence of regulatory changes to avoid detection by the host immune system, hence well-tuned gene regulation is essential for survival. However, how regulatory adaptation enables pathogens to overcome host resistance and cause damage is poorly understood. Here, we used *Zymoseptoria tritici*, one of the most important pathogens of wheat, to generate a

genome-wide map of genetic and epigenetic regulatory polymorphism governing gene expression. For this, we performed expression quantitative trait loci (eQTL) mapping on 146 con-specific strains. We identified cis-eQTLs for 65.3% of all genes and the majority of all eQTL were within 2kb of the transcription start site. Core genes were more likely to segregate eQTLs compared to accessory genes. We also found that insertion-deletion polymorphisms are more likely to act as a cis-eQTL and had a higher effect size than SNPs. Next, we contrasted the amount of cis-eQTL mapped across categories of pathogenicity-related genes. Effector genes were less likely to present cis-eQTLs compared to other genes including genes encoding CAZymes. This suggests that regulatory variation in effector genes is governed rather by epigenetic factors than by genetic polymorphism. This is consistent with pathogenicity genes tending to overlap regions of heterochromatin compared to other gene categories. To better understand epigenetic variation in the genome, we analyzed the transcriptional activity of individual copies of transposable elements (TEs) across isolates. We found 23 TE insertion loci with regulatory variation explained by cis-eQTLs. Furthermore, TE insertion polymorphism was associated with variation in pathogenicity traits among isolates. Our study establishes the first genome-wide map of genetic and epigenetic variation underpinning transcriptional plasticity and trait variation in a fungal pathogen. The extensive regulatory polymorphism is likely to fuel rapid adaptation to resistant hosts and environmental changes.

632W GWAS for investigating laccase expression in *Ceratocystis albifundus* Vinolia Danki[1,2], Emma Steenkamp[1], Lieschen De Vos[1], Benedicta Swalarsk-Parry[1], Farai Muchadeyi[2], Nokuthula Mchunu[2], Åke Olsen[3], Brenda Wingfield[1], *Magriet van der Nest*[1,2] 1) Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa; 2) Biotechnology Platform, Agricultural Research Council, Onderstepoort Campus, Pretoria, South Africa; 3) Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Ceratocystis albifundus is an economically important pathogen of non-native *Acacia mearnsii* in South Africa. Phenotypic analyses of a *C. albifundus* population using alpha-naphthol staining showed that laccase production represents a quantitative trait in this fungus. Although the molecular basis of this trait is not well-understood in *C. albifundus*, laccases are known to catalyse a wide range of substrates from phenols to non-phenolic compounds and are known to influence the outcome of plant-pathogen interactions. Given the value of genome wide association studies (GWAS) for identifying genes associated with important phenotypic traits (e.g., growth and virulence) in fungal pathogens, we used this tool to find genes or genomic regions associated with laccase production in *C. albifundus*. For this purpose, a set of *C. albifundus* isolates, originating from a wide geographic range, was subjected to low-coverage genome sequencing using Ion Torrent sequencing technology. Correlation between the single nucleotide polymorphism data for the isolates and their corresponding phenotype information allowed identification of collection of genomic regions that were significantly (P-value < 0.05) associated with laccase production. For example, one of the regions contained a gene that codes for a protein in the multicopper oxidase superfamily, of which laccase is a member. Given thatlaccase production is often correlated with fitness traits in fungal pathogens, our future research will thus use GWAS to further explore the possible link between laccase production and virulence in *C. albifundus*, with the ultimate goal, of improving our knowledge regarding pathogenesis in this economically important fungus.

633T Overexpression of *BnNAC19* in *Brassica napus* enhances resistance to *Leptosphaeria maculans*, the blackleg pathogen of canola *Zhongwei Zou*[1], Dilantha Fernando[1] 1) University of Manitoba, Winnipeg, Manitoba, Canada.

Leptosphaeria maculans is a fungal pathogen that causes blackleg disease in canola (*Brassica napus*), resulting in significant yield and economic losses in Canada and many parts of the world. Plant *NAC (NAM:* no apical meristem*, ATAF1/2: Arabidopsis thaliana* transcription activation factor*, CUC2:* cup-shaped cotyledon*)* transcription factors play critical roles in plant development and response to biotic or abiotic stress. In this study, we identified and characterized a *BnNAC19* gene from *Brassica napus*. The overexpression of *BnNAC19* contributed to the improvement of seedling resistance in transgenic canola plants against *L. maculans* infection. The growth and production of *L. maculans* pycnidiospores and mycelia were shown to be inhibited by the GFP-tagged strain of *L. maculans* in the overexpression of *BnNAC19* transgenic canola plants. In addition, the *BnNAC19* overexpression in the canola transgenic line showed increased disease resistance in the adult plant, which was determined by the quantitative resistance. Both increased seedling and adult plant disease resistance in overexpressed *BnNAC19* canola transgenic plants indicates that the *BnNAC19* gene plays a positive effect against *L. maculans* infection. The expression pattern of *BnNAC19* downstream genes, which participate in plant defense pathways, will be investigated to elucidate the *B. napus* resistance mechanisms to *L. maculans* infection, hence for the long-term blackleg disease-resistant breeding programs.

634F Relevance of copper homeostasis in *Fusarium oxysporum* pathogenicity *Rafael Palos Fernández*[1], Antonio Di Pietro[1], Manuel Sánchez López-Berges[1] 1) Departamento de Genética, Facultad de Ciencias, Universidad de Córdoba, Campus de Excelencia Agroalimentario (ceiA3), Córdoba, E-14071, Spain..

The soil-borne fungus *Fusarium oxysporum* causes vascular wilt disease on more than one hundred plant species and opportunistic infections in humans, making this species an excellent model for the study the genetic basis of fungal pathogenicity in hosts of different kingdoms.
Copper is an essential micronutrient for all living organisms that acts as a cofactor for enzymes that are involved in crucial processes such as cellular respiration (cytochrome *c* oxidase) and free radical detoxification (superoxide dismutase). On the other hand, its excess is enormously toxic to cells, so its intracellular concentration must be strictly regulated and remain in what is called the homeostatic window.
Here we have characterized MacA, a key regulator of copper homeostasis that has been described to orchestrate the transcriptional response to copper limiting conditions in fungi. Predictably, under copper limitation, targeted deletion of *macA* (*macA*Δ) in *F. oxysporum* caused deactivation of genes required for copper acquisition, resulting in the inability to grow both on solid and liquid cultures. Importantly, MacA has been described as a virulence factor in human pathogens such as *Candida albicans* and *Aspergillus fumigatus* but, surprisingly, its role in plant pathogenesis is poorly understood. Our infection assays show that MacA is essential for virulence of *F. oxysporum* on tomato plants. Quantification of fungal burden 3- and 7-days post inoculation indicates that *macA*Δ does not efficiently colonize the plants. Interestingly, the expression of *ctr* genes, encoding copper membrane high affinity transporters, is low during plant infection, suggesting that the role of MacA in virulence is not linked to copper limitation. Further supporting this hypothesis,

plants infected with *macA*Δ displayed similar mortality rates regardless of the presence or absence of copper in the irrigation water. RNA-seq analysis show that several genes that are induced in the wild-type during plant infection (and not in absence of cooper) are downregulated in *macA*Δ, which could explain the virulence phenotype of this mutant.

635W Exclusively RNAi-based antimicrobial drug resistance is inherited after meiosis in the mucormycosis pathogen *Mucor circinelloides* *Carlos Pérez-Arques*[1], María Isabel Navarro-Mendoza[1], Joseph Heitman[1] 1) Department of Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, NC, USA.

Heritable, epigenetic modifications that alter gene expression are a widespread phenomenon in eukaryotic organisms. These are known as epimutations and may arise from RNAi, DNA methylation, and/or heterochromatin modifications, often resulting in gene silencing. Recently, epimutations were identified as a novel mechanism conferring antimicrobial drug resistance, one of the gravest threats to public health. Epimutations were discovered for the first time in two species of the early-diverging fungus *Mucor* and result in small RNA (sRNA) silencing of the gene *fkbA* encoding the FK506 target FKBP12. This silencing results in transient, unstable drug resistance that reverts after several mitotic growth cycles in the absence of FK506. We have discovered that epimutations conferring drug resistance in *Mucor* species are exclusively RNAi-based and post-transcriptional, as demonstrated by the absence of cytosine DNA methylation (5mC) or histone H3 lysine 9 (H3K9) di- or trimethylation but the presence of RNA polymerase II and complementary antisense sRNAs directed against epimutationally-silenced loci.
Mucor epimutations are sufficiently stable to be trans-generationally inherited following sexual reproduction and meiosis, despite lacking heterochromatin marks frequently associated with epigenetic inheritance. We have identified new, RNAi-based epimutants in the pathogenic *Mucor circinelloides* phylogenetic species 15 (PS15) that is able to undergo a complete sexual cycle including the production of viable meiotic progeny after zygospore germination. Epimutations were found to be inherited stochastically in the progeny of the *fkbA*-epimutant and an opposite mating-type wildtype parent, in contrast to the mendelian ratio (1:1) observed in the progeny from an *fkbA*- mutant and wildtype cross. Similar to the FK506-resistant epimutant parental isolate, the epimutant progeny are resistant to FK506 and harbor antisense sRNAs targeting *fkbA*, and following passage in the absence of drug both FK506-resistance and sRNAs targeting *fkbA* of the epimutant progeny were lost. Our findings demonstrate that epimutations are broadly present across the *Mucor* species complex and act exclusively through post-transcriptional gene silencing to control gene expression. Although epimutations are stable through both mitosis and meiosis, their detection may pose a challenge to typical culture methods employed in clinical diagnostics given that these involve growth in the absence of drug selective pressure. Understanding how epimutations arise and the mechanisms via which they confer resistance may enable their detection in clinical settings and provide solutions to combating the challenge of antimicrobial drug resistance.

636T *Identification and deep analysis of the target genes of an RNAi mechanism involved in virulence of Mucor lusitanicus.* *Ghizlane Tahiri*[1], Francisco Esteban Nicolás Molina[1], Eusebio Navarro Ros[1], Victoriano Garre Mula[1] 1) Murcia University ESQ3018001B.

Mucor lusitanicus is a human pathogen that has been extensively used as a model organism of the infection known as mucormycosis. In this fungus, an RNA degradation mechanism, called as Non-canonical RNA interference pathway (NCRIP) because it uses the atypical ribonuclease III R3B2, operates controlling multiple physiological processes from genome integrity to virulence. Here in this work, we have discovered and described the direct targets of the NCRIP by sequencing and analysing small RNAs and messenger RNAs from a wild-type and an *r3b2* mutant strains in saprophytic conditions and in early interaction with macrophages. Most of the identified direct target genes are transposable elements, including LTR transposons. For the first time, we have described the presence of this kind of transposons in *Mucor* genome. We used direct targets as reporters to determine the regulation of NCRIP using different stresses found in the phagosome, establishing iron restriction causes an activation of the RNAi pathway. In addition, we have characterized the complex gene network controlled by the early phagocytosis and/ or by the NCRIP. Our results indicate that both in non-stressful conditions and during the early phagocytosis of macrophages, this RNAi pathway controls mainly metabolic processes, which makes sense since this mechanism is involved in virulence and because metabolic processes play a pivotal role during infection. Additionally, we have studied the expression of the direct targets by analyzing the bioluminescence emission of strains containing the fusion of the mentioned genes to the luciferase *locus*, confirming that NCRIP regulates gene expression by degrading the mRNA of target genes and establishing a new method for studying gene expression in Mucor. The causative agents of mucormycosis are highly resistant to common antifungals. Consequently, the discovery of the direct targets of this RNA degradation mechanism, which is only conserved in Mucorales, could contribute to the development of specific drugs to attack the pathogen.

637F The development of siRNA-mediated mRNA knockdown in *Batrachochytrium dendrobatidis* *Rebecca Webb*[1], Lee Berger[2], Catherine Rush[1], Lee Skerratt[2], Alexandra Roberts[1] 1) James Cook University, Queensland, Australia; 2) University of Melbourne, Victoria, Australia.

The amphibian chytrid fungus, *Batrachochytrium dendrobatidis,* is the cause of amphibian chytridiomycosis, one of the worst wildlife diseases in recorded history. It has spread globally since escaping from Asia in the early 20th century, resulting in the decline or extinction of at least 500 amphibian species so far. Thus, there is a dire need to develop strategies to mitigate this pathogen and prevent further loss of biodiversity. The development of functional genomic techniques for *B. dendrobatidis* would be beneficial, not only to help understand what makes this pathogen so virulent, but also as a targeted tool to reduce virulence. RNA interference (RNAi) is a functional genomic technique in which short interfering RNA (siRNA) is used to target a gene of interest, resulting in transient knockdown of target mRNA. In this study we investigated whether siRNA could be used to manipulate gene expression in *B. dendrobatidis.* We designed two siRNA with different modifications to target gamma-glutamylcysteine synthetase, the first rate-limiting step in glutathione synthesis. The siRNA was delivered to zoospores by electroporation, and mRNA levels monitored for 48 h. Both types of siRNA triggered a significant reduction (~50%) in target transcripts, with a maximal knock-down at 36-42 h. Our results show for the first time that RNAi is possible in *B. dendrobatidis,* and gene expression can be manipulated in this pathogen. However, knockdown of mRNA did not produce robust phenotypic changes, highlighting the need for further optimisation of siRNA delivery and target gene selection.

638W Arginine Methylation of RNA Binding Proteins in Cryptococcal Virulence and Antifungal Resistance *Murat Can Kalem*[1], Harini Subbiah[1], Jan Nasser Kaur[1], Shichen Shen[1], Jun Qu[1], John C. Panepinto[1] 1) SUNY University at Buffalo.

Fine-tuning of complex molecular processes is necessary for pathogenic microbes to respond to dynamic environmental changes during infection. Post-translational modifications (PTMs) are fast and reversible, and can activate or suppress function while catalyzing transitions between multiple functional states. While the role of phosphorylation is more commonly studied, we know very little about the role of arginine methylation in fungal pathogenesis. Arginine methylation (R methylation) is a common modification of RNA-binding proteins (RBPs) and is catalyzed by R methyltransferases (RMTs). The methylation status of RBPs alters their RNA binding ability, leading to changes in the RNA metabolism.

Cryptococcus neoformans encodes 5 putative RMTs identified by sequence homology. Functional characterization of the human PRMT5 ortholog Rmt5 revealed that it is important for fungal cell morphology, thermotolerance, cell wall remodeling, growth under high CO_2, and hypoxia. Interactome analysis of Rmt5, determined by proximity-dependent biotinylation – TurboID, revealed interactions with many RBPs and translation factors. One of the Rmt5 interactome proteins was the pumilio/FBF domain containing RBP, Puf4. Our recent work highlighted the role of Puf4 in posttranscriptional regulation of caspofungin resistance. Puf4 binds to cell wall biosynthesis mRNAs including *FKS1*, leading to increased stability and reduced translation. Puf4 deletion causes enhanced resistance to caspofungin mainly through posttranscriptionally modulated cell wall remodeling. The *puf4Δ* is also resistant to tunicamycin-induced ER stress. Since Puf4 interacts with Rmt5, we investigated the PTMs of Puf4. We identified methylation and phosphorylation sites using IP/MS. To investigate the functional roles of methylated R residues, we created R to K methyl-deficient mutants of 2 sites: a) within the Pumilio domain b) within the RG-rich domain adjacent to the Pumilio domain. We complemented the *puf4Δ* using mutant constructs. Investigation of Puf4-related phenotypes revelated that R to K mutant of the RG-rich domain restores the tunicamycin resistance phenotype while the mutant carrying a mutation within the Pumilio domain does not. Interestingly, both R to K mutants did not restore the caspofungin resistance phenotype.

Our current efforts include the identification of methylation-dependent Puf4 localization and RNA binding using microscopy and RIP-seq. Overall, we propose a model in which Puf4 exists in multiple functional states based on unique combinations of the PTMs it carries. Post-transcriptional regulation of key fungal processes reveals nuances that are often neglected, yet may reveal novel antifungal targets.

639T The multi-KH domain RNA binding protein Khd4 orchestrates membrane trafficking to promote polar growth of infectious hyphae *Srimeenakshi Sankaranarayanan*[1], Carl Haag[1], Michael Feldbrügge[1] 1) Institute of Microbiology, Heinrich Heine University of Duesseldorf.

The virulence of many fungal pathogens is tightly linked to the formation of polarised hyphae. In maize smut fungus *Ustilago maydis*, loss of the multi-KH domain RNA binding protein *khd4* causes a profound defect in hyphal polar growth and pathogenicity. RNA binding proteins (RBP) play a key role in achieving the precise control of protein expression in a spatiotemporal manner. Here we demonstrate that Khd4 regulates the stability of a distinct set of mRNAs to orchestrate the membrane trafficking process during polar growth of infectious hyphae.
We successfully used RNA-editing based hyperTRIBE technique in *U. maydis* to identify *in vivo* mRNA targets of Khd4. Khd4 binds to a specific set of mRNAs enriched for GTP binding proteins and mRNAs associated with the membrane trafficking process. Upon *khd4* deletion, nearly 40% of the target mRNAs show differential abundance with the most being upregulated. These upregulated mRNAs display high enrichment of Khd4 binding site AUACCC at the 3´UTR. Subsequent examination of Khd4 targets such as small GTPases *arl1*, endosomal cargo adapter *hok1*, and the vacuolar ATPase assembly factor *vma21*, revealed that the target mRNA expression is strongly reduced when Khd4 binds to AUACCC binding motif present in the 3´UTR. Interestingly, hyphal specific transcriptomics analysis indicated that in comparison to wild-type hyphal cells, the vesicular transport pathway is differentially regulated in *khd4Δ* hyphae. Indeed, loss of *khd4* causes adverse effects on endocytosis and vacuolar biogenesis in cells. The aberrant vacuoles were minuscule, found scattered throughout the hyphae and importantly, missorted to the cell cortex upon *khd4* deletion. Thus, the RBP Khd4 is important for post-transcriptional regulation of mRNA, failure of which impairs the endomembrane system and cause defective vacuolar biogenesis.
Hence, our study illustrates that the membrane trafficking mediated vacuolar biogenesis is regulated at the post-transcriptional level and it is necessary for the polar growth during fungal infection.

640F An RNA-binding protein that evolved a change in function to control fungal growth: the surprising history, structure, and function of Ssd1 *Edward W. J. Wallace*[1], Rosemary A, Bayne[1], Elizabeth R. Ballou[2], Uma Jayachandran[1], Aleksandra Kasprowicz[1], Stefan Bresson[1], David Tollervey[1], Atlanta G. Cook[1] 1) The University of Edinburgh; 2) The University of Exeter.

Regulatory pathways evolve to enable organisms to adapt to their environment. In ascomycete fungi, homologous Ssd1/Sts5/gul-1 RNA-binding proteins regulate translation and affect cell growth, cytokinesis, and fungal pathogenicity. The domain structure of Ssd1 resembles that of proteins with a different function: the RNase II/Dis3 family of 3′-5′ exoribonucleases, which play essential roles in RNA degradation. Ssd1 itself has no nuclease activity, making it a “pseudonuclease”. However, the evolutionary origins of Ssd1-like pseudonucleases are unknown: what sequence of evolutionary and structural events led to their novel function, and when did these events occur?

Here, we show how Ssd1-like pseudonucleases are descended from active enzymes in the Dis3L2 subfamily. During fungal evolution, active site mutations in Dis3L2 homologs have arisen at least four times, in some cases following gene duplication. Our new crystal structure of Ssd1 shows that the ancestral RNA-binding “funnel” leading to the active site is blocked by loop insertions, implying emergence of a novel RNA-binding site. In contrast, N-terminal cold-shock domains and regulatory features are conserved across diverse dikarya and mucoromycota. We map the RNA-binding sites of Ssd1 by UV crosslinking and high-throughput sequencing. Our finding that Ssd1 binds near start codons at 5’ ends of mRNA emphasises a different mode of RNA binding from the 3’ terminal interaction

reported for Dis3L2. We map the novel RNA-binding site to the cold-shock domains of Ssd1 by showing that mutations to a conserved surface reduce RNA binding *in vitro* and cause cell wall stress sensitivity *in vivo*.

We also show that in the basidiomycete pathogenic yeast Cryptococcus neoformans, the single Ssd1/Dis3L2 homolog is required for cell separation from polyploid "titan" growth stages. This phenotype is consistent with those of inactive fungal pseudonucleases, yet the protein retains an active site sequence signature. We propose that a nuclease-independent function for Dis3L2 arose in an ancestral hyphae-forming fungus, involving RNA-binding on the surface of the cold shock domains. Our work more generally indicates the power of fungal genetics for studying the evolution of proteins and their regulatory functions.

641W A-to-I mRNA editing is catalyzed by FgTad2 and FgTad3 ADAT in *Fusarium graminearum* *Zhuyun Bian*[1], Kaiyun Xin[2], Jingwen Zou[2], Chanjing Feng[2], Chaohui Li[2], Huiquan Liu[2], Jin-Rong Xu[1] 1) Purdue University, West Lafayette, IN; 2) Northwest A&F University, Yangling, Shaanxi, China.

Adenosine-to-inosine (A-to-I) editing of mRNAs in animals is catalyzed by adenosine deaminase acting on RNA (ADAR) enzymes. Filamentous ascomycetes lack ADAR homologs but have genome-wide editing events specifically during sexual reproduction. To characterize the underlying molecular mechanisms, we functionally characterized all the adenosine/cytidine deaminase genes in *Fusarium graminearum* and found that all but two essential genes orthologous to yeast *TAD2* and *TAD3* ADAT (adenosine deaminase acting on tRNA) are dispensable for mRNA editing. FgTad2 and FgTad2 interacted with each other in vivo and their expression levels were significantly increased during sexual reproduction. Like yeast Tad3, FgTad3 may lack deaminase activities due to the E-to-V change in the catalytic core only function as a facilitator of FgTad2 in RNA editing. Interestingly, both *FgTAD2* and *FgTAD3* had two transcript isoforms and the short isoforms had stage-specific expression and were the major forms during sexual reproduction. We then used the RIP (repeat-induced point nutation) approach to generate ascospore progeny that were normal in vegetative growth but defective in sexual reproduction. Selected missense and nonsense RIP mutations in *FgTAD2* and *FgTAD3* were then confirmed by site-directed mutagenesis for their specific effects on ascosporogenesis and mRNA editing. Furthermore, we developed an in vitro assay with FgTad2-His protein affinity-purified from perithecia and showed its enzymatic deaminase activity on in vitro synthesized mRNA substrates. Under the same conditions, FgTad2-His protein purified from vegetative hyphae of the same strain had no A-to-I editing activities with our mRNA substrates. These genetic and biochemical data showed that FgTad2 is responsible for A-to-I mRNA editing in *F. graminearum*, likely by forming a heterodimer with FgTad3 and involving stage-specific cofactors expressed in perithecia.

642T The role of R3B2 in the RNAi mechanisms of *Mucor lusitanicus* is driven by both double-stranded RNA binding domains *José Tomás Cánovas-Márquez*[1], Ghizlane Tahiri[1], Pablo Carrillo-Marín[1], Eusebio Navarro[1], Ulrike Binder[2], Álvaro Sánchez-Ferrer[3], Victoriano Garre[1] 1) Department of Genetics and Microbiology (Associated Unit to IQFR-CSIC), Faculty of Biology, University of Murcia, Murcia, Spain; 2) Institute of Hygiene & Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria; 3) Department of Biochemistry and Molecular Biology-A, Faculty of Biology, University of Murcia, Murcia, Spain.

The filamentous fungus *Mucor lusitanicus* exhibit a complex RNAi network that modulate the accumulation of mRNAs. Among the different RNAi mechanisms, the canonical pathway, including the epimutational pathway conferring transient resistant to antifungal drugs, is negatively regulated by the non-canonical RNAi pathway or NCRIP. In the NCRIP, the role of Dicer is replaced by R3B2, an unusual RNase with one RNase III domain and two double-stranded RNA-binding domains (dsRBDs). Our recently published results show that both dsRBDs are necessary for RNA binding, but neither for the RNase activity of R3B2. Here, we reported that these dsRBDs interact with the dicer proteins of the canonical RNAi machinery, suggesting that the link between the RNAi mechanisms of the fungus could rely on these dsRBDs. To delve into the crosstalk of the canonical RNAi and the NCRIP, we constructed strains of *M. lusitanicus* carrying alleles of *r3b2* with mutations in the dsRBDs. The ability of these strains to trigger the canonical RNAi mechanism and the NCRIP was analyzed. The strains that express a truncated form of R3B2, where the second dsRBD was absent, or an allele with a deletion in the first dsRBD, were unable to complement the RNAi mechanisms. Conversely, the allele with point mutations in the second dsRBD, whose activity was abolished *in vitro*, was able to complement the wild-type allele. Thus, these results indicate that both dsRBDs of R3B2 are necessary for the RNAi mechanisms of *M. lusitanicus*. Based on the structure prediction of Dicer and R3B2 proteins, and their molecular docking, we dissected the interaction between these proteins by using yeast two-hybrids analysis. The results obtained indicated two different types of interactions between R3B2 and Dicer-1 or Dicer-2 from *M. lusitanicus* regarding the dsRBDs of the first involved. While the interaction with Dicer-1 occurs through both dsRBDs of R3B2 positioned in opposite parts of Dicer, which suppose a possible steric hindrance to its RNase catalytic center, the interaction with Dicer-2 is produced by only one of the dsRBDs of R3B2. Considering the antagonistic functions of the canonical pathway and NCRIP and their physical interactions through their key RNases, is tempting to speculate a regulatory function of R3B2 that solves most of the complex regulation network present in *M. lusitanicus*.

643F MERLIN unlocks the secrets to chitin signaling: Using gene-network inference to predict mediators of fungal response to lipo-chitooligosaccharides *Cristobal Carrera Carriel*[1], Spencer Halberg-Spencer[5], Saptarshi Pyne[5,6], Jean-Michel Ané[3,4], Nancy P. Keller[2,3], Sushmita Roy[5,6] 1) Department of Genetics, University of Wisconsin, Madison, WI, USA; 2) Department of Medical Microbiology and Immunology, University of Wisconsin, Madison,WI, USA; 3) Department of Bacteriology, University of Wisconsin, Madison, WI, USA; 4) Department of Agronomy, University of Wisconsin, Madison, WI, USA; 5) Biostatistics and Medical Informatics, Madison, WI, USA; 6) Wisconsin Institute for Discovery, Madison, WI, USA.

Chitin is a naturally occurring polymer composed of *N*-acetylglucosamine and is synthesized by many organisms, including fungi. Although chitin is mainly considered a structural component, derivatives can also serve as signaling molecules. A 2020 study found that all fungi produce a lipid-containing derivative of chitin called a lipo-chitooligosaccharide (LCO), and that treatment of the filamentous fungus *Aspergillus fumigatus* with LCOs increases germination and reduces hyphal branching. To investigate the gene networks important for LCO response, we used the MERLIN algorithm to infer regulatory gene networks for *A. fumigatus* using publicly available RNA-seq datasets. MERLIN implicated transcription factor AtfA as an important regulator of many genes that were differentially regulated in response to LCOs response, and we hypothesized that *atfA* is important for mediating *Aspergillus*' phenotypic response to LCOs.

Studies of *aftA* deletion and overexpression mutants reveal that *atfA* is required for *A. fumigatus* germination and hypo-branching responses to LCOs. Our work here is the first to uncover, using gene-network predictions, a transcription factor responsible for a fungus regulatory response to LCOs. Future work will investigate if LCO perception and response occurs through the high-osmolarity glycerol (HOG) pathway as further predicted by MERLIN.

644W Ccr4 and Gcn2 contribute differentially to stress-specific translational repression in *C. neoformans* *Corey Knowles*[1], John Panepinto[1] 1) Department of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY, USA.

Cryptococcus neoformans is a ubiquitous environmental fungus and opportunistic human pathogen, primarily impacting immuno-compromised hosts such as those living with HIV/AIDS. One of *C. neoformans* key virulence traits is its ability to undergo the rapid transition from surviving in its environmental niche, to surviving the harsh environment inside of the human lung. Here, it is subject to the sudden temperature shift to the human core temperature of 37°C, and oxidative stress from resident lung macrophages, among other stressors. In a wild type (WT) strain, exposure to these stressors results in ribosome collision accompanied by a repression in translation, and rapid decay of abundant homeostatic mRNAs, many of which code for ribosomal proteins. This response results in a translatome reprogramming that promotes translation of mRNAs encoding stress response effectors. Our previous work has identified Ccr4-dependent mRNA decay to be a requirement for translatome reprogramming in response to stress in *C. neoformans,* and as a result, a *ccr4*Δ mutant is broadly stress sensitive. This observation has led us to investigate additional paths to translational repression, testing the hypothesis that stress responsive translatome reprogramming will require regulation of translation at the ribosome. Gcn2, the sole kinase of eIF2α in *C. neoformans*, is required for translational repression and subsequent RP mRNA decay during oxidative stress from H_2O_2, but is completely dispensable for adaptation to host temperature stress of 37°C. Additionally, a *gcn2*Δ mutant is sensitive to oxidative stress, and exhibits persistent disome accumulation. Interestingly, the defect in temperature-induced translational repression in a *ccr4*Δ mutant is rescued in minimal medium by robust phosphorylation of eIF2α. This rescue is dependent on Gcn2, and is abrogated in a *ccr4*Δ*gcn2*Δ mutant. These results point to deadenylation-dependent decay as a convergence point for translatome reprogramming in *C. neoformans*, and suggest that individual stressors and their magnitude contribute to the translational response to stress in this important pathogen through different ribosome associated pathways. Future work will determine which components of the ribosome quality control machinery are necessary for recognizing and resolving these changes in translation in response to environmental stressors relevant to host adaptation.

645T White-opaque switching in *Candida albicans* as a model system for the quantitative and molecular analysis of stochastic cell fate switching *Naomi Ziv*[1], Lucas Brenes[1], Alexander Johnson[1] 1) University of California San Francisco, San Francisco, CA.

Cellular identity is not static; gene expression programs change in response to external and internal stimuli. Many transcriptional networks can generate distinct transcriptional states and stably maintain these states across cell divisions. Switching between states can be stochastic, occurring in a small subset of cells of an isogenic population in a seemingly homogenous environment. Given the scarcity and unpredictability of switching in these cases, investigating the determining molecular events is challenging. Moreover, in eukaryotes, regulatory networks are large and complex, typically containing many transcription factors forming multiple interconnected feedback loops. The fungal species *Candida albicans* undergoes a stochastic epigenetic switch between two distinct types of cells, referred to as white and opaque. We use microfluidics combined with fluorescent reporters to directly observe rare switching events between the white and opaque states. We investigate the stochastic nature of switching by beginning with white cells and monitoring the activation of Wor1, a master regulator and marker for the opaque state, in single cells and throughout cell pedigrees. Our results indicate that switching requires two steps; first an event occurs that predisposes a lineage of cells to switch. In the second step, some but not all, of those predisposed cells rapidly express high levels of Wor1 and commit to the opaque state. Using a synthetic inducible system in *Saccharomyces cerevisiae*, we investigate the function of white-opaque regulators and characterize specific regulatory interactions, such as Wor1 autoregulation. Taken together, our results suggest that reaching a threshold level of a master regulator is sufficient to drive cell type switching in single cells and that an earlier molecular event increases the probability of reaching that threshold in certain small lineages of cells. Quantitative molecular analysis of the white-opaque circuit can serve as a model for the general understanding of complex circuits.

646F Identifying global regulators of effector gene expression in the rice blast fungus *Magnaporthe oryzae* *Camilla Molinari*[1], Bozeng Tang[1], Xia Yan[1], Matthew J. Moscou[1], Nicholas J. Talbot[1] 1) The Sainsbury Laboratory.

Global rice production is threatened by rice blast a devastating disease caused by the filamentous fungus *Magnaporthe oryzae*. To invade its host the fungus secretes a battery of effectors which are differentially expressed during infection with distinct functions and sub-cellular targets. Little is known, however, regarding the specific mechanisms by which *M. oryzae* regulates effector gene expression. Typically, effector-encoding genes are not expressed when the fungus is not growing in a plant and show enhanced expression during host infection. Based on this simple concept, we have designed a series of forward genetic screens to discover novel transcriptional regulators that control effector expression in the rice blast fungus. We have selected representative effectors that are expressed at different *M. oryzae* infection stages– *SLP1, AVR-PIK, AVR-PITA, MEP4* and *BAS1*. Then we constructed promoter fusions of each effector to the hygromycin phosphotransferase gene and expressed these in *M. oryzae.* Later we selected hygromycin resistant mutants, which must result from a mutation that leads to constitutive effector gene expression. Using a combination of bioinformatic analysis and gene mapping experiments we are identifying the corresponding regulators. Previously, using a similar screen we identified the G-protein Rgs1 as a transcriptional repressor of effector gene expression in *M. oryzae*. Result which validates this approach as a means of identifying novel transcriptional regulators in the rice blast fungus.

647V Elucidating the composition and functions of the Remodels the Structure of Chromatin (RSC) complex in *Candida albicans* *Santanu Ghosh*[1] 1) Indian Institute of Technology Bombay.

Morphogenic transitions, genome plasticity, and the ability to form biofilm are the strong suits for *Candida albicans*, one of the most

prevalent human fungal pathogens, to survive in various host niches. The exhibition of these traits is linked with a specific gene expression pattern that is epigenetically governed by chromatin architectural changes mediated by histone modifiers and ATP-dependent chromatin remodelers. We are pioneered to investigate the functions of one of the conserved chromatin remodelers, the RSC (Remodels the Structure of Chromatin) complex in *C. albicans*. We observed that the loss of Sth1, the catalytic subunit of the RSC complex, perturbs cell cycle progression and causes cell lethality due to defects in centromere clustering, spindle morphology, sister chromatid cohesion, and chromosome segregation. Affinity purification of Sth1 revealed that the *C. albicans* RSC (CaRSC) complex consists of 13 subunits, including two novel non-essential subunits Nri1 and Nri2 (Novel RSC Interactors), that exist only in the CTG clade fungi. Study of CaRSC subunits essentiality compared to model fungi suggests that the complex has both conserved and distinct features in *C. albicans*. Transcriptomic and proteomic profiling of *STH1* conditional mutant illustrated broad roles of the CaRSC complex in the processes such as stress response, morphogenic switching, and pathogenesis. For instance, deletion of *RSC4* or *NRI1* genes resulted in abnormal filamentation and stress responses, and genetic interaction was observed among these genes. *rsc4* mutant also showed attenuated virulence in the murine model of systemic infection. Undergoing investigation of the Nri proteins revealed that the deletion of the *NRI1* gene resulted in a significantly higher number of multibudded cells in the cycling population, indicating possible cell separation defects. Although the *nri* mutants grown on various carbon sources displayed no growth defects, the *nri1* colonies showed rough morphology when grown on N-acetyl glucosamine. Interestingly, microscopic examination revealed the presence of opaque cells in these colonies even though the strain is heterozygous for the MTL locus suggesting that the mutants may be predisposed to white-opaque switching. High mortality of invasive *Candida* infections and increased drug resistance cases emphasize a dire need for novel therapeutic targets to treat *Candida* infections. Given the presence of fungal-specific subunits, broad-range functions, virulence attenuation, and essentiality for cell viability, the RSC complex has the potential to be future therapeutic target.

648V Functional analysis of the conserved chromatin modifier ASF1 in the filamentous ascomycete *Sordaria macrospora* *Jan Breuer*[1], Minou Nowrousian[1], Ramona Lütkenhaus[1] 1) Ruhr-Universität Bochum.

ASF1 is a conserved eukaryotic histone chaperone and is involved in the assembly and disassembly of nucleosomes during transcription, replication and DNA-repair. Its importance is underscored by studies showing that all non-DNA-bound histones are bound to ASF1. *S. macrospora* is one of the very few multicellular organisms where *asf1* deletions are viable which makes it exceptionally useful for *in vivo* analysis of ASF1 functions. Prior studies of our group have shown that *asf1* deletions lead to sterility and vegetative growth defects but don't affect nucleosome positioning.
We focused on mapping areas of ASF1's highly conserved core and divergent C-terminal tail that are relevant for histone interaction and sexual development of *S. macrospora*. Furthermore, we studied the effect of ASF1 on histone modification and tested its relevance for genomic stability.
By using Co-IP and complementation analysis we were able to show that substitutions at the conserved core of ASF1 abolish histone binding and lead to strains resembling a deletion mutant. A mutation in a putative HIRA-binding site didn't inhibit histone binding, but produced strains with growth aberrations. Truncations of the C-terminal tail after position 210 had no effect on histone interaction or sexual development, while truncations at positions 185 and 152 severely disturbed histone binding and inhibited development. Δasf1 strains proved to be sensitive to the DNA-damaging agent MMS, while complementation strains retained the wild type resistance despite the inability of ASF1 variants to interact with histones. Semiquantitative western blots showed that the deletion of *asf1* leads to a significant increase in overall H3K27Me3 and a significant decrease in H3K56Ac compared to the wild type.
In summary our data indicates that interaction of ASF1 with histones is an essential factor for sexual development in *S. macrospora*. The divergent C-terminal region is also essential for histone binding as truncations before position 210 abolish this function. Interaction with histones isn't necessary for protection against DNA damage, but the exact mechanism remains elusive. A connection with histone modifications which undergo a significant change in Δasf1 seems likely.

649V A fungal ING protein regulates H3 acetylation and H4 deacetylation by interacting with two distinct histone modifying complexes Meng Ye[1], Huaijian Xu[1], Aliang Xia[1], Hang Jiang[1], Panpan Huang[1], Huiquan Liu[1], Qinhu Wang[1], *Cong Jiang*[1] 1) Northwest A&F University, Yangling, Shaanxi, China.

Members of the ING (INhibitor of Growth) family, defined as candidate tumor suppressors, are evolutionarily conserved from yeast to human. The roles of ING proteins depend on their association with histone acetyltransferases (HATs) or histone deacetylases (HDACs) complexes, but the molecular basis of their interaction remains unknown. In this study, the *FNG3* ING gene was functionally characterized in the filamentous plant pathogenic fungus *Fusarium graminearum*. The *fng3* deletion mutant was defective in vegetative growth, sexual development, pathogenicity and DON biosynthesis. Yeast two-hybrid and bimolecular fluorescence complementation (BiFC) assays showed that Fng3 is associated with the NuA3 HAT complex and modulates H3 acetylation by interacting with FgNto1, a well-conserved subunit of the NuA3 complex. In addition, Fng3 shares overlapping functions with another ING protein Fng2 in the regulation of H4 deacetylation through association with the Rpd3 HDAC complex. Whereas Fng2 is associated with the Rpd3 complex via FgRxt2, Fng3 interacts with the C-terminal tail region of FgRpd3 that is absent in yeast Rpd3. Domain swapping analysis showed that the ING domain of Fng2 and Fng3 is responsible for protein-protein interactions and their functional specificities. Taken together, our data showed that Fng3 interacts with two distinct histone modification complexes, which represents a novel regulatory mechanism of a fungal ING protein. Fng3, likely serves as a hub to orchestrate the balance between histone acetylation and deacetylation that is important for fungal development and pathogenicity.

650V Understanding the histone dynamics and regulatory role of lysine methyltransferases Set2, Ash1, and PRC2 in *Magnaporthe oryzae* *David Rowe*[1], David Cook[1] 1) Kansas State University.

Histone lysine methyltransferases are a crucial component of gene regulation from organism development to pathogenesis. Methylation of H3K36 is widely considered to be an activating mark that is enriched at regions actively transcribed by Pol II based on work in metazoan systems. Recent studies in model fungi revealed that SET2 catalyzes 80-95% of total H3K36me3, which is associated with actively transcribed genes consistent with data in animals. However, the other 5-20% of H3K36me3, derived from ASH1, largely co-localized with the repressive mark H3K27me3, catalyzed by PRC2, in regions of the genome characterized as heterochromatic.

Interestingly, while the histone modification catalyzed by SET2 and ASH1 is the same, there is evidence of clear delineation between genomic regions modified by either of the two enzymes. The impact and causation of crosstalk between H3K36me3 deposited by ASH1 and H3K27me3 deposited by PRC2 remains unclear. For instance, in *Fusarium fujikuroi*, Δash1 resulted in increased H3K27me3 in tested regions that were previously methylated by ASH1, suggesting a repressive role. Conversely, in *Neurospora crassa*, Δash1 resulted in a loss of H3K27me3 in associated ASH1-regulated regions of the genome, suggesting that H3K36me3 serves a positive role in the deposition of H3K27me3. Recently published work from our lab in *Magnaporthe oryzae*, showed that ΔMokmt6, an ortholog of PRC2, resulted in a specific reduction of gene body H3K36me3 at regions previously marked by H3K27me3. To further understand how H3K36me3 deposited by two separate enzymes associates with opposing genomic functions, and how this is impacted by cross-talk with PRC2 mediated H3K27me3, we are conducting genetic and epigenomic assays in the plant pathogenic fungus *M. oryzae*. We hypothesize that a complex epigenetic regulatory network involving H3K36me3 and H3K27me3 exists to regulate gene expression and genome stability. Results from these experiments can further define the regulatory mechanisms determining the deposition of these important histone marks and address the biological impact on genome regulation.

651V The histone code of the fungal genus *Aspergillus* uncovered by evolutionary and proteomic analyses *Xin Zhang*[1,2], Roberta Noberini[3], Tiziana Bonaldi Bonaldi[3], Jérôme Collemare[2], Michael Seidl[1] 1) Theoretical Biology & Bioinformatics Group, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, the Netherlands; 2) Westerdijk Fungal Biodiversity Institute, Uppsalalaan 8, 3584 CT Utrecht, the Netherlands; 3) Department of Experimental Oncology, IEO, European Institute of Oncology IRCCS, Milan, Italy.

Chemical modifications of DNA and histone proteins can impact the organization of chromatin within the nucleus. Changes in these modifications, catalyzed by different chromatin-modifying enzymes, influence chromatin organization, which in turn is thought to impact the spatial and temporal regulation of gene expression. While combinations of different histone modifications, the histone code, has been studied in a few model species, we know very little about histone modifications in the fungal genus *Aspergillus*, whose members are generally well-studied due to their importance as models in cell and molecular biology as well as their medical and biotechnological relevance. Here, we used phylogenetic analyses in 94 Aspergilli as well as other fungi to uncover the occurrence and evolutionary trajectories of enzymes and protein complexes with roles in chromatin modifications or regulation. We found that these enzymes and complexes are highly conserved in Aspergilli, pointing towards a complex repertoire of chromatin modifications in Aspergilli. *SET7* and other components of the PRC2 complex, which is responsible for trimethylation on histone H3 at lysine 27 in many eukaryotes including fungi, is absent in Aspergilli as well as in closely related *Penicillium* species, suggesting that these lost the capacity for this modification. Recent other individual losses or duplications were also found, pointing at a few species to further study to understand the role of specific modifications. Finally, we performed unbiased mass spectrometry in three Aspergilli (*A. nidulans*, *A. niger*, and *A. fumigatus*) to confirm our computational predictions, and to uncover and quantify histone modifications that constitute the histone code in these species. In summary, we here provide, to our knowledge, the first systematic analysis of the histone code throughout the fungal genus *Aspergillus*, which will pave the way for future research into the complexity of the histone code and its functional implications on genome architecture and gene regulation in fungi.

652V Sirtuins in the control of virulence in the plant pathogen fungus *Ustilago maydis*. *Blanca Navarrete*[1], José Ignacio Ibeas Corcelles*[1], Ramón Ramos Barrales*[1] 1) Andalusian Centre for Developmental Biology, Pablo de Olavide University, Seville, Spain.

Ustilago maydis is a pathogenic fungus that causes smut disease on maize. During the infection, it secretes effector proteins at different stages of the process to colonize the plant and avoid the immune response. Many transcription factors involved in controlling the correct expression pattern of these effectors have been widely described in this fungus (Kämper *et al*.,2006), however, how chromatin modification may affect this, has not been well established. A previous study from our group systematically characterized Class I and Class II histone deacetylases and found one of them to be involved in pathogenesis through controlling the expression of a transcription factor essential for virulence (Elías-Villalobos *et al*.,2015). Now, we are focused on the other group of histone deacetylases, the Class III (sirtuins) and its role controlling gene expression during pathogenesis.
We have identified five sirtuins homologs in the *U. maydis* genome, named Sir2, Hst2 and Hst4 to 6. By microscopy studies, we have observed that Hst5 and Hst6 localize in the mitochondria, Hst2 in the cytoplasm and Hst4 and Sir2 in the nucleus. As deletion of *hst4* was lethal, we focused on Sir2 for further analysis. Deletion of *sir2* produced an earlier filament formation when cells were grown in filament-induced medium but not in axenic condition, interestingly almost no differences in gene expression were found by RNA-seq in the *sir2* mutant compared with the wild-type strain in both axenic and filamentation conditions. These data suggest that deletion of *sir2*, although may not be avoiding expression of the pathogenic program in axenic condition, makes the cells more susceptible for subsequent inductions. In agreement with this, the artificial induction of *sir2* during filamentation and the pathogenic process, where *sir2* is repressed, caused a reduction in filamentation and in the infection symptoms observed in the plant. An RNA-seq analysis performed in this over-expressed strain during the infection process showed a lack of induction of a pull of the virulence-induced genes. Intriguingly, we have observed that the repressive effect by Sir2 in this fungus is not due to the deacetylation of the most described target of this protein in other fungi, H4K16 or H3K9. Current work is focused on determine whether its target is a different histone residue or has a non-histone target.

653V The epigenetic regulatory mechanisms of effector genes in the wheat pathogen *Zymoseptoria tritici* *Marta Suárez Fernández*[1], Sofia Lopez-Solis[1], Andrea Sanchez-Vallet[1] 1) Universidad Politecnica de Madrid.

Zymoseptoria tritici is a filamentous fungus responsible for septoria tritici leaf blotch, one of the most important diseases of wheat, causing significant yield losses worldwide. Similarly to other fungal plant pathogens, *Z. tritici* harbours effectors, which are molecules required for host colonization. Different effector genes are involved in distinct stages of infection and, therefore, they are tightly regulated. Effector genes are frequently located in heterochromatic regions of the genome, in which genes are typically silenced. Thus, modifications in the chromatin structure are needed for effector gene induction during the interaction of the pathogen with the host. Enzymes that chemically modify chromatin structure - such as Histone Acetyltransferases (KATs) and Demethylases (KDMs) – are proposed to be responsible for enabling the expression of effector genes. We have identified 9 putative HATs and 12 putative KDMs

in *Z. tritici*. Based on their expression pattern during plant infection, we selected 6 KATs and 1 KDM for functional characterization. We will investigate the effect of loss of function of these chromatin-modifying enzymes on effector gene expression, fungal growth and wheat infection. Analyzing the behaviour of the obtained mutants will provide us with new insights into the mechanisms involved in the regulation of the infection machinery of plant pathogens.

654V The Elp3 GNAT superfamily protein modulates development, cell cycle progression and virulence in the fungal insect pathogen, *Beauveria bassiana* *Qing Cai*[1,2], Juanjuan Wang[3], Jiatao Xie[2], Daohong Jiang[2], Nemat Keyhani[1] 1) Department of Microbiology and Cell Science, University of Florida, Gainesville, USA; 2) College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, China; 3) School of Biological Science and Biotechnology, University of Jinan, Jinan, China.

Chromatin remodeling is mediated partly by post-translational acetylation/deacetylation modifications of histones. Histone acetyltransferases (HATs), e.g., members of the GNAT/MYST superfamily, activate gene transcription via promotion of euchromatin formation. Here, we characterize a GNAT family HAT, that is also the catalytic subunit of elongator complex, Elp3 (BbElp3) in the environmentally and economically important fungal insect pathogen, *Beauveria bassiana.* Targeted gene knockout of *BbElp3* resulted in impaired asexual development and morphogenesis, reduced abilities to utilize various carbon/nitrogen sources, reduced tolerance to osmotic/oxidative stress, fungicides and DNA damage stress, and attenuated virulence. The *ΔBbElp3* mutant showed disrupted cell cycle development and abnormal hyphal septation patterns. Transcriptome analyses of wild type and *ΔBbElp3* cells revealed the differential expression of 775 genes, including 336 downregulated and 438 upregulated genes. Bioinformatic analyses revealed downregulated genes to be enriched in pathways involved in DNA processing and transcription, cell cycle control, cellular transportation, cell defense and virulence, while upregulated gene were mainly enriched in carbohydrate metabolism and amino acid metabolism. Downregulated virulence genes included hydrophobins, cellular transporters (ABC and MFS multidrug transporters) and insect cuticular degrading enzymes. These data indicate broad effects of BbElp3 on fungal development, multi-stress response and virulence.

655V Functional analyses of predicted G-protein-coupled receptors in nematode-trapping fungus, *Arthrobotrys oligospora* *Chih-Yen Kuo*[1,2], Guillermo Vidal-Diez de Ulzurrun[1], Yen-Ping Hsueh[1,2] 1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; 2) Molecular Cell Biology, Taiwan International Graduate Program, Academia Sinica and National Defense Medical Center, Taipei, Taiwan.

The ability to sense the environments and to respond appropriately is essential for any organism. Nematode-trapping fungi (NTF) are carnivorous microbes that develop specialized trap structures to capture and consume nematodes when the nutrients in the environment are limited. Previous study demonstrated that mitogen-activated protein kinase (MAPK) pathways are essential for prey sensing in the nematode-trapping fungus *Arthrobotrys oligospora*. The conserved transcription factor Ste12 acting downstream of the pheromone-response pathway also plays a vital role in the predation of *A. oligospora*. Besides, an *A. oligospora* mutant line lacking the G-protein β subunit (GPB1) exhibited defective in sensing nematodes. To identify upstream receptors of these well-conserved signaling pathways, we systematically study the G-protein-coupled receptors (GPCRs) in *A. oligospora*. The *A. oligospora* genome encodes 83 putative GPCR genes, and the largest predicted GPCR class in *A. oligospora* is the Pth11-related family, with orthologs required for plant infection in the plant pathogen *Magnaporthe oryzae*. Time-course RNAseq analysis identified 21 GPCRs of the Pth11 gene family up-regulated after nematode exposure. Interestingly, transcriptional profiling of a *ste12* mutant identified 9 Pth11-related GPCRs are Ste12 dependent. To unravel the function of Pth11-related GPCRs in *A. oligospora*, we characterized the phenotypes of 6 Pth11-related GPCR mutants. None of these GPCRs were required for vegetative growth, but mutation in three GPCR genes resulted in impaired trap development and nematode predation. These results demonstrate that these Pth11-related GPCRs are required for the virulence of *A. oligospora.*

656V Systematic Analysis of Host-derived Cues for the Regulation of Pathogenicity-associated Transcription factors in *Cryptococcus neoformans* *Seong-Ryong Yu*[1], Minjae Lee[1], Kyung-Tae Lee[1], Yong-Sun Bahn[1] 1) Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University.

Cryptococcus neoformans is a causative agent of global fungal meningoencephalitis, which results in more than 180,000 deaths annually. However, its treatment option is limited mainly due to a lack of complete understanding of how the pathogen interacts with the host during infection and disease progression. Although several signaling components and pathways involved in the pathogenicity of *C. neoformans* have been characterized past decades, it remains elusive how complex signaling pathways are coordinated and regulated during the whole infection process. To analyze this, we previously performed NanoString-based *in vivo* transcription profiling to monitor 180 transcription factors during the entire infection process at diverse infected tissues, including lungs, kidneys, spleen, and brain. Here, we focused on 23 transcription factors (TFs) whose *in vivo* expression was highly induced during host infection and deletion significantly decreased pathogenicity of *C. neoformans* in signature-tagged mutagenesis-based murine infectivity assay. To further elucidate their regulatory mechanisms during host infection, the expression patterns of the 23 TF genes were analyzed under in vitro host mimic condition (HMC) – RPMI media supplemented with 10% fetal bovine serum incubated at 37°C under 5% CO_2. Among these, the expression of 12 transcription factors was strongly induced in HMC. To classify which host factor causes the induction of gene during infection, HMC signals were further dissected into three distinct cues: temperature, carbon starvation, and nitrogen starvation. Notably, we found that all three distinct cues made a significant contribution to the regulation of their expression. For example, expression of *PDR802*, *FZC39*, *FZC30*, *BZP4*. *ZNF2* and *HLH1* were markedly induced by temperature upshift from 30°C to 37°C. Supporting this, the double deletion of *PDR802* and *FZC39* caused a reduced growth rate at 37°C. On the other hand, the expression of *FZC30*, *GAT201*, *PDR802*, *BZP4*, *MLN1,* and *STB4* was highly induced by glucose starvation, whereas *PDR802, ZNF2, SRE1, HLH1, STB4, FZC39, FZC30, BZP4*, and *MLN1* was highly induced by nitrogen starvation. Among these, we further focused on *MLN1*, because its expression is induced by both glucose and nitrogen starvation. Supporting this finding, deletion of *MLN1 caused growth defects supplemented with maltose or ammonium sulfate in a nutrient starvation medium.* In conclusion, we systematically dissected host-signaling cues that affect *in vivo* expression of pathogenicity-related transcription factors in *C. neoformans,* providing further insight into complex signaling pathways modulating the host-pathogen interactions of *C. neoformans*.

657V Unveiling the Roles of the Casein Kinase 2 Complex in the Growth, Differentiation, Stress Responses, and Pathogenicity of *Cryptococcus neoformans* *Yeseul Choi*[1], Yong-Sun Bahn[1] 1) Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University.

The basidiomycete human fungal pathogen *Cryptococcus neoformans* causes fatal meningoencephalitis both in immunocompromised patients and immunocompetent individuals. However, the therapeutic options for treatment of cryptococcosis are currently highly limited. As a potential antifungal drug target, kinases have been considered to be good candidates as some of them play critical roles in cellular mechanisms and virulence of fungal pathogens. In our previous studies, we demonstrated that Cka1, which is a serine/threonine kinase and the catalytic subunit of the casein kinase 2 (CK2) complex, is involved in controlling the growth, morphology, and pathogenicity of *C. neoformans*. In this study, we aim to further characterize the functions and regulatory mechanism of the whole CK2 complex in *C. neoformans*. The cryptococcal CK2 complex consists of the catalytic subunit Cka1 and two regulatory subunits, Ckb1 and Ckb2. The *ckb1*Δ, *ckb2*Δ, and *ckb1*Δ *ckb2*Δ mutants exhibited increased susceptibility to antifungal drugs, oxidative stress, and DNA damaging agents, albeit to a lesser extent to the *cka1*Δ mutant, indicating that Ckb1 and Ckb2 play accessary roles for Cka1. Notably, however, the *cka1*Δ *ckb1*Δ *ckb2*Δ triple mutants showed more severe growth defects and greater stress susceptibility than the *cka1*Δ mutants, indicating that the two regulatory subunits may have Cka1-independent functions. Supporting this, we found that the CK2 complex is required for maintaining normal cell cycle and morphology. Coimmunoprecipitation assay revealed physical interactions between Cka1 and Ckb1, Cka1 and Ckb2, and Ckb1 and Ckb2, suggesting that the CK2 complex has a heterotetramer structure (Cka1-Ckb1-Ckb2-Cka1). Considering pleiotropic roles of the CK2 complex in *C. neoformans*, we elucidated its downstream effector genes and proteins through RNAseq-based transcriptomics and mass spectrometry-based proteomics analyses, respectively. In conclusion, this study provides a comprehensive insight into the function and regulatory mechanism of the fungal CK2 complex.

658V Cpk1, Mpk1, and Hog1, MAPK Pathways Coordinately Regulate the Growth, Thermotolerance, and Cell Wall Integrity of *Cryptococcus neoformans* *Yu-Byeong Jang*[1], Yong-Sun Bahn[1] 1) Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University.

Cryptococcus neoformans causes meningoencephalitis mainly in immunocompromised individuals and is responsible for approximately 180,000 deaths annually. In this basidiomycetous human fungal pathogen, three mitogen-activated protein kinases (MAPKs), Cpk1, Mpk1 and Hog1, play central roles in growth, differentiation, and stress response and adaptation. Nevertheless, it remains elusive how these three major MAPK pathways crosstalk with each other and are coordinately regulated to regulate the growth, differentiation, stress response and adaptation, and the of *C. neoformans*. To address this question, here we constructed a series of double and triple MAPK deletion mutants (*mpk1*Δ *cpk1*Δ, *mpk1*Δ *hog1*Δ, *cpk1*Δ *hog1*Δ, and *mpk1*Δ *cpk1*Δ *hog1*Δ) and comparatively analyzed their in vitro and in vivo phenotypic traits. Deletion of *CPK1*, *MPK1*, and *HOG1* caused only a minor growth defect at 30°C under unstressed conditions, but markedly reduced the growth of *C. neoformans* at 37-39°C, even more than any double mutants, suggesting that all three MAPKs contribute to thermotolerance. Supporting it, we found that deletion of *CPK1* and *MPK1* alter Hog1 phosphorylation under temperature upshift from 30°C to 37°C. Although Mpk1 plays more important roles in thermotolerance than Cpk1 and Hog1, Hog1 has a major role in regulating thermotolerance-related transcription factors, including *HSF1, CRZ1, PZF1*, and *MBS2*. In contrast to the shared roles of the three MAPKs in thermotolerance, we found that Mpk1 and Cpk1 play major and minor roles, respectively, in promoting tolerance to cell wall damaging agents, such as calcofluor white (CFW) and Congo red (CR), but Hog1 has a negative role. As CFW and CR binds to fungal chitin, we examined the role of the three MAPKs in regulation of 14 chitin synthesis related genes (*CHS1-CHS8*, *CSR1-CSR3*, and *CDA1-CDA3*). We found that the three MAPKs play redundant and distinct roles in CFW-mediated induction of the following chitin synthesis and modification genes: Mpk1 for *CHS4, CHS5, CHS7,* and *CDA1*, Hog1 for *CHS7* and *CDA1*, and Cpk1 for *CSR2*. Under 37℃, Hog1 mainly regulates the mRNA expression level and Mpk1 and Cpk1 regulate minor. Notably, we found that the three MAPKs regulate thermotolerance by altering cell wall composition. When we stained MAPK mutants with CFW, which non-specifically binds to chitin and cellulose of cell wall, and wheat germ agglutinin (WGA), which selectively binds to β-GlcNAc oligomers and chitin-like oligomer of bud necks rather than chitin, we found that deletion of *MPK1* leads to increased WGA signal at 37°C, whereas deletion of *HOG1* decreased chitin exposure. In conclusion, the three major MAPKs, Mpk1, Cpk1, and Hog1, coordinately regulate growth, thermotolerance, and cell wall integrity in *C. neoformans*.

659V Identification of novel transcription factors involved in *Aspergillus fumigatus* adherence Shuai Liu[1], Yazan Abu Yousef[2,3], Kevin Amalfi[2,3], Donald Sheppard[2,3], Shizhu Zhang[1], *Francois Le Mauff*[2,3] 1) Jiangsu Key Laboratory for Microbes and Functional Genomics, Jiangsu Engineering and Technology Research Center for Microbiology, College of Life Sciences, Nanjing Normal University, Nanjing, China; 2) Microbiology and Immunology department, Faculty of Medicine and Health Sciences, McGill University, Montreal, Quebec, Canada; 3) Infectious Diseases and Immunity in Global Heath program, Research Institute of the McGill University Health Center, Montreal, Quebec, Canada.

Background:
Adherence to cells is a key step in fungal pathogenesis. In *Aspergillus fumigatus*, hyphal adherence to host cells is mediated by the exopolysaccharide galactosaminogalactan (GAG). While several studies have identified genes whose product is required for GAG biosynthesis, little is known about the genetic regulation of GAG production.

Methods:
A library of 400 *A. fumigatus* transcription factor knock-outs was screened for their capacity to form adherent biofilms using the crystal violet assay. Transcription factor mutants with impaired biofilm-forming capacity were re-constructed to confirm the role of each candidate gene in the regulation of adherence. Mutants were then tested for potential growth defects by visual observation and XTT metabolic activity. GAG synthesis was quantified by ELISA and immunofluorescence microscopy. Cell wall composition was assessed by gas chromatography/mass spectrometry.

Results:
Out of 400 transcription factor knockouts, 9 strains exhibited a reduction of > 50% in biofilm formation as compared with the parent

strain Ku80. After reconstruction of the 9 deletions, the simultaneous study of biofilm adherence and growth allowed the classification of these mutant strains into 4 categories: 1 mutant had no growth defect and exhibited impaired formation of adherent biofilms throughout the growth period, 4 mutants had no growth defect, and reduced biofilm formation that could be restored with longer incubation, 3 mutants exhibited both a growth defect and a defect in biofilm formation that persisted despite prolonged incubation. Finally, 1 mutant displayed a severe germination defect and was excluded from further study. Interestingly, all strains except one produced both cell wall-associated or secreted GAG. Further studies of the cell wall polysaccharides in these mutants suggested a wider dysregulation of cell wall biosynthesis.

Conclusion:
This study highlights the role of several novel transcription factors in the regulation of *A. fumigatus* adherence and cell wall synthesis. Further, the inability of several of these strains to form adherent biofilms despite the production of GAG may provide insights into other GAG-interacting or independent factors required for fungal adhesion and biofilm formation. The identification of these new adherence actors and their precise role may identify new therapeutic targets to prevent the development of *A. fumigatus* biofilms.

660V Transcriptional and strain-dependent impact of *C. Albicans HGC1,* the hyphal-specific G1 cyclin *Anupam Mahto*[1], Manning Huang[2], Norma Solis[4], Frederick Lanni[3], Scott Filler[4], Aaron Mitchell[1] 1) University of Georgia, Athens, GA, USA; 2) University of California San Francisco, San Francisco, CA , USA; 3) Carnegie Mellon University, Pittsburgh, PA , USA; 4) Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, CA , USA..

The virulence of *Candida albicans* depends on its ability to form filamentous hyphae during infection. Hyphal morphogenesis requires coordination of cellular functions that include cell polarity, membrane trafficking, and exocytosis. Prior studies showed that the hyphal-specific cyclin, Hgc1, partners with cyclin-dependent kinase Cdc28 to regulate these processes. In this study, we have asked whether Hgc1 is associated with strain-dependent phenotypes, whether Hgc1 is required for transcriptional features associated with hyphal growth, and whether Hgc1-dependent transcriptional responses may impact phenotype. We created *hgc1Δ/Δ* mutants in 5 *C. Albicans* clinical isolates: SC5314, P76067, P57055, GC75, and 19F. The *hgc1Δ/Δ* genotype had the most significant impact on hyphal growth, biofilm formation and endothelial cell damage ability in P57055, GC75, and 19F, which are weaker filamentation strains. In all strains, biofilms of *hgc1Δ/Δ* mutants showed a “piebald” growth pattern, with decreased surface area coverage compared to the wild-type strain. Hgc1 is known to be required virulence in a mouse disseminated infection model, and we have found that Hgc1 is required for maximal virulence in a mouse oropharyngeal candidiasis model as well.
To assay the impact of Hgc1 on gene expression, we conducted RNA seq analysis of *hgc1Δ/Δ*mutants and wild-type strains under hyphal-inducing conditions, and of *TDH3-HGC1* overexpression strains and wild-type strains under non-hyphal inducing conditions. *hgc1Δ/Δ* mutants had altered expression of 38 and 147 genes in the SC5314 and P57055 backgrounds, respectively. Overexpression of *HGC1* in SC5314 promoted pseudohyphal growth under non-inducing conditions and altered the expression of 101 genes. There was little overlap among genes affected by *TDH3-HGC1* and *hgc1Δ/Δ* genotypes. However, common GO terms among affected genes included “cell wall organization,” an indication that cell wall biogenesis may respond to modulated filamentation. We found that overexpression of *HGC1* affected cell wall integrity, as these cells were highly susceptible to the cell wall targeting antifungal drug caspofungin under hyphal inducing conditions. Our findings indicate that Hgc1 activity impacts gene expression, and connect gene expression responses to biological properties.

661V Role of calcineurin signaling components in cryptococcal Titan cell formation *Julia Reuwsaat*[1], Heryk Motta[1], Andrea Tavanti[1], Tamara Doering[2], Charley Staats[1,3], Livia Kmetzsch[1,3] 1) Biotechnology Center, Universidade Federal do Rio Grande do Sul, Brazil; 2) Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri, USA; 3) Department of Molecular Biology and Biotechnology, Universidade Federal do Rio Grande do Sul, Brazil..

Cryptococcus neoformans is the etiological agent of cryptococcosis, an infectious disease that affects mainly immunocompromised patients and causes 180,000 deaths worldwide each year. Once inside the host, cryptococcal cells undergo a variety of adaptative processes that are regulated by a network of transcription factors (TFs). One of these is the TF Pdr802, which has been already shown as an important regulator of *C. neoformans* pathogenicity. In our recent work, we demonstrated that the *PDR802* expression is highly induced under host-like conditions *in vitro* and its deletion impairs *C. neoformans* survival in a mammalian host, mouse serum, and tissue culture media. Two important cryptococcal virulence determinants are negatively regulated by Pdr802: the polysaccharide capsule and Titan cells (TC) production. Using ChIP-Seq and RNA-Seq, we identified direct targets of Pdr802, which include the calcineurin-regulated proteins Had1 and Crz1. The calcineurin pathway is important for *C. neoformans* growth at 37°C, cell wall remodeling, and virulence. Upon intracellular calcium influx, calcineurin dephosphorylates the transcription factor Crz1, which then translocates to the nucleus and regulates gene expression. We found that Pdr802 binds the *CRZ1* gene promoter and positively regulates its expression. Since Pdr802 is the major negative regulator of cryptococcal titanisation and Crz1 the main effector of the calcineurin signaling pathway, we aim to describe the possible interaction between these TFs during TC formation. To understand the role of Pdr802 in gene expression regulation of calcineurin targets, *pdr802*-null mutant cells were subjected to TC induction conditions *in vitro,* and total RNA was extracted and sequenced. In this condition, Pdr802 negatively regulates *CRZ1* expression, while no other classical component of the calcineurin signaling pathway was differently expressed. However, gene products involved in cell wall remodeling, cell membrane integrity, autophagy, gene expression regulation, and copper/iron metabolism were differentially expressed in the *pdr802* mutant. In parallel, a screening of TC formation by mutants of the calcineurin-signaling pathway was conducted *in vitro* and our results show that deletion of *CAM1*, *CRZ1*, *VCX1*, and double deletion of *PMC1*/*VCX1* and *NCS1*/*MID1* affect cryptococcal total cell size, cell body diameter, and cell viability.

662V Natural variation in the control of *Candida albicans* iron acquisition *Liping Xiong*[1], Aaron Mitchell[1] 1) University of Georgia, UGA, Athens, GA.

Candida albicans is part of our natural fungal microflora and can cause lethal infection in susceptible patients. This organism expresses numerous virulence-associated traits, including its abilities to form biofilms, damage host cells, and acquire nutrients in

the host environment. The pathogenic potential of *C. albicans*, like that of all microbes, depends upon acquisition of scarce iron in the bloodstream and tissue. Three transcription factors – Sef1, Hap43, and Sfu1 – work in concert to control iron acquisition and utilization genes, based on studies with the *C. albicans* type strain SC5314 and derivatives. Recent studies of biofilm formation from our laboratory have shown that biofilm regulatory relationships vary extensively among clinical *C. albicans* isolates and have raised the possibility that iron regulatory relationships will vary among clinical isolates as well. Moreover, the fact that there is overlap among targets of Sef1, Hap43, and Sfu1 presents a situation in which changes in activity of one regulator may be compensated by changes in activity of the others. We hypothesize that iron regulatory networks are subject to within-species rewiring and rebalancing. We constructed *sef1Δ/Δ, hap43Δ/Δ* and *sfu1Δ/Δ* mutants in five *C. albicans* clinical isolates with distinct lineages, then we assayed their growth properties under iron replete/ deplete conditions and cell wall integrity of Caspofungin sensitivity and Calcofluor white resistance. Our findings indicate that both Sef1 and Hap43 are required for iron acquisition and cell wall integrity in all strains tested. However, the severity of mutant phenotypes varies with strain background, and Sef1 plays a more prominent role than Hap43 in those phenotypes. We defined gene expression features of the Sef1 regulatory circuit in these five clinical isolates under iron replete and low iron conditions through Nanostring RNA assay, interestingly our data demonstrates that in low iron condition Sef1 regulatory circuit is generally uniform among the clinical isolates, though the expression of biofilm genes exhibits significant variation among *sef1* mutants. Overall, our data indicates that the iron regulatory network is much more uniform among clinical isolates than the biofilm regulatory network.

663V Leveraging machine learning essentiality predictions and chemogenomic interactions to identify antifungal targets *Ci Fu*[1], Xiang Zhang[2], Amanda O. Veri[1], Kali R. Iyer[1], Emma Lash[1], Alice Xue[1], Huijuan Yan[3], Cassandra Wong[4], Zhen-Yuan Lin[4], BenJamin VanderSluis[2], Jing Hou[1,5], Yoko Yashiroda[6], Anne-Claude Gingras[1,4], Charles Boone[1,5,6], Teresa R. O'Meara[7], Matthew J.O'Meara[8], Suzanne Noble[3], Nicole Robbins[1], Chad L. Myers[2], Leah E. Cowen[1] 1) Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada; 2) Department of Computer Science and Engineering, University of Minnesota, Minneapolis, MN; 3) Department of Microbiology and Immunology, UCSF School of Medicine, San Francisco, CA; 4) Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada; 5) Donnelly Center, University of Toronto, Toronto, ON, Canada; 6) RIKEN Center for Sustainable Resource Science, Wako, Saitama, Japan; 7) Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI; 8) Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI .

Fungal pathogens pose a global threat to human health, with Candida albicans among the leading killers. Systematic analysis of essential genes provides a powerful strategy to discover potential antifungal targets. Here, we build a machine learning model to generate genomewide gene essentiality predictions for C. albicans and expand the largest functional genomics resource in this pathogen (the GRACE collection) by 866 genes. Using this model and chemogenomic analyses, we define the function of three uncharacterized essential genes with roles in kinetochore function, mitochondrial integrity, and translation, and identify the glutaminyl-tRNA synthetase Gln4 as the target of N-pyrimidinyl-β-thiophenylacrylamide (NP-BTA), an antifungal compound.

664V Fungal A-to-I mRNA editing controls lethality of a fungal meiotic drive gene in homologous and heterologous expression systems *Jessica Lohmar*[1], Nicholas Rhoades[2], Tejas Patel[2], Robert Proctor[1], Thomas Hammond[2], Brown Daren[1] 1) USDA-ARS; 2) Illinois State University.

Spore killers are meiotic drive elements that block development of sexual spores in certain species of the fungal phylum Ascomycota. In the maize ear rot and mycotoxin-producing fungus *Fusarium verticillioides*, the meiotically defined spore killer locus *Sk* has been localized to a 102-kb interval of chromosome V. Here, we show that a gene within this interval, *SKC1*, is required for the spore killing-mediated meiotic drive. We also demonstrate that *SKC1* is associated with at least four transcripts, two sense (sense-SKC1a and sense-SKC1b) and two antisense (antisense-SKC1a and antisense-SKC1b). Both antisense *SKC1* transcripts lack obvious protein-coding sequences and thus appear to be non-coding RNAs. In contrast, sense-SKC1a is a protein-coding transcript that undergoes A-to-I editing to sense-SKC1b in sexual tissue. Translation of sense-SKC1a produces a 70 amino acid protein (Skc1a), whereas translation of sense-SKC1b produces an 84 amino acid protein (Skc1b). Heterologous expression analysis of *SKC1* transcripts shows that sense-SKC1a also undergoes A-to-I editing to sense-SKC1b during the *Neurospora crassa* sexual cycle, and that Skc1b induces most meiotic cells to die. Finally, we report that *SKC1* homologs are present in over 20 *Fusarium* species. Overall, our results demonstrate that fungal meiotic drive elements like *SKC1* can influence the outcome of meiosis by hijacking a cell's A-to-I editing machinery and that the involvement of A-to-I editing in a fungal meiotic drive system does not necessarily preclude its horizontal transfer to a distantly related species.

665V Blue Mold's Clues: Comparative transcriptomics of blue mold fungi clue into biochemical processes associated with fungal aggressiveness and conidial germination in *Penicillium* spp. *Holly Bartholomew*[1], Franz Lichtner[2], Michael Bradshaw[3], Verneta Gaskins[1], Jorge Fonseca[1], Joan Bennett[4], Wayne Jurick II[1] 1) Food Quality Laboratory, USDA-ARS, Beltsville, MD; 2) U.S. Army Corps of Engineers Engineer Research & Development Center, Cold Regions Research & Engineering Lab, Hanover, NH; 3) Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA ; 4) Department of Plant Biology and Pathology, Rutgers, The State University of New Jersey, New Brunswick, NJ .

Blue mold decay, caused by *Penicillium* species, is a devastating postharvest disease of pome fruit resulting in reduced quality, mycotoxin contamination, and significant economic losses. Our aim is to elucidate critical molecular aspects that mediate blue mold development to design novel postharvest decay mitigation strategies. A transcriptomic approach determined fungal genes related to pathogen aggressiveness in apple and identified loci important for conidial germination in *Penicillium* species. Total RNA was isolated from ungerminated conidia and decayed apple fruit infected with *P. expansum* R19 (highly aggressive) or *P. polonicum* RS1 (less aggressive), for RNA-Seq and qRT-PCR analyses. There were 2,442 genes differentially expressed between R19 and RS1 in apple. Comparisons between apple and conidia for R19 revealed 4,404 Differentially Expressed Genes (DEGs) while RS1 had 2,935 DEGs. Gene ontology of transcriptomic data revealed differential regulation in hundreds of fungal transport and metabolism genes during apple fruit decay, suggesting changes in nutrient acquisition and detoxification strategies (i.e. efflux). In R19, 108 iron-binding, 11 copper-binding, and an additional 862 oxidoreductase genes were differentially expressed in decayed apple fruit verses ungerminated conidia.

This suggests that redox regulation during apple fruit decay is a critical component of the pathogen's ability to colonize its host. The glutathione detoxification system had 14 genes differentially expressed in R19 vs. RS1 in apple and has been previously associated as the primary mechanism of patulin detoxification in human cell lines. Interestingly, there were 34 genes exclusively found and expressed in the R19 genome. Among these were loci encoding a transcription factor, a ureohydrolase, a heat shock protein, a cell wall synthase, a secreted protein, and 18 hypothetical proteins (some having no detectable functional domains, few having well characterized motifs). Currently we are targeting 22 single copy loci for functional analysis to further understand their roles in apple fruit decay and conidial germination. Our ultimate goals are to identify novel drug targets for new chemical controls, develop antisporulants, and to generate biological control agents to abate blue mold decay and maintain pome fruit quality in storage.

666V The role of RNA helicases during *Ustilago maydis* teliospore dormancy and germination *Amanda Seto*[1], Barry Saville[1] 1) Trent University, Peterborough, ON, Canada.

Plant diseases caused by fungal pathogens are major threats to sustainable crop production. These fungal pathogens can persist in the environment through the formation of resistant spores. The spread of fungal diseases is achieved through spore dormancy, dispersal, and germination. We use *Ustilago maydis* teliospores as a model for studying the transition from dormancy to germination in fungal plant-pathogens. Previous research indicated that fungal spores store the components required for germination during dormancy. Among these components, stored mRNAs would need to be stabilized in some way during dormancy. This could be through the formation of structures that impart stability, forming double-stranded RNAs with antisense RNAs, and/or forming RNA-protein complexes that contain RNA helicases. During germination, we hypothesize that stored mRNAs are translated following being unwound or released from ribonucleoproteins through processes that include the activity of RNA helicases. RNA helicases are highly conserved enzymes that are found in all eukaryotes and are capable of: forming RNA clamps, unwinding RNA-RNA duplexes, and displacing proteins. We identified 46 RNA helicases in *U. maydis*, five, of these had levels increased in dormant teliospores, and these transcript levels decreased during germination. This suggested these RNA helicases may have a role in germination and we have begun functional analyses to assess this. Genes for the *U. maydis* orthologs to the *Saccharomyces cerevisiae DED1* and *DBP3* RNA helicases were deleted. Deletions strains were assessed for growth defects, stress response, their ability to form the filamentous dikaryon, pathogenesis, and their ability to develop teliospores. Further functional analyses will include the expression of an antisense RNA in *U. maydis* strains where the RNA helicase has been expressed at an elevated level and identifying potential target gene transcripts for RNA helicase activity based on their interaction with secondary structures. Understanding the role of RNA helicases in fungal spores can provide insights into the mechanisms involved during fungal spore dormancy and germination.

667V Role of Nuclear mRNA Degradation Pathway in the Regulation of Telomere Length in *Saccharomyces cerevisiae* *Mayukh Banerjea*[1], Biswadip Das[1] 1) Jadavpur University, Kolkata, West Bengal, India.

Nuclear mRNA surveillance pathway is one of the most crucial cellular systems ensuring the survival of *Saccharomyces cerevisiae* by preventing the accumulation of aberrant messages produced during mRNA biogenesis. With the help of its co-factors, this machinery detects distinct classes of defective messages that are produced co- and post-transcriptionally inside the nucleus. Interestingly, it also targets certain non-aberrant normal messages (also known as special messages) thereby controlling their cellular repertoire.
A previously published microarray data revealed that upon the disruption of the surveillance pathway, the expression levels of several genes involved in telomere length maintenance were altered significantly. In an effort to investigate this phenomenon, we used the principle of telomere silencing to detect changes in telomere length with the help of a simple growth test. We also employed real-time PCR to detect changes in expression levels of specific telomere length regulatory genes of interest.
Our experiments revealed that upon the deletion of essential components of the surveillance pathway, the growth test results indicate a significant increase in telomere length as compared to cells with functional surveillance. While the increase in length is quite possibly the result of altered expression across several genes, we have identified three messages whose expression levels have been reported to be positively correlated to changes in telomere length.
While two of the genes (*EST2*, *TEL1*) represent some of the most well defined and crucial components of the telomere complex, the functional role of the third (*MTC7*) remains elusive. We aim to establish this phenomenon firmly with the help of Telomere restriction fragment length analysis and measuring the protein levels of our genes of interest in the surveillance defective strains. Furthermore, the roles of the surveillance cofactors will also be investigated in parallel to unveil the exact mechanism by which the surveillance pathway regulates telomere length in *Saccharomyces cerevisiae.*
Findings from this study will shed light on a novel mechanism of telomere length regulation which could be utilized for further studies in telomere biology and possibly, also provide an avenue for potential therapeutic approaches.

668V Characterization of N6-methyladenosine RNA methylation factors in *Fusarium graminearum* Hyeonjae Kim[1], *Wonyong Kim*[1] 1) Korean Lichen Research Institute, Sunchon National University, Suncheon, South Korea.

N6-methyladenosine (m6A) is one of the most abundant form of internal mRNA modification in higher eukaryotes. m6A modification is regulated reversibly by three m6A RNA methylation factors (writer, reader, and eraser). In filamentous fungi, little is known about the roles of m6A modification in developmental processes. To characterize the function of m6A factors in a filamentous fungus, *Fusarium graminearum*, we attempted to generate knockout mutants lacking an m6A writer (a homolog of yeast *IME4*), an m6A reader (a homolog of yeast *PHO92*), or a putative m6A eraser. Knockout mutants lacking the *PHO92* homolog or the putative m6A eraser formed normal perithecia and ascospores, indicating that these m6A factors are dispensable for sexual development. Despite our extensive efforts, we were unable to obtain knockout mutants lacking the *IME4* homolog. To investigate the involvement of the *IME4* homolog in m6A modification process as an m6A writer, we generated *IME4*-overexpressing strains by introducing another copy of *IME4* gene under the control of a constitutive promoter. The relative *IME4* expression level and cellular m6A level in an *IME4*-overexpressing strain were significantly higher than those in the wild type, indicating that the *IME4* homolog is an authentic m6A writer in *F. graminearum*. The *IME4*-overexpressing strain exhibited slower growth rate and lower hyphal density, compared to the wild type. Moreover, sexual development was significantly delayed in the *IME4*-overexpressing strain, suggesting that the m6A writer plays important roles for vegetative growth and sexual reproduction. These results suggested that proper regulation of m6A status is critical not only for timely sexual

development, but also normal hyphal growth in *F. graminearum*. For further studies, we are currently investigating transcripts that show drastic changes in m6A levels during vegetative growth and sexual development.

669V Identification of a stage-specific co-factor required for A-to-I mRNA editing during sexual reproduction in fungi Chanjing Feng[1], Kaiyun Xin[1], Zhuyun Bian[2], Jingwen Zou[1], Yanfei Du[1], Jin-Rong Xu[2], *Huiquan Liu*[1] 1) State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, China; 2) Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, United States of America.

Adenosine-to-inosine (A-to-I) editing of mRNAs catalyzed by double-stranded RNA-specific adenosine deaminase (ADAR) enzymes is an important post-transcriptional modification in animals. In the filamentous ascomycete *Fusarium graminearum* that lacks ADARs but has genome-wide A-to-I editing, two genes orthologous to yeast *TAD2* and *TAD3* encoding two subunits of tRNA-specific adenosine-34 deaminase, respectively, have been implicated in catalyzing A-to-I mRNA editing specifically during sexual reproduction. RNA immunoprecipitation sequencing analysis revealed that edited RNAs were more likely to be bound by FgTad2 during sexual reproduction. By characterizing 34 genes expressed specifically during sexual reproduction, we found one of them is essential for A-to-I mRNA editing in *F. graminearum* (named *AME1* for activator of mRNA editing). *AME1* is highly conserved in Sordariomycetes but not present in other fungi. It encodes a protein with a domain of unknown function (DUF). Deletion of *AME1* had no effects on growth, conidiation, and pathogenesis but resulted in the abolishment of A-to-I mRNA editing during sexual reproduction. Perithecia formed by the Δ*ame1* mutant had no asci/ascospores and no detectable RNA editing events. Interestingly, when the *AME1* gene was expressed with a constitutive promoter, approximately two-thousands of mRNA editing sites were detected in vegetative hyphae. The physical interaction between Ame1 and FgTad2/FgTad3 and their functional relationship during A-to-I mRNA are being characterized and will be presented. In addition, we identified a gene encoding an RNA-binding protein that represses mRNA editing during sexual reproduction. Taken together, our results showed that Ame1 functions as a stage-specific co-factor of FgTad2 and FgTad3 for A-to-I mRNA editing in fungi during sexual reproduction.

670V Exploring the role of natural antisense transcripts in the stress response of *Ustilago maydis* *Monique Lariviere*[1], Barry Saville[1] 1) Trent University, Peterborough, ON.

As climate changes at an accelerated rate and the global population continues to rise, global food security has become an increasing cause of concern. Advances are being made to mitigate this concern through increasing crop yields; however, the threat of evolving pathogens must also be considered. Fungi are one of the most economically harmful groups of plant pathogens as they are responsible for severe losses of cereal crops. As the climate changes, fungal pathogens can adapt at a faster rate compared to their hosts due to their greater variety of adaptive mechanisms and shorter generation times. We hypothesize that RNA-mediated mechanisms enhance the fungal adaptation to stress and propose exploring the modulation of stress response in the model fungus *Ustilago maydis* through natural antisense transcripts (NATs). NATs are a subset of non-coding RNAs which have regions of their sequence complementary to an mRNA. Functional characterization of NATs in *U. maydis* has revealed roles in modulating mRNA stability, pathogenesis, and mitochondrial function. An RNA-seq comparison of three smut fungi revealed that 349 of the 2617 NATs found in *U. maydis* are conserved among all three smut species. The conservation of NATs among these smut species suggests that they have important functional roles. Genes complementary to these conserved NATs were investigated to identify those with previously documented roles in stress response. *U. maydis* haploid strain 521 was exposed to oxidative, osmotic, nitrogen, and carbon stressors separately, and the mRNA and NAT transcript levels of the identified genes were determined through RT-PCR. Additionally, we investigated the impact of combined sequential stresses on growth and transcript level change. This screen revealed that five of the 28 NATs investigated had altered expression levels in more than one stressed environment. The expression level changes of these NATs were quantified by RT-qPCR. Antisense transcript expression vectors have been created for selected NATs and will be transformed into *U. maydis* haploid cells to assess the impact of antisense expression on the complementary mRNA levels, the structure of *U. maydis* haploid cells, and their growth in response to stress. Identifying and describing these RNA-mediated responses to environmental stress may provide a better understanding of the increasing prevalence and severity of fungal diseases.

671V Gad1 functions as a negative regulator of A-to-I mRNA editing during sexual reproduction *Zeyi Wang*[1], Zhuyun Bian[1], Yang Li[1], Huiquan Liu[2], Jin-Rong Xu[1] 1) Department of Botany and Plant Pathology, Purdue University; 2) State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University.

The FgTad2 and FgTad3 ADATs have been shown to catalyze sexual stage-specific A-to-I mRNA editing in *Fusarium graminearum*. However, both *FgTAD2* and *FgTAD3* are constitutively expressed and lack dsRNA binding domains present in human ADARs, it is likely that they are facilitated by stage-specific co-factors for A-to-I editing during sexual reproduction. To identify putative stage specific co-factors of FgTad2 and FgTad3, in this study we used the affinity purification and mass spectrometry analysis approach to identify proteins that specifically interact with FgTad2 and/or FgTad3 in perithecia. In comparison with vegetative hyphae, sixty-eight FgTad2-interacting and thirty-four FgTad3-interacitng proteins were found to be specific to perithecia. Eleven of them (one specific for FgTad2, ten interacting with both FgTad2 and FgTad3) were selected for functional characterization based on their expression profiles and predicted functions. Mutants deleted of the FgTad2- and eight FgTad2/FgTad3-interacting genes had no detectable phenotypes. For the other two, deletion of FGSG_10943 only resulted in a minor defect in ascospore discharge but mutants deleted of FGSG_09556 (named *GAD1* for growth and ascospore defect 1) had defects in both vegetative growth and sexual reproduction. The *gad1* mutant still formed normal, melanized perithecia. However, most of *gad1* asci were aborted or had less than 8 ascospores. The *gad1* ascospores also tended to have morphological defects. In co-immunoprecipitation assays, Gad1 interacted with FgTad2 in perithecia but not in vegetative hyphae, suggesting a stage-specific interaction. The *gad1* mutant was defective in the distribution of nuclei in ascospores but normal in conidia. Gad1 contains two RNA-recognition motifs (RRMs) and deletion analysis showed that both of them are important for its function during sexual reproduction but dispensable for vegetative growth. Interestingly, the number of editing sites and editing levels were increased in the *gad1* mutant in comparison with the wild type. Overall, our results indicated that Gad1 interacts with FgTad2 specifically during sexual reproduction and likely functions as a negative regulator of A-to-I mRNA editing in *F. graminearum*.

672V The role of COP9 signalosome complex in secondary metabolism in *Fusarium* *Massimo Ferrara*[1], Cecilia Lasorella[1], Miriam Haidukowski[1], Christopher Toomajian[2], Nik M. I. Mohamed Nor[2,3], Wei Yue[2], Antonio F. Logrieco[1], John F. Leslie[2], Giuseppina Mulè[1] 1) Institute of Sciences of Food Production, National Research Council, Bari, Italy; 2) Department of Plant Pathology, Kansas State University, Manhattan, Kansas USA; 3) School of Biological Sciences, University Sains Malaysia, Penang, Malaysia.

Fusarium is a cosmopolitan genus that includes plant pathogens of many important cereal crops. Within the genus, the *Fusarium fujikuroi* species complex is one of the best studied. *F. fujikuroi*, and *F. proliferatum* are sister species in this species complex. Although *F. fujikuroi* and *F. proliferatum* are very closely related, the two species can be recovered from very different plant hosts and also differ in their secondary metabolite production profiles. *F. fujikuroi* strains commonly produce gibberellic acid (GA), while *F. proliferatum* strains can produce multiple mycotoxins including fumonisins (FUMs). Some strains are cross-fertile with members of the other species and those crosses produce a few viable progeny. A cross was made between a *F. fujikuroi* strain that produces GA but not FUMs and a *F. proliferatum* strain that produces FUMs but not GA. Amongst the progeny novel pathogenicity and secondary metabolite combinations were observed, including variation for GA and FUMs production. GBS analysis and QTL mapping of GA production levels detected one major QTL on chromosome 5, hosting the well-known GA gene cluster, and two minor QTLs on chromosome 1. This latter genomic region was 17 Kb in length and contained five genes. One gene was identified as a probable COP9 signalosome complex subunit 2, which is involved in the regulation of protein degradation and secondary metabolism. Deletion of this gene by CRISPR/Cas9 approach did not alter colony morphology, growth, or pathogenicity towards onion, with GA production being slightly reduced. FUMs production, however, was drastically reduced, respect to the *F. proliferatum* parent. These results suggest a new role for the COP9 signalosome complex in FUMs biosynthesis regulation and suggest that this QTL may have a role in the regulation of the *FUM* cluster.

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673V Culturing *Aspergillus nidulans* in soil microcosm elucidates its ecological behavior and interaction with soil microbiota Marina Takata[1], Moriyuki Kawauchi[2], Kiminori Shimizu[3], Keishi Senoo[2,4], Yasuo Ohnishi[2,4], Syun-ichi Urayama[5,6], *Daisuke Hagiwara*[5,6] 1) Grad. Sch. Life and Env. Sci., Univ. of Tsukuba; 2) Grad. Sch. Agr. and Life Sci., The Univ. of Tokyo; 3) Fac. of Adv. Eng., Tokyo Univ. of Sci.; 4) CRIIM, The Univ. of Tokyo; 5) Fac. of Life and Env. Sci., Univ. of Tsukuba; 6) MiCS, University of Tsukuba.

Fungi profoundly inhabit in soil and play a key role in degrading biomass and consequent material cycle in soil biome. Despite their importance, fungal behavior in the community of soil microorganisms is poorly understood at a molecular level. To investigate fungal physiology and its effect on soil microbiome, we developed a soil microcosm where the model filamentous fungus *Aspergillus nidulans* was cultured. The germinated conidia of *A. nidulans* were inoculated in sterilized and unsterilized soils and incubated for 14 days. The DNA of *A. nidulans* was extracted from the soil and subjected to quantitative PCR to measure the biomass content. Maximum growth was shown on the first day in both sterilized and unsterilized soils, and then the growth declined. This result suggests that *A. nidulans* hyphae autolyzed as the culture progressed. The amounts of cells in unsterilized soil were apparently smaller than those in sterilized soil, suggesting a competition in the microbial community.
Transcriptome analysis by RNA-sequencing revealed a set of *A. nidulans* genes that were highly expressed in the soil but not in conventional media such as PDA and PDB. Chitinase and glucosidase genes, which are associated with autolysis, showed high expression levels in the soil. Several genes related to secondary metabolism were expressed in both sterilized and unsterilized soils, but not in the conventional media. To assess if fungal secondary metabolism affects the soil microbiome, the deletion and overexpression mutant strains of *laeA* encoding a master regulator for secondary metabolism were cultured in the soil microcosm. Growth level of the mutants was decreased compared with that of the WT strain in PDA and soil microcosm. Then, the effects of *A. nidulans* on the soil microbiome and mycobiome were analyzed by 16S rDNA and ITS amplicon sequencing, respectively. A slight but significant difference in bacterial and fungal community structures was observed between the soil inoculated with and without *A. nidulans*. Notably, the fungal community structures were significantly different between the soil inoculated with *laeA* deletion mutant and that with the WT strain after 8 weeks incubation. These results suggest that *A. nidulans* growing in the soil affects the surrounding fungal community through LaeA-dependent secondary metabolism.

674V Identification of co-culture responsive biosynthetic gene cluster in *Aspergillus niger* and *Penicillium* species Haruka Tsuji[1], Akihiro Ninomiya[2], Norio Takeshita[2,3], Kenji Kai[4], Syun-ichi Urayama[2,3], *Daisuke Hagiwara*[2,3] 1) Grad. Sch. Life and Env. Sci., Univ. of Tsukuba; 2) Fac. of Life and Env. Sci., Univ. of Tsukuba; 3) MiCS, Univ. of Tsukuba; 4) Grad. Sch. Life and Env. Sci. Osaka Pref. Univ..

Co-culturing multiple fungi have drawn much attention to potentiate fungal ability to produce unidentified secondary metabolites. The molecular mechanisms underlying co-culture dependent metabolite production are poorly understood. To address the issue, co-culture responsive metabolite production was sought using different species of *Aspergillus* fungi. When co-cultured with other *Aspergillus* strains, *Aspergillus niger* accumulated much amount of yellow pigments in the mycelia. Molecular mass of the co-culture responsive yellow pigment (CRP) was determined by HR-ESIMS. Based on molecular mass, the spatial production in *A. niger* colony confronted with partner fungi was confirmed using Imaging mass spectrometry. The CRP was produced when co-cultured with *Penicillium* species as well as *Aspergillus* species, although the close relative *A. luchuensis* did not stimulate the production. Transcriptome analysis comparing between the mono- and co-cultures showed that one biosynthetic gene cluster (BGC) of *A. niger* was coordinately activated in co-culture. The BGC presumably contains 12 genes including those encoding PKS-NRPS (*crp2*) and NRPS (*crp7*). The disruption mutants of *crp2* and *crp7* did not produce the metabolite in co-culture, indicating that the cluster was responsible for CRP biosynthesis. The *crp* cluster is conserved in the genome of closely related species such as *A. luchuensis*, *A. phoenicis*, and *A. neoniger*, as well as the species in different genus such as *Penicillium expansum* and *P. camenverti*. In co-culture with *A. fumigatus, A. phoenics* and *A. neoniger,* but not *A. luchuensis*, were able to produce the CRP. Meanwhile, co-culture responsive or unresponsive and constitutive production of yellow pigments were observed in the strains of *P. expansum* and *P. camenverti*. These findings suggested that the BGC is shared by several fungal species, and the responsiveness to other fungi is partly conserved. This work provided ecological view and

evolutionary insight into secondary metabolism in fungal kingdom.

675V Characterization of conidia-specific transcription factor CsgA in *Aspergillus* spp. *He jin Cho*[1], Ye eun Son[1], Hee soo Park[1,2] 1) School of Food Science and Biotechnology, Kyungpook National University , Daegu, Republic of Korea; 2) Department of Integrative Biology, Kyungpook National University, Daegu, Republic of Korea.

Aspergillus spp. mainly reproduce through asexual reproduction, producing the asexual spore called conidia. The process of conidia formation (conidiation) is controlled by various transcription factors. Among them, BrlA, AbaA, and WetA have been defined as the central regulators which regulate gene expressions related to conidiation. Our previous transcriptomic analysis identified twenty novel conidia-specific transcription factors. In this study, we characterized one of the conidia-specific transcription factors CsgA, the Zn_2Cys_6 transcription factor containing the GAL4-like zinc-finger domain. The roles of CsgA were investigated in two *Aspergillus* species, the model organism *Aspergillus nidulans* and the aflatoxin producer *Aspergillus flavus*. In *A. nidulans*, the Δ*csgA* strain showed an increase in conidiation and fungal growth. The expression levels of *brlA* in the Δ*csgA* strain increased in the early stage of conidiation. Deletion of *csgA* exhibited a defect in sexual growth. Overexpression of *csgA* resulted in decreased conidiation and increased sexual development, suggesting that CsgA plays a role in maintaining the balance between asexual and sexual development in *A. nidulans*. In conidia, deletion of *csgA* resulted in increased trehalose content and higher tolerance to thermal, oxidative, and UV stresses. In ascospore (sexual spore), the absence of *csgA* showed higher trehalose content and stress tolerance compared to control. Germination ability of ascospore was lower in the Δ*csgA* strain compared to control. The production of sterigmatocystin increased in the Δ*csgA* conidia and ascospore. In *A. flavus*, deletion of *csgA* showed a decrease in fungal growth but an increase in conidiation. The Δ*csgA* strain exhibited abnormal sexual development. Deletion of *csgA* resulted in increased trehalose content and higher tolerance in thermal and oxidative stresses. The aflatoxin B1 production was lower in the Δ*csgA* conidia compared to control. Overall, these results suggest that CsgA plays a crucial role in proper fungal development and mycotoxin production in *A. nidulans* and *A. flavus*.

676V Transcriptome-based functional analysis of spore-specific transcription factors in *Aspergillus species* *Ye-Eun Son*[1], He-Jin Cho[1], Hee-Soo Park[1,2] 1) School of Food Science and Biotechnology, Kyungpook National University, Daegu, Republic of Korea; 2) Department of Integrative Biology, Kyungpook National University, Daegu, Republic of Korea.

Aspergillus, a filamentous fungus that makes up the majority of airborne fungi, reproduces primarily by forming asexual spores called conidia. The process of making conidia is regulated by various transcription factors (TFs). Although previous studies have shown that some TFs, such as VosA, VelB, and WetA, mediate conidia formation and maturation, there are still unexplored TFs for conidiogenesis. Therefore, we performed transcriptome analysis of conidia and hyphae in three *Aspergillus spps* and subsequently analyzed the function of putative spore-specific TFs in *Aspergillus nidulans*. Afterwards, we identified twenty-two spore-specific TFs and each deletion mutant was phenotypically analyzed in *A. nidulans*. Among them, we characterized one of the **s**pore-**s**pecific-$\mathbf{C}_2H_2$ zinc finger A SscA in *A. nidulans*. The Δ*sscA* mutant showed defective conidiation, sexual development, and reduced conidia viability in *A. nidulans*. The Δ*sscA* mutant conidia were more sensitive to various stresses than wild-type conidia. And the amount of trehalose in the Δ*sscA* mutant was decreased compared to that of the WT. On the other hand, deletion of *sscA* caused induced germ tube formation with or without glucose and increased the amount of β-glucan in Δ*sscA* mutant conidia compared to wild-type conidia. Absence of *sscA* led to increase the amount of stematocystin in conidia. Furthermore, transcriptome data suggested that SscA affected the mRNA expression of various genes in *A. nidulans* conidia. Interestingly, deletion of *sscA* resulted in alterations of gene expression involved in the response of conidia to stimuli and stress. The mRNA levels of β-glucan biosynthesis gene and stematocystin gene cluster were upregulated in *sscA* mutant conidia. In addition, we confirmed that the roles of SscA in conidia were conserved in *A. flavus* and *A. fumgiatus*. Overall, these results suggest that SscA is a spore-specific transcription factor, essential for proper asexual and sexual development, conidia maturation, conidia stress tolerance and secondary metabolites in *A. nidulans*. And the functions of SscA in conidia are conserved in three representative *Aspergillus* spp.

677W Probing the role of N6-methyladenine DNA modification within the *Rhizopus microsporus* and *Mycetohabitans* symbiosis *Margaret Branine*[1], Imperio Real-Ramirez[1], Sue Hoseon Choi[2], Stephen Mondo[3], Teresa Pawlowska[2] 1) Graduate Field of Microbiology, Cornell University, Ithaca, NY; 2) School of Integrative Plant Science, Cornell University, Ithaca, NY; 3) US Department of Energy Joint Genome Institute, Berkeley, CA .

The early-diverging fungal phylum Mucoromycota displays a high degree of coevolution with bacteria as representatives from each of the three subphyla (Glomeromycotina, Mortierellomycotina, and Mucoromycotina) commonly harbor bacterial endosymbionts. It is not clear why members of Mucoromycota so commonly and intimately associate with bacteria relative to other fungal lineages. We hypothesize establishment and maintenance of the symbiosis is mediated by a shared epigenetic DNA modification of the two partners, N6-methyladenine (6mA). This hypothesis stems from the recent discovery that, unlike in most eukaryotes, 6mA is the predominant methylation mark in early-diverging fungi, a feature shared with bacteria. For this study, we focused on the symbiosis between *Rhizopus microsporus* (Mucorales) and its endosymbiont *Mycetohabitans* (Burkholderiales). The reproductive addiction (asexual and sexual) of host strains of *R. microsporus* to its endosymbiont along with the existence of strains naturally free of endosymbionts (i.e., non-hosts) permit comparative investigations into symbiosis factors. To this end, we assessed vegetative growth of two host and one non-host isolates when exposed to the small molecule DNA adenine methyltransferase inhibitor pyrimidinedione. While pyrimidinedione inhibited growth for each isolate, the effect of inhibition did not differ depending on host status. When we cured one host strain of its endosymbiont and repeated the inhibitor experiment, we observed growth of the cured strain was significantly inhibited by pyrimidinedione after 2 days. Furthermore, when we mated two compatible host strains in the presence of the inhibitor, we qualitatively observed little difference in sexual sporulation compared to control conditions. Taken together, our inhibitor experiments suggest endobacteria protect their hosts from the negative effects of the methylation inhibitor; however, we cannot exclude several confounding factors, namely rapid inhibitor degradation. To address these limitations, we are currently creating adenine methyltransferase mutants in *Mycetohabitans* to determine the role of bacterial 6mA modifications within the symbiosis. Additionally, we present our in-progress analysis of 6mA methylation from PacBio sequences of wildtype and cured host strains ATCC52813 and ATCC52814 and their endosymbionts, *Mycetohabitans* sp. B13 and B14, respectively.

678T Hijacking time: How *Ophiocordyceps* fungi could be using ant host clocks to manipulate behavior *Charissa de Bekker*[1], Biplabendu Das[1], Roos Brouns[3], Andreas Brachmann[2] 1) University of Central Florida, Orlando, Florida, USA; 2) Ludwig Maximilians Universität, Munich, Germany; 3) Universiteit Utrecht, Utrecht, the Netherlands.

Ophiocordyceps fungi adaptively manipulate the behavior of their ant hosts as a strategy to increase transmission. We have observed conspicuous changes in the daily timing of disease phenotypes that suggest that *Ophiocordyceps* could be hijacking the host clock. Climbing and biting to fix the host at elevated positions (i.e., summiting), following death, and spore release display time-of-day synchronization. Prior to this, the daily activity patterns of infected ants are significantly disrupted. Moreover, light appears to play an important role in the final positioning of manipulated individuals. Using a transcriptomics approach, we investigate if the biological clocks of *Ophiocordyceps* fungi and their hosts could be involved in establishing infection, dysregulation of host activity and following manipulated summiting behavior. Time course transcriptomics data for the parasite suggest that *Ophiocordyceps* has a light-entrainable clock that could be driving daily expression of secreted effectors and candidate manipulation genes. Host time course data demonstrated that rhythms in ant gene expression are seemingly highly plastic and involved in behavioral division of labor, which could make them susceptible to parasite hijacking. To provisionally test if the expression of ant behavioral plasticity and rhythmicity genes could be affected by fungal manipulation, we performed a gene co-expression network analysis on the ant time course data and linked it to our behavioral manipulation transcriptomics data. We found that behavioral plasticity genes reside in the same modules as those affected during fungal manipulation. These modules showed significant connectivity with rhythmic gene modules, suggesting that *Ophiocordyceps* could be indirectly affecting the expression of those genes as well. As the next step in our aim to discover how parasite and host clocks might be involved in infection and manipulation, we have performed time-course transcriptomics on *Ophiocordyceps*-infected individuals. We are currently analyzing the data to investigate how fungus and ant host clocks are ticking at a time during infection when host daily activity is significantly disturbed.

679F Characterizing variation within the European *Batrachochytrium salamandrivorans* epidemic *Moira Kelly*[1], Frank Pasmans[1], Jose Muñoz[2], Terrance Shea[2], Matthew Gray[3], Christina Cuomo[2], An Martel[1] 1) Ghent University; 2) Broad Institute of MIT and Harvard; 3) University of Tennessee Institute of Agriculture, Knoxville, Tennessee.

Pathogens rarely drive their hosts to extinction. The chytrid fungus *Batrachochytrium dendrobatidis (Bd)*, however, is frequently associated with population extinctions in amphibians, resulting in the most biodiversity-devastating epidemic in recorded history. In 2013, a closely related chytrid, *Batrachochytrium salamandrivorans* (*Bsal*), was discovered in association with the collapse of salamander populations in northern Europe. Analyses of host-pathogen dynamics suggest Bsal poses a similar extinction threat as its sister fungus *Bd*. However, all studies of Bsal to date have focussed on a single isolate, assuming the European epidemic to be homogenous. Through genomic and phenotypic analyses of *Bsal* strains isolated from across the European *Bsal* epidemic, we identified highly divergent genomic landscapes, rapid evolutionary rates and isolate-specific gene family expansions and acquisitions. Phenotypic analyses found this genomic variation to be associated with surprising levels of phenotypic variation, including isolate-specific metabolic capacities, a saprotrophic lifecycle, and highly variable thermal ranges, which have important implications for developing effective mitigation strategies. We employed comparative genomic analyses of a high passage isolate displaying species-specific reduction in virulence, compared to the index site isolate, to characterise active mechanisms of genomic evolution, and biological processes and molecular functions that may be important in determining pathogenicity.

680W Deciphering the potential niche of novel black yeast fungal isolates in a biological soil crust based on genomes, phenotyping, and melanin regulation *Erin Carr*[1], Quin Barton[1], Sarah Grambo[2], Mitchell Sullivan[1], Cecile Renfro[1], Alan Kuo[3], Jasmyn Pangilinan[3], Anna Lipzen[3], Keykhosrow Keymanesh[3], Emily Savage[3], Kerrie Barry[3], Igor Grigoriev[3,4], Wayne Riekhof[1], Steven Harris[2] 1) University of Nebraska - Lincoln, Lincoln, NE; 2) Iowa State University, Ames, Iowa; 3) US Department of Energy Joint Genome Institute Lawrence Berkley National Laboratory, Berkley California; 4) Department of Plant and Microbial Biology, University of California Berkeley, Berkeley, California .

Black yeasts are polyextremotolerant fungi that contain high amounts of melanin in their cell wall and maintain a primarily yeast form. These fungi grow in xeric, nutrient deplete environments which implies that they require highly flexible metabolisms and the ability to form lichen-like mutualisms with nearby algae and bacteria. However, the exact ecological niche and interactions between these fungi and their surrounding community is not well understood. We have isolated and described two novel black yeast fungi of the genus *Exophiala*: JF 03-3F “Goopy” *E. viscosium* and JF 03-4F “Slimy” *E. limosus*, which are from dryland biological soil crusts. A combination of whole genome sequencing and various phenotyping experiments have been performed on these isolates to determine their fundamental niches within the biological soil crust consortium. Our results reveal that these *Exophiala* spp. are capable of utilizing a wide variety of carbon and nitrogen sources potentially from symbiotic microbes, they can withstand many abiotic stresses, and can potentially provide UV resistance to the crust community in the form of secreted melanin. Besides the identification of two novel species within the genus *Exophiala*, our study also provides new insight into the production and regulation of melanin in extremotolerant fungi.

681T Human p11-mediated re-direction of phagosomes to the recycling endosome-expulsion pathway induced by fungal pathogen *Leijie Jia*[1], Muhammad Rafiq[1,2], Lukáš Radosa[1], Peter Hortschansky[1], Thomas Krüger[1], Franziska Schmidt[1], Thorsten Heinekamp[1], Maria Stassbruger[3], Olaf Kniemeyer[1], Axel Brakhage[1,2] 1) Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Jena, Germany; 2) Department of Microbiology and Molecular Biology, Institute of Microbiology, Friedrich Schiller University, Jena, Germany; 3) Transfer Group Anti-infectives, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Jena, Germany.

The saprotrophic fungus *Aspergillus fumigatus* is an opportunistic fungal pathogen, which causes invasive pneumonia and disseminated infections in immunocompromised patients. The fungus produces hydrophobic spores (conidia) that are released into the air and are continuously inhaled. Because of their small size of 2–3 µm, conidia can reach the alveoli, where they are eliminated by phagocytes and intracellularly by phagolysosomal killing. Without an effective immune response, inhaled conidia invade pulmonary epithelial cells by induced phagocytosis which can lead to the onset of life-threatening infection.
Although dihydroxynaphthalene melanin on fungal conidia has been shown to inhibit phagosome maturation, a proportion of mela-

nin-lacking mutant conidia still escaped killing by phagosomes, implying that additional mechanisms must be in place. The analysis of host pathogen interactions bears the potential to discover novel pathogenicity mechanisms and also to obtain novel insights into fundamental mechanisms of cell biology. In this study, we obtained unprecedented insight under both aspects. We discovered a novel function for the so far uncharacterized fungal surface protein HscA: it binds to the host p11 protein and acts as a fungal effector protein that induces expulsion of conidia. Also, we found that the human p11 protein is a decisive factor for targeting phagosomes either to the degradative or secretory pathway and that this factor is manipulated by HscA. Specifically, after phagocytosis of pathogens, phagosomes undergo a series of maturations steps to develop into phagolysosomes. A key step is the recruitment of Rab7 to phagosomal membranes. *A. fumigatus* induces accumulation of the p11-Annexin A2 tetramer (A2t) on phagocytic cups. The conidial surface effector protein HscA anchors the A2t complex to the membranes of phagocytic cups and phagosomes. This excludes Rab7 recruitment but rather triggers recruitment of Rab11, which is a marker for recycling endosome, and thereby interferes with phagosome maturation. As a consequence, conidia escape phagolysosomal killing by germinating inside of Rab7-negative phagosome or these conidia-containing phagosomes are translocated to the surface of host cells and thereby released to the medium or even transferred to other cells.

682F *Cryptococcus neoformans* transcytosis of human brain endothelial cells likely begins with macropinocytosis. *Dylan Lanser*[1], Amelia Bennett[1], Suvidha Menon[1], Kiem Vu[1], Angie Gelli[1] 1) University of California, Department of Pharmacology SOM, Davis.

Infections of the central nervous system (CNS) caused by fungi have the highest morbidity and mortality when compared to other causative agents of CNS infections. *Cryptococcus neoformans* (*Cn*) is a basidiomycete yeast responsible for hundreds of thousands of deaths annually, primarily among immunosuppressed individuals who develop infections of the CNS. The remarkable ability of *Cn* to cross the blood-brain barrier (BBB) is the basis for its unique neurotropism among fungal pathogens. *Cn* crosses the BBB via either Trojan macrophages or direct transcytosis of brain endothelial cells (BECs). Although the latter crossing method is extensively documented both *in vitro* and *in vivo*, how BECs endocytose *Cn* is unknown. Macropinocytosis is a non-selective form of endocytosis in which actin-supported projections of the cell membrane engulf extracellular material. Based on our observations of plasma membrane ruffles and upregulation of numerous proteins associated with cytoskeleton and membrane rearrangement on BECs exposed to *Cn*, we hypothesize that *Cn* invades BECs by macropinocytosis. Amiloride, a World Health Organization Essential Medicine which blocks Na^+/H^+ exchange, was used to chemically inhibit macropinocytosis in an *in vitro* model of the BBB consisting of BECs grown to confluence on transwell membranes. *Cn* were introduced to the apical chamber at a multiplicity of infection of 5. We quantified transcytosis at 12 h following *Cn* introduction as the concentration of *Cn* colony-forming units beneath the transwell membrane. We found that 250μM amiloride significantly reduced *Cn* crossing relative to carrier control across three independent trials. *Cn* growth was not inhibited by amiloride at the clinically relevant concentration used in this study, indicating these results were not due to amiloride acting on *Cn*. Amiloride also did not inhibit transcytosis of transferrin, which enters BECs by receptor-mediated endocytosis. Our studies suggest that during transcytosis of the BBB, *Cn* invades BECs by inducing macropinocytosis. Additionally, the curbing of *Cn* crossing in our *in vitro* BBB model by a common medicine opens new avenues of research to combat this fungal pathogen, as the current repertoire of antifungal agents are mostly inadequate against CNS-disseminated infections. Current studies are aimed at resolving the spatial and temporal dynamics of *Cn* during macropinocytosis and identifying the regulators facilitating the transcytosis of *Cn* across the BBB.

683W Unmasking chitin in *C. neoformans*: Panic or protection? *Rajendra Upadhya*[1], Woei, C Lam[1], Jennifer, K Lodge[1] 1) Department of Molecular Microbiology, Washington University School of Meidicne, St. Louis.

Chitosan is an important component of the cell wall of *Cryptococcus neoformans*. It is essential for maintaining the integrity of the cell wall during *in vitro* growth and under a variety of environmental stress conditions. Most importantly, it is required for fungal virulence. Three distinct isoforms of chitin deacetylase (CDA) have been identified as being responsible for chitin «masking» via deacetylation to chitosan. The choice of a specific deacetylase for virulence in mammalian infection depends on the species of *Cryptococcus*; in *C. neoformans*, Cda1 is the major deacetylase, whereas in *C. gattii*, Cda3 plays an important role in the conversion of chitin to chitosan during infection. Furthermore, the involvement of specific deacetylases is dependent on the environment in which cryptococcal cells grow. While all three CDAs are dispensable during *in vitro* growth, coordinated activity of both Cda1 and Cda2 is required for fungal virulence in *C. neoformans*, whereas in *C. gattii*, Cda3 alone is sufficient for causing virulence. We show that the culture medium has a significant effect on chitosan biosynthesis. When compared to yeast extract, peptone and dextrose (YPD) grown cells, yeast grown in unbuffered yeast nitrogen base (YNB-U) medium had a 90% reduction in chitosan. As we discovered, *C. neoformans* also alters the pH of the medium during growth. When grown in unbuffered YPD, it raises the pH to alkalinity, but when grown in YNB-U, it lowers the pH to acidity. When YNB-U grown cells were compared to YPD or YNB, pH 7, the decrease in chitosan was associated with a significant increase in pathogen-associated molecular patterns (PAMPs) on the cell surface. When tested in a murine infection model, the altered cell wall architecture resulted in a significant reduction in virulence. Furthermore, when heat-killed cells were used for infection, KN99 grown in YNB-U caused an abnormal hyper-inflammatory response in the lungs, resulting in the death of the animals. Heat-killed KN99 cells grown in YNB, pH 7, on the other hand, caused little to no inflammatory response in the host lung, but when used as a vaccine, they conferred a robust protective response against a subsequent challenge infection with the virulent KN99 cells. These findings highlight the importance of chitin and its chitosan derivative in shaping the organization of the *C. neoformans* cell wall, impacting fungal virulence and pathogenicity.

684T Connecting fungal genomes with the behavioral phenomes of ants, manipulated by *Ophiocordyceps* *Charissa de Bekker*[1], Ian Will[1], William Beckerson[1], Devin Burris[1] 1) University of Central Florida, Orlando, Florida, USA.

Transmission-promoting summiting behavior is a common parasitic manipulation, observed in a wide range of insect species infected by zombie-making parasites, including fungi. Yet, the molecular mechanisms that the fungi have evolved to hijack host behavior and the affected host pathways that give rise to altered behavioral phenotypes remain largely unknown. To provide a mechanistic perspective, we have developed *Ophiocordyceps camponoti-floridani* and its carpenter ant host as a model. Through our infection assays we found that *Ophiocordyceps*-infected individuals lose their ability to forage effectively, demonstrate a reduced communication, and undergo full-body tremors. Subsequently, towards the end if the infection, the ants climb towards an elevated position in which they latch on with their mandibles to facilitate fungal fruiting body formation and spore dispersal. To begin to unravel the fungal compounds that are

involved in establishing these extended phenotypes, we combine comparative transcriptomics and metabolomics, with quantitative behavioral studies, micro-CT analyses and functional genetics assays. As such, we have begun to identify various candidate fungal compounds and ant host pathways that appear to be involved in the manipulated summiting of *Ophiocordyceps*-infected carpenter ants. These candidates include secreted enterotoxins, a protein-tyrosine phosphatase known to induce manipulations in caterpillars, an aflatrem derivative, and various novel small secreted proteins. We are currently testing the functions and involvement in manipulation of these compounds through knock outs, heterologous gene expression and protein production. As such, our integrative efforts are beginning to connect behavioral phenotypes of infected ants with the underlying fungal genes that give rise to those phenotypes.

685F Pyricularia HAG effector family interactions with rice candidate target proteins *Nicholas Farmer*[1], Meilian Chen[2], Guodong Lu[3], Zonghua Wang[2,3], Daniel Ebbole[1] 1) Texas A&M University; 2) Minjiang University; 3) Fujian Agriculture and Forestry University.

Plant pathogen effectors play important roles in parasitism, including countering plant immunity. However, investigation of the diversification of fungal effectors is limited. Previously we described a 21-member gene family of the rice blast fungus Pyricularia oryzae that we named host-adapted genes (HAGs). Most AVR/effector genes of P. oryzae are either unique or have few paralogs. The presence of such a high number of paralogous HAG effectors suggests the potential for both redundancy and diversification in effector function. Redundancy may allow for the loss of some gene family members without loss of virulence activity. In fact, most members of the gene family display presence/absence polymorphism in the rice infecting population. Redundancy may also allow for more precise regulation of effector expression or adaptation to allelic variation of host targets. On the other hand, divergence can allow for expansion of effector target repertoires that can also lead to increased fitness. Closely related Pyricularia species contain orthologous gene family members. However, in many cases the sequence divergence of orthologs is as great as is found between paralogs. One view is that orthologs would display conservation of host target interactions and paralogs would display diversification. We have begun to test these assumptions using Yeast Two-Hybrid assays to identify candidate rice target proteins that interact with members of the HAG effector family. Putative targets identified via Yeast Two-Hybrid were cross tested with the paralogous effectors from P. oryzae as well as orthologous effectors from other closely related Pyricularia species, allowing us to define overlap in the target repertoires of these effectors.

686W Validation and characterization of *Pyrenophora teres* f. *teres* effectors VR1 and VR2 conferring virulence on Rika barley *jinling li*[1,3], Nathan Wyatt[1,3], Robert Brueggeman[2], Timothy Friesen[3] 1) Department of Plant Pathology, North Dakota State University; 2) Department of Crop and Soil Science, Washington State University, Pullman, WA, USA; 3) Cereals Research Unit, US Department of Agriculture-Agricultural Research Service, Fargo, ND, USA.

The fungal pathogen *Pyrenophora teres* f. *teres* causes the barley foliar disease net form net blotch (NFNB), a devastating disease with the potential to cause substantial yield loss across barley growing regions worldwide. Although *P. teres* f. *teres* has been increasingly recognized as an economically important barley pathogen, little is known of the molecular mechanisms involved in *P. teres* f. *teres* virulence. A previous study using a biparental population of *P. teres* f. *teres* isolates 15A and 6A identified two quantitative trait loci (QTL), namely, *VR1* and *VR2*, each conferring virulence contributed by isolate 6A on Rika barley, however, the specific genes underlying fungal virulence remain unknown. In this study, we identified several candidate genes, with priority given to genes encoding small, secreted proteins (SSPs), that were polymorphic between the parental isolates. CRISPR-Cas9 based gene disruption of one of the candidate genes within the *VR1* QTL region made a virulent progeny isolate harbouring *VR1* but not *VR2* avirulent on Rika barley. Also, an isolate avirulent on Rika complemented with *VR1* made this new strain virulent on Rika barley, further supporting this gene as the *VR1* effector gene contributing virulence on Rika barley. Five candidate genes were also identified within the *VR2* QTL region. Gene disruption of one of these candidate genes made a virulent isolate avirulent on Rika barley, indicating that this gene was *VR2*. Additional gene characterization is underway using CRISPR-Cas9-based gene editing of *VR1* and *VR2* in their native positions.

687T Unravelling the role of CRZ1 dependent F-BAR protein in mediating virulence of *Ascochyta rabiei* *Ankita Shree*[1], Praveen K. Verma[1,2] 1) National Institute of Plant Genome Research, New Delhi, India; 2) School of Life Sciences, Jawaharlal Nehru University, New Delhi, India.

Filamentous fungi respond to various environmental stimuli through the perception of signal cues. The association of the pathogen with its cognate host initiates downstream signalling cascade which results in an intracellular response. Previous reports suggest that endocytosis in fungi is critical for the uptake of nutrients from the host, polarized hyphal growth, receptor internalization, and activation of downstream signalling cascades. Furthermore, coordinated polarized hyphal growth in filamentous fungi is prerequisite for its host penetration and colonization. In this study, we have identified a unique *ArF-BAR* gene from the necrotrophic fungal pathogen *Ascochyta rabiei* (*A. rabiei*) as a potential determinant of fungal virulence. *A. rabiei* enforces a huge challenge in the productivity of its host legume crop *Cicer arietinum*. The functional role of *ArF-BAR* is being further established using gene-knockout (Δ*arf-bar)*, as Δ*arf-bar* strain exhibited reduced virulence on its host. However, the complemented strain (Δ*arf-bar/ArF-BAR*) phenocopies the virulence of wild-type *A. rabiei*. The Δ*arf-bar* mutant have compromised endocytosis, exhibits delayed formation of apical septa, and impairment in secretion of the fungal effector. Additionally, we unravelled a stress-induced zinc-finger transcription factor ArCRZ1, as an upstream transcriptional regulator of *ArF-BAR.* This study explores the novel mechanism by which fungal pathogen regulates and targets key pathways involved in fungal endocytosis and virulence crucial for host colonization. Overall, the functional implication of ArF-BAR protein in modulating the fungal virulence via endocytosis, actin remodelling and effector secretion will be discussed.

688F Calcineurin regulates ß(1,3)-glucan exposure in *Candida albicans* *Andrew Wagner*[1], Stephen Lumsdaine[1], Mikayla Mangrum[1], Ainsley King[1], Todd Reynolds[1] 1) University of Tennessee, Knoxville.

The ability to evade the host immune response is an essential virulence factor deployed by pathogenic fungi to successfully cause disease. For the opportunistic human fungal pathogen *Candida albicans*, this is achieved in part by masking the immunogenic cell wall epitope ß(1,3)-glucan under an outer layer of mannosylated glycoproteins. Consequently, increased ß(1,3)-glucan exposure (unmasking) reveals *C. albicans* to the host's immune system and attenuates its virulence. As drug resistance and other limitations continue to be an issue with the current antifungal regiment, leveraging unmasking as an alternative therapeutic approach is beginning to gain at-

tention. Yet, in order to effectively employ this approach as an immunotherapeutic strategy, an understanding of how *C. albicans* actively regulates its levels of exposed ß(1,3)-glucan is needed. We previously reported that activation of the Cek1 mitogen activated protein kinase (MAPK) pathway by a hyperactive upstream kinase mutant (*STE11$^{\Delta N467}$*) induces unmasking, enhances macrophage recognition and cytokine production *in vitro* and reduces kidney fungal burden by ~33-fold during systemic infection in mice. Furthermore, we have shown that activation of Cek1 stimulates a second signal transduction pathway via the cell wall sensor Dfi1, and this second pathway is required for full unmasking by *STE11$^{\Delta N467}$* expression. Here, we demonstrate that the Dfi1-mediated unmasking is acting through the calmodulin-calcineurin signaling pathway, as both chemical and genetic interruptions within this pathway successfully reduced Ste11$^{\Delta N467}$-induced ß(1,3)-glucan exposure. Moreover, we found that calcineurin plays a role in a more general unmasking phenomena, as inhibition of calcineurin, but not the MAPK Cek1, also impaired unmasking induced by the antifungal caspofungin. In order to identify the mechanism driving calcineurin-induced changes in ß(1,3)-glucan exposure, we next utilized public datasets to identify conserved genes differentially regulated during both *STE11$^{\Delta N467}$* expression and caspofungin exposure. In doing so, we identified two cell wall proteins, Fgr41 and C1_11990W_A, that are down-regulated in each condition. Genetic deletion of either of these targets causes ß(1,3)-glucan exposure, suggesting that downregulation of these proteins may contribute to regulating unmasking. Collectively, our data implicate calcineurin and its downstream regulon as general mediators of unmasking in response to multiple stimuli.

689W Elucidating Fungal Immune Receptors and Testing the Potential Role of Nucleotide-binding Domain Leucine-rich Repeat-like Proteins (NLR-like) Against Bacterial Antagonists. *Frances Stark*[1], Ksenia Krasileva[1], N. Louise Glass [1] 1) University of California, Berkeley .

Filamentous fungi are hosts to pathogens such as viruses, bacteria, parasitic fungi, and grazing nematodes. Besides RNAi to protect fungal genomes from mycoviruses, a fungal inducible defense upon recognition of bacteria has yet to be fully described. Genes encoding nucleotide-binding domain Leucine-rich repeat-like (NLR-like) proteins are present in abundance in the genomes of filamentous fungi. NLRs are intracellular receptors known to mediate cross-kingdom, antagonistic communication in plants and metazoans. Although a role for NLR-like proteins in fungi has been described for allorecognition known as heterokaryon incompatibility, evidence of cross-kingdom surveillance of fungal NLR-like proteins is lacking. In order to investigate if fungal NLR-like proteins participate in an inducible response like plant and animal NLRs, I utilize Neurospora crassa and various bacteria with a primary focus on the seventeen putative NLR-like proteins encoded in the N. crassa genome. I show that exposure of N. crassa to bacteria and bacterial secretions results in an environmental-dependent response including growth defects, increased growth rate, macroconidia production, and cell death. These results suggest that N. crassa is initiating many physiological changes, including programmed cell death upon recognition of bacteria that might be constituting a putative immune response. In order to investigate these responses, I plan on conducting RNAseq, reverse genetics of NLR-like genes, and Genome Wide Association studies of N. crassa environmental isolates. The discovery of genes underlying an immune-like response within the kingdom of fungi will not only lead to a better understanding of basic fungal biology but possibly identify novel tiggers of programmed cell death pathways to target destructive fungi or bacterial/fungal relationships.

690T Targeted delivery of antifungal liposomes to *Rhizopus delemar* *Quanita Choudhury*[1], Suresh Ambati[1], Xiaorong Lin[1], Zachary Lewis[1], Richard Meagher[1] 1) University of Georgia, Athens, GA.

Rhizopus delemar is an opportunistic fungal pathogen and the primary causative agent of mucormycosis, an invasive infection with mortality rates often exceeding 50%. Hallmarks of mucormycosis include angioinvasion and the production of a ricin-like toxin (mucoricin). Treatment usually involves repeated injections of fungicidal amphotericin B (AmB). However, AmB's extended use is restricted by its severe toxicity concerns. Our research group has developed a novel technology in which Dectin immune receptors, which recognize fungal cell oligosaccharides, are incorporated onto the outer surface of an antifungal-loaded liposome (DectiSomes). The Dectin receptors guide the liposomes to fungal cells, concentrating them away from human cells and thus minimizing potential toxicity effects. I am currently evaluating the efficacy of these DectiSomes against *R. delemar*. DectiSomes bind to *R. delemar* hyphae at least orders of magnitude more effectively than uncoated antifungal-loaded liposomes. Preliminary data indicate that DectiSomes also inhibit fungal metabolic activity in vitro and reduce lung fungal burden in a neutropenic mouse model of pulmonary mucormycosis. We continue to explore the potential of this exciting novel therapeutic for treating mucormycosis.

691F Dectisomes Target Antifungal Drugs for Fungal Cells Suresh Ambati[1], Quanita Choudhury[1], Xiaorong Lin[1], *Zachary Lewis*[1], Richard Meagher[1] 1) University of Georgia.

Candidiasis, aspergillosis, cryptococcosis are three of the four most life-threatening invasive fungal diseases with several million new cases annually. Their mortality rates following antifungal drug therapy generally exceed 25 to 50%. Annual medical costs to treat these invasive fungal diseases in the U.S. top several billion dollars. The risk of acquiring invasive mycoses have steadily increased for the last 4 decades from increases in the numbers of HIV AIDS patients and for individuals taking immunosuppressants as part of stem cell or organ transplants or implantation of medical devices. Current antifungal drug therapies are not achieving satisfactory efficacy because (1) they do not provide sufficient fungal clearance to prevent relapses, (2) most antifungals become toxic with extended use, (3) drug resistant fungal isolates are rapidly emerging, and (4) only one new class of antifungal drugs has been approved for clinical use in the last two decades. In our innovative design of DectiSomes, anti-infective drug loaded lipid nanoparticles (e.g., liposomes) are coated with pathogen receptor protein that targets them to pathogenic cells. In particular, we have constructed Amphotericin B loaded liposomes (AmB-LLs) and coated them with the carbohydrate recognition domains of three C-type lectins, Dectin-1, Dectin-2 and DC-SIGN, which collectively bind to oligoglucans, oligomannans, and oligolipomannans present in fungal cell walls and their extracellular matrixes. Relative to untargeted AmB-LL, one or more of these DectiSomes show order of magnitude increases in the binding and in the killing of *C. albicans*, *C. neoformans*, and *A. fumigatus* in vitro. The various DectiSomes have shown similarly improved efficacy in mouse models of pulmonary aspergillosis and invasive candidiasis. Mice treated with DectiSomes showed dramatically reduced organ fungal burden and prolonged survival compared to those treated with untargeted liposomes. Thus, DectiSomes have the potential to usher in a new antifungal drug treatment paradigm and provide the badly needed leap forward in antifungal drug development.

692W *Saccharomyces cerevisiae* var. *'boulardii'* host interactions and the virulence-related gene heme oxygenase-1

(*HMX1*) *Alexandra Imre*[1], Renátó Kovács[1], László Majoros[1], Zsigmond Benkő[1], István Pócsi[1], Walter Pfliegler[1] 1) University of Debrecen, Debrecen, Hungary.

Saccharomyces yeast probiotics (*Saccharomyces 'boulardii'*) have long been successfully applied in the treatment of several gastrointestinal conditions, including *Clostridium difficile* infection and diarrhea, in microbiome management, and have gained traction in animal husbandry/nourishment. However, these products sometimes cause fungaemia in patients treated with probiotics. The occurrence of such cases is likely to be underestimated, so research on the pathomechanism and adaptive properties of probiotic yeast is important. The potential virulence attributes of *S. 'boulardii'* as well as its interactions with the human immune system have thus been in the focus of various studies. Nevertheless, no information is available on how their phenotypes and virulence factors may change under the selective pressure exerted by the human host's body upon infection or colonization.
To extend observations to yeasts that underwent potential in-host selection, we obtained both commercial and clinical isolates of probiotic yeasts and compared their general phenotypic and genomic characteristics, virulence factors, immunological interactions, and pathogenicity (in BALB/c immunosuppressed mouse model). We also exploited CRISPR/Cas9 technology to conduct gene deletions to determine the effects of potential virulence genes, including the gene heme oxygenase-1 (*HMX1*), involved in iron recycling and utilization. The tolerance of wild-type isolates and deletion mutants to iron starvation, their hemolytic activity, and *in vivo* pathogenicity of the strains were also investigated.
Our results showed that in-host selection is not directed towards refining and higher expression of virulence factors commonly associated with pathogenic yeast. Instead, the probiotic yeasts in products already possess characteristics that enable them to act as pathogens upon permissive conditions. Furthermore, the deletion of the *HMX1* gene significantly ($p = 5.5 \times 10^{-14}$) increased the survival of *S. 'boulardii'* strains in the bloodstream, but it did not affect the survival rate of the mice. These results call attention to the need for caution in the development of designer yeast probiotics, as, surprisingly, the lossoffunction of even one gene can increase viability in the bloodstream, which increases the risk of fungemia caused by probiotics. A more thorough assessment will thus be needed in potential future probiotics, especially in an era when genetically modified, designer probiotics are gaining momentum.

693T Pathogen carbon metabolism influences host immune response during infection by *Cryptococcus neoformans* *Hannah Berguson*[1], Lauren Caulfield[2], Lori Neal[3], Michal Olszewski[3,4], Michael Price[1,5] 1) Department of Molecular and Cellular Sciences, College of Osteopathic Medicine, Liberty University, Lynchburg, Virginia USA; 2) Department of Biology and Chemistry, Liberty University, Lynchburg, Virginia, USA; 3) Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan, USA; 4) VA Ann Arbor Health System, Ann Arbor, Michigan, USA; 5) Department of Medicine, Duke University School of Medicine, Durham, North Carolina, USA.

Cryptococcus neoformans is an emerging fungal pathogen with worldwide distribution and significant mortality in sub-Saharan Africa. The fungus initially colonizes in the lungs then spreads to the central nervous system, causing meningoencephalitis. Colonizing these two very different host niches requires utilization of unique carbon sources and evasion of host defenses. Blocking key genes in carbon utilization can profoundly affect the virulence of *C. neoformans* while seemingly protecting the pathogen from host clearance. Further understanding of the relationship between *C. neoformans* carbon metabolism and communication with host immunity is crucial to understanding its ability to cause disease. This study explores the signaling between *C. neoformans* and sentinel immune cells during initial colonization and the alteration of immunological recognition in response to colonization of the lung by carbon utilization deficient strains. We found that the *pyk1Δ* mutant of *C. neoformans* causes significantly decreased cytokine levels compared to wild-type in vivo. This was true for both protective and non-protective cytokines. These results further characterize the relationship between carbon utilization and immunogenicity of *C. neoformans*.

694F Linkage analysis of clinical isolates in the *Cryptococcus neoformans* ST93 clade reveals two non-recombining populations with different *in vivo* disease manifestations *Katrina Jackson*[1], Peter Tiffin[1], Kirsten Nielsen[1] 1) University of Minnesota.

Cryptococcus neoformans is a human pathogenic basidiomycete yeast that can cause cryptococcal meningitis, predominantly in immunocompromised individuals. Patient outcome depends on both host and pathogen specific factors, including the genetics of *C. neoformans*. Sequencing has revealed over 100 sequence types (ST) of *C. neoformans* that are associated with both geographic location and clinical outcome in patients. In the sub-Saharan African country of Uganda, which has high rates of cryptococcal meningitis, the most frequently isolated clinical isolate is ST93 – a common sequence type found globally. In a previous study, we performed whole genome sequencing on 38 ST93 Ugandan clinical isolates. We identified 652 unique SNPs in this ST93 population compared to the H99 reference genome. We also showed that ST93 contained two subpopulations: ST9A and ST93B. In the current study, we further analyzed the genetic and virulence differences between ST93A and ST93B. We performed a linkage disequilibrium analysis on the 652 ST93 SNPs and found that 30% of the SNPs were in long range linkage disequilibrium (LRLD) when mapped to the H99 genome. Examining the LRLD in the subpopulations, 30% of SNPs in ST93B were in LRLD but only 15% of SNPs in ST93A were in LRLD Additionally, we showed that many of the groups of linked SNPs were specific to either ST93A or ST93B, indicating that ST93A and ST93B are evolutionarily distinct, non-recombining populations. The linkage groups tracked with phylogeny and had a defined linkage structure in ST93B but not ST93A, suggesting that ST93A isolates may be recombining at a higher frequency than ST93B isolates. We investigated chromosomal changes and large structural variations (SVs) between ST93A and ST93B using both long read sequencing and pulsed field gel electrophoresis and identified a chromosomal translocation event wherein chromosome 2 and chromosome 11 had swapped arms. Finally, we were interested in whether there were any differences in virulence between the ST93A and ST93B subpopulations. We infected mice with 35 isolates from ST93A and ST93B and found that lethal non-CNS disease occurs more frequently with ST93B isolates, whereas infections with ST93A isolates more frequently resulted in either meningitis or an avirulent controlled infection. Taken together, our mouse virulence studies suggest that population structure can be linked to virulence in some instances, but that recombination in some *C. neoformans* sub-populations enhances isolate diversity and makes identification of genetic virulence determinants difficult.

695W Roles of candidalysin of *Candida albicans* in the gut permeability and brain pathology *Courtney Smith*[1], Eun Young Huh[2], Jenny Hsieh[3,4], Soo Chan Lee[1,4] 1) South Texas Center for Emerging Infectious Diseases (STCEID), Department of Molecular

Microbiology and Immunology, University of Texas, San Antonio, TX; 2) Naval Research Unit – San Antonio (NAMRU-SA), Maxillofacial Injury and Disease Department; 3) Brain Health Consortium, University of Texas, San Antonio, TX; 4) Department of Neuroscience, Developmental and Regenerative Biology, University of Texas, San Antonio, TX.

Candida albicans is one of the most researched and clinically isolated commensal fungal pathogens that has a major public health impact. In the past few decades, there have been increasing amount of data that shows neurodegenerative diseases, such as Alzheimer's disease (AD), may have a microbial origin. AD is characterized as a progressive neurological disorder that that destroys memory along with other important mental functions; it is also associated with the accumulation of amyloid plaques. It has been documented that *C. albican*s has colonized approximately 89% of AD patient's brain biopsies. However, the mechanism as to how *C. albicans* migrates to the brain and what fungal factor(s) contribute to AD pathology remains unknown. Our data for both *in vivo* mouse and *in vitro* cell line models, it is suggested that a toxin secreted by *C. albicans*, called candidalysin, increases gut permeability of the epithelial barrier permitting the fungus to migrate from the gut to the brain. These results strongly suggest that candidalysin plays a key role in the migration of *C. albicans*. Currently, we are testing how candidalysin affects brain pathology and endothelial cell permeability. Therefore, this will improve our knowledge of fungal pathogenesis and its contribution of commensal fungi have on AD pathology.

696T Elucidating key interactions between *Coccidiodies* and macrophages *Jane Symington*[1], Allison Cohen[1], Anita Sil[1] 1) UCSF, San Francisco, CA.

Coccidioidomycosis or Valley Fever is a fungal infection caused by *Coccidioides* spp. with potentially life-threatening sequelae. Infection is caused after inhalation of spore (arthroconidia) from the environment, which develop into spherules that contain internal cells called endospores which can in turn differentiate into new spherules after their release. There is a wide range of outcomes of infection, from asymptomatic infection to meningitis. The host and fungal factors that lead to the stark differences in outcomes remain unknown. We are interested in understanding the role of innate immune cells in the early host response to infection, specifically the role of macrophages in host response *Coccidioides* arthroconidia. Although macrophages are key players in the host response to many other fungal infections in the lung and can be subverted as a niche by some of those pathogens, little is known about the interaction between coccidioides and macrophages. We use a facile cell culture model (infection of murine bone marrow derived macrophages) to define key events that occur during macrophage infection with *Coccidioides* arthroconidia. We show that bone marrow derived macrophages phagocytose *Coccidioides* arthroconidia. Early phagocytosis of arthroconidia is decreased in C3aR1-/- bone marrow derived macrophages. Additionally, arthroconidia can transition to spherules in the presence of macrophages in culture and that small spherules can be found within macrophages. We are currently using transcriptional profiling to identify and characterize host pathways that play key roles in the macrophage response to *Coccidioides* in vitro as arthroconidia develop into spherules.

697F Roles of *Candida albicans* chromosome instability in the host *Huijuan Yan*[1], Suzanne Noble[1] 1) University of California, San Francisco.

Candida albicans is a fungal pathobiont that colonizes the gastrointestinal tract of most healthy humans. It is also the most common cause of fungal infectious disease. Recently, our lab discovered that *C. albicans* maintains two histone-based systems (*H2A.1* and *H2A.2*) that promote chromosome instability under in vitro conditions. I hypothesize that *C. albicans* noncanonical *H2A.1* improves fungal fitness in at least one host niche. Consistent with this hypothesis, I found that WT *C. albicans*, which expresses two copies of noncanonical *H2A.1* and two copies of canonical *H2A.2*, is more virulent in a mouse bloodstream infection model than an isogenic all-*H2A.2* strain that exhibits more accurate chromosome segregation under in vitro conditions. In line with this, the all-*H2A.2* strain is also outcompeted by the WT strain in infected kidneys. However, WT and the all-*H2A.2* strains exhibit similar fitness in a mouse model of gut colonization, suggesting that moderate chromosome instability promotes virulence but not commensalism in *C. albicans*. In parallel with this work, I propose to test the hypothesis that *H2A.1* promotes virulence in the bloodstream model by facilitating the acquisition of adaptive aneuploidies in kidneys, the major target organ in this infection model. Monotypic infections will be performed with WT or the all-*H2A.2* strain, and *C. Albicans* recovered from dissociated kidneys will be evaluated using single-cell RNA-seq to identify aneuploidies. This study will reveal a role for chromosome instability in fungal adaptation to the host and will identify specific genetic changes that correlate with fitness.

698W Identification of the genetic basis of novel azole resistance mechanisms in *Aspergillus fumigatus* *Asmaa Alghamdi*[1], Felicia Stanford[1], Paul Dyer[1] 1) School of Life Sciences, University of Nottingham NG7 2RD.

Aspergillus fumigatus is a ubiquitous saprotrophic fungus that has a primary ecological role in the breakdown and recycling of organic matter. Importantly, *A. fumigatus* is also an opportunistic pathogen responsible for invasive aspergillosis in immunocompromised individuals. The broad ecological niche of *A. fumigatus* is of clinical concern as: (i) the small conidia (2-3µm) produced are easily air dispersed and inhaled into the respiratory track; and (ii) the global emergence of azole resistant isolates in both clinical and environmental settings has contributed to higher mortality rates. Genome sequencing and bioinformatic analysis has identified key mutations in the promoter and coding regions of the cytochrome P450 (*cyp51A*) gene that can be responsible for resistance to azole antifungals. However, a rising number of azole resistant isolates have been reported which exhibit non-*cyp51A* based resistance. Here we applied Mendelian genetic analysis and bulk segregant analysis (BSA) in tandem with next-generation sequencing to investigate the genetic basis of azole resistance in *A. fumigatus*. In our study, we analysed twelve clinical isolates with supposed non-*cyp51A* mechanisms of azole resistance. The majority of isolates showed monogenic patterns of inheritance for resistance, with only a few isolates exhibiting polygenic (linked to multiple genes) inheritance. One subset of isolates were found to contain a previously undescribed unique 2-4 fold tandem repeat in the *cyp51A* promotor region. Meanwhile, one isolate (C286) that exhibited high resistance to itraconazole, with an unknown monogenic resistance mechanism, was chosen as a candidate for bulk segregant analysis. A sexual cross was set up between C286 and an azole sensitive parent strain of complementary mating type. Multiple rounds of backcrossing between resistant progeny and the sensitive parent were then undertaken. Finally, DNA from progeny were pooled into resistant and sensitive bulks and next-generation sequencing combined with a dedicated bioinformatic pipeline was used to identify candidate resistance genes. Ongoing results will be presented. Our studies highlight the importance of understanding the genetic basis and underlying molecular mechanisms of such mutations in order to facilitate the design of diagnostics to improve treatment outcome

in those suffering from invasive aspergillosis. Collectively, this work has the potential to aid in the research and development of novel therapies.

699T A longitudinal study investigating patient acquisition of azole resistant *Aspergillus fumigatus* (AR*Af*) *Amelie Brackin*[1], Darius Armstrong-James[1], Matthew Fisher [1], Anand Shah[1] 1) Imperial College London.

Aspergillus fumigatus is an environmental saprophytic fungus and primary etiological agent of a spectrum of diseases collectively known as aspergillosis. In chronic respiratory disease, there is a significant burden of Aspergillus related infections with high morbidity and mortality. Azole antifungals are the leading frontline therapy for aspergillosis infections, however, increasing evidence suggests that extensive dual-use of azoles in agriculture and the clinic has facilitated the global emergence and escalation of azole resistant *A. fumigatus* (AR*Af*). To monitor the risk posed by environmental sources of AR*Af,* this study has employed a longitudinal surveillance strategy that screened sputum samples from patients diagnosed with chronic respiratory diseases, patient homes and hospital environments.

Since September 2020, >900 fungal colonies have been isolated from >240 sputum samples from 60 patients diagnosed with chronic respiratory diseases. *A. fumigatus* has been isolated from 45% of patients of which 62% of isolates are resistant to at least one of the leading clinical azoles. We have identified a prevalence of 3% AR*Af* from hospital air samples indicating a potential source of nosocomial acquisition. Furthermore, we have identified heterogenous Aspergillus populations within individual sputum samples that contain both azole susceptible and azole resistant phenotypes, indicting multiple phenotype colonisations or clonal replication and emergence of triazole resistance.

Through whole genome sequencing, we will further characterise the genetic backgrounds of isolates to determine the frequency of resistance alleles and whether patients have been infected by azole-resistant Aspergillus from their environment or acquired during azole therapy. We will examine population structures to determine whether AR*Af* infections are transient or persisting within the host. Lastly, genomic data will feed into a global dataset of >1000 sequenced Aspergillus genomes to explore clonality of environmental and clinical AR*Af* genotypes to investigate potential sources of infection.

700F Involvement of kinase genes in antifungal tolerance in the pathogenic yeast *Candida glabrata* *Colin Clairet*[1], Jeanne Chiaravalli[2], Fabrice Agou[2], Christophe D'Enfert[1] 1) Institut Pasteur, Université de Paris, INRA USC2019, Fungal Biology and Pathogenicity Unit, Paris, France. ; 2) Institut Pasteur, Chemogenomics plateforme, Paris, France..

Candidemia-inducing Candida species are difficult to treat in immunocompromised patients due to several mechanisms including biofilm formation and resistance to existing molecules. Three major classes of antifungals are currently used for treatment of invasive fungal infection (IFI). Polyene antibiotics such as Amphothericin B form complex with ergosterol, a major component of fungal cytoplasmic membranes and disrupt the fungal plasma membrane that results in the leakage of the cytoplasmic content. Azoles, the most widely used antifungals, block the synthesis of ergosterol. Echinocandins are targeting the (1,3)-ß-D-glucan synthase, which produces an essential component of the cell wall. *Candida glabrata,* the second most prevalent species in IFI after *Candida albicans*, is naturally tolerant to fluconazoles and is able to rapidly acquire resistance against other antifungals. New classes of molecules used alone or in combination with existing treatments are therefore urgently needed. Protein kinases hold a key position in the regulation of multiple metabolic processes notably in chemical sensing and adaptation but less than 30% have been functionally characterized in fungal pathogens of humans. Our study combines molecular approaches to decipher the function of protein kinases in antifungal resistance and tolerance together with the identification and characterization of kinase inhibitors with relevant activities in the investigated species. On one hand, *C. glabrata* kinase-encoding genes have been systematically deleted and the resulting mutants have been characterized for their sensitivity to existing antifungals. Seventeen and fifteen kinases involved in respectively azole and caspofungin tolerance have been identified. On the other hand, 10,000 kinase inhibitor molecules have been screened in combination with existing antifungal molecules including azoles and echinocandins. Fifty molecules including known and new scaffolds have been selected for further confirmation. These two complementary approaches will highlight preferred kinases to target as well as potential synergistic molecules to be used in combination with existing treatments.

701W Elucidation of Intrinsic Echinocandin Drug Resistance Mechanisms in Mucorales Fungi *Alexis Garcia*[1], Eun Young Huh[2], Soo Chan Lee[1] 1) The university of Texas at San Antonio; 2) Naval Research Unit San Antonio (NAMRU-SA), Maxillofacial Injury and Disease Department.

Mucormycosis is an emerging infection caused by fungi in the order Mucorales. Due to resistance of Mucorales fungi to antifungal drugs, typical treatments for mucormycosis infections are highly toxic and may lead to the surgical disfiguration of patients as a result of debridement. Even more alarmingly, the mortality resulting from mucormycosis remains unacceptably high reaching up to 90-100% among disseminated infections. Mucormycosis results in life-threatening risks to immunocompromised patients globally. Currently, there is little or no knowledge regarding the mechanisms underlying this antifungal drug resistance. This study is to elucidate the genetic mechanisms underlying the intrinsic resistance of Mucorales to the antifungal drug class echinocandins. Echinocandins are the newest antifungal drug class and act as non-competitive inhibitors of the enzyme β-(1,3)-D-glucan synthase. Mucorales exhibit a resistance to this class of antifungal drugs despite harboring the *fks* gene family which encode the target of this drug. Our study found that the model Mucorales species, *Mucor circinelloides* (denoted *Mucor*), carries three copies of the echinocandin drug target gene (*fksA, fksB,* and *fksC*). A phylogenetic analysis of the *fks* genes revealed that *fksA* and *fksB* were converged from a segmental duplication in an early divergence of Mucorales and *fksC* is distinctly related to the other two. Unlike what we observe in other fungal organisms, the *fksA* and *fksB* genes were found to be essential. Interestingly, we found that, in the presence of the echinocandin micafungin the *fksA* and *fksB* genes were overexpressed. Our study further found that the serine/threonine phosphatase, calcineurin, regulates the overexpression of the *fksA and fksB* genes in response to micafungin as deletion of calcineurin results in a decrease in expression of all the *fks* genes and a lower minimal inhibitory concentration (MIC) to micafungin. Furthermore, the heterokaryotic mutants of the *fksA* and *fksB* exhibit higher sensitivity to micafungin when compared to the wildtype strain. Taken together, this study demonstrates that the *fks* gene duplication and overexpression by calcineurin contribute to the intrinsic resistance to echinocandins in *Mucor*.

702T Immunoprotection against cryptococcosis offered by Znf2 depends on capsule and the hyphal morphology Jianfeng Lin[1], *Nhu Pham*[1], Kenton Hipsher[1], Nathan Glueck[1], Yumeng Fan[1], Xiaorong Lin[1] 1) University of Georgia.

Cryptococcosis is a fatal disease with a morbidity rate of 80% even with antifungal therapies. There is no vaccine to prevent cryptococcosis. Previously, we discovered a transcription factor Znf2, that drives the yeast-to-hyphal transition in *Cryptococcus neoformans.* We found Znf2 to have a dramatic effect on its interaction with the host. Overexpression of *ZNF2* drives filamentous growth, attenuates cryptococcal virulence, and elicits protective host immune responses. Interestingly, vaccination of mice with cryptococcal cells overexpressing *ZNF2* in either heat-inactivated or live form provides significant protection to the host from a lethal challenge by the wild-type clinical isolate H99. We hypothesize that cellular components enriched in *ZNF2*^{oe} cells are immunoprotective. Here, we found that serum from protected mice vaccinated with heat-inactivated *ZNF2*^{oe} cells recognizes cryptococcal antigens that reside within the capsule. Consistently, capsule is required for immunoprotection offered by *ZNF2*^{oe} cells. As expected, serum from protected mice also recognizes antigens from wild-type yeast cells but at a much lower level compared to *ZNF2*^{oe}. Accordingly, a high vaccination dose of the heat-inactivated wild-type cells also provides protection. We have found disrupting Brf1, a chromatin remodeling factor that is important for the initiation of filamentation by Znf2, reduces the antigen level in *ZNF2*^{oe} cells. Deletion of *BRF1* also drastically reduces the protective effect of *ZNF2*^{oe} cells regardless of live or inactive forms, even when the *ZNF2*^{oe}*brf1*Δ strain is avirulent. We questioned whether vaccinating mice with inactivated *ZNF2*^{oe} cells will provide protection from subsequent lethal challenge with CD4 T cells depletion, simulating the situation with AIDs patients. To test this, we first vaccinated mice with inactivated *ZNF2*^{oe} cells at a low and high dose and inactivated H99 cells at a high dose, and we then depleted CD4 T cells right before challenge. The preliminary results indicate that CD4 T cells, but not CD8 T cells are required for the protection. Collectively, our finding confirms the importance of CD4 T cells in the protection against cryptococcosis and emphasizes the need of identifying the cryptococcal surface factors that benefit the host.

703F Copper homeostasis and *Cryptococcus neoformans* cell surface architecture *Corinna Probst*[1], Sarela Garcia-Santamarina[2], Jake Brooks[3], Inge van der Kloet[1], Andrew Alspaugh[1] 1) Duke University School of Medicine: Department of Medicine, Department of Molecular Genetics and Microbiology, Durham USA; 2) Instituto de Tecnologia Quimica e Biologica, Universidade Nova de Lisboa, Oeiras; 3) University of North Carolina at Chapel Hill, Department of Physics and Astronomy, Chapel Hill USA.

The trace element Copper (Cu) is an essential micronutrient, serving as a catalytic cofactor that drives iron uptake and distribution, cellular respiration, ROS detoxification and other activities important for cell proliferation and survival. However, elevated levels of free non-bound copper are cytotoxic, causing ROS damage and mis-metalation of proteins. Therefore, functional copper homeostasis is essential for pathogenic microbes to quickly adapt to changing copper levels within their host during infection. In the opportunistic fungal pathogen *Cryptococcus neoformans* (*Cn*), the Cuf1 transcription factor is the central switch regulating Cu-responsive genes.
The newly identified Cuf1-regulated copper-binding and release protein *Cn* Cbi1/Bim1 is a GPI-anchored copper-associated protein. Highly induced in copper limitation, Cbi1 plays a role in copper uptake through the copper transporter Ctr1. However, the mechanism of Cbi1 action is unknown. Interestingly, deletion of the *CBI1* gene affects cell wall integrity and architecture leading to altered macrophage activation and changes in the expression of specific virulence-associated phenotypes. The copper-deficient *cbi1*Δ mutant strain possesses an aberrant cell wall gene transcriptional signature as well as defects in chitin and chitosan deposition, cell wall carbohydrates previously shown to bind copper ions. In line with this finding, *Cn* strains defective in chitosan biosynthesis show an altered resistance profile when presented with copper stress. Based upon these findings, we suggest a new role for the fungal cell wall in regulating cellular copper levels, shielding the cell from states of excess copper, while serving as a copper storage site during conditions of extracellular copper limitation. Given its ability to bind and release copper and its localization on the cell surface of *Cn*, we further suggest that the Cbi1 protein is likely functioning in shuttling copper from the cell wall to the copper transporter for regulated copper uptake.

704W The Fungal Granuloma: Mechanisms of Fungal Containment and Persistence *Calla L. Telzrow*[1], Shannon Esher Righi[2], Natalia Castro-Lopez[3,4], Althea Campuzano[3], Jacob T. Brooks[5], John M. Carney[1], Floyd L. Wormley Jr.[3,4], J. Andrew Alspaugh[1] 1) Duke University, Durham, NC; 2) Tulane University, New Orleans, LA; 3) University of Texas at San Antonio, San Antonio, TX; 4) Texas Christian University, Fort Worth, TX; 5) University of North Carolina at Chapel Hill, Chapel Hill, NC.

Many successful pathogens cause latent infections in which they persist in the human host long-term but retain the ability to cause disease. The opportunistic human fungal pathogen *Cryptococcus neoformans* establishes latent pulmonary infections upon inhalation of cells or spores from the environment. These latent infections are characterized by granulomas, or foci of chronic lung inflammation that contain dormant fungal cells. Immunocompetent hosts typically maintain these granulomas and do not develop disease during this stage of latency. However, latent infections can reactivate upon immunosuppression, causing these granulomas to break down and release persistent fungal cells that proliferate and disseminate, causing lethal cryptococcosis. Promoting fungal containment within granulomas to prevent reactivation would greatly reduce the incidence and severity of cryptococcal disease. Unfortunately, this characteristic course of *C. neoformans* latency and reactivation is understudied due to limited models, as chronic pulmonary granulomas do not typically form in mouse models of cryptococcal infection. We have developed a novel murine model of cryptococcal granuloma formation. Chronic pulmonary granulomas form in mice inoculated with the *mar1*Δ loss-of-function mutant strain. From the host perspective, granuloma formation in this model requires functional host GM-CSF signaling. From the fungal perspective, the increased immunogenicity of the *mar1*Δ strain, caused by cell wall defects, induces a robust immune response that contains the slow-growing *mar1*Δ cells within granulomas. We are exploring the function of the Mar1 protein to identify fungal processes that contribute to containment and persistence within granulomas. Mar1 localizes to the mitochondria and is required for normal mitochondrial structure and function. Because *C. neoformans* is an obligate aerobe, disrupted mitochondrial function likely drives the cell wall defects and slow growth rate of the *mar1*Δ strain. Mitochondrial defects likely also explain another phenotype of the *mar1*Δ strain, increased fluconazole tolerance, as tolerant cells have altered mitochondrial structure. We propose that its enhanced drug tolerance capacity suggests that the *mar1*Δ strain may also have enhanced "host tolerance", or tolerance to the stressors of the host environment, enabling it to grow slowly and persist within granulomas. This model will advance the understanding of the typical course of human cryptococcal disease progression.

705T Unfolded protein response is critical for the corneal pathogenesis of *Aspergillus fumigatus* *Manali Kamath*[1], Jorge

Lightfoot[1], Emily Adams[1], Kevin Fuller[1,2] 1) Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2) Department of Ophthalmology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Aspergillus fumigatus is a predominant agent of fungal keratitis (FK), an ocular infection resulting in long-term visual impairment and blindness worldwide. The high treatment failure rate for FK underscores the need to identify fungal pathways that could serve as targets for novel antifungals. The site of infection for FK, the avascular cornea, is comprised of a dense collagen matrix that is ostensibly poor in free soluble nutrients. We, therefore, hypothesized that fungi experience a nutrient limitation in the cornea and must utilize collagen (protein) catabolic pathways to support infection. To test this, we infected murine cornea with WT *A. fumigatus* using the corticosteroid epithelial abrasion model of keratitis and found that genes encoding various secreted proteases as well as protein chaperones were up-regulated 48h post-infection relative to baseline *in vitro* conditions (glucose minimal media). These results support a model in which *A. fumigatus* upregulates secreted hydrolases in the nutrient-poor cornea, which in turn places the fungus under endoplasmic reticulum (ER) stress. As it is known that the unfolded protein response (UPR) is critical for maintaining the secretory capacity and growth of *A. fumigatus* during ER stress, we next hypothesized that UPR is essential for corneal virulence. To test this, we first deleted the *A. fumigatus hacA* gene, encoding the bZIP transcription factor that is critical for UPR function. Consistent with previous reports, the *A. fumigatus hacA* KO displayed hypersensitivity to stresses that induce protein misfolding, including increased temperature, DTT, and tunicamycin. The mutant was also deficient in secreted collagenase activity and had severe growth defect on biological tissue *ex vivo*. We then tested the virulence of this mutant in the above-described corneal infection model. In contrast to WT-infected corneas, those infected with the *hacA*-KO failed to develop signs of corneal disease (opacification), displayed normal corneal structure (optical coherence tomography), and did not harbor viable fungus (CFUs). This corresponded with the histopathology and flow cytometry analyses which demonstrated reduced inflammation in *hacA*-KO infected corneas. Our results are consistent with our hypothesis that the UPR is essential for *A. fumigatus* nutrient acquisition on protein-rich substrates as well as virulence in a murine model of FK. These data suggest that the fungal UPR could serve as a target for antifungal intervention.

706F Fungal hypoxia adaptation is critical for the establishment of keratitis *Jorge Lightfoot*[1], Emily Adams[1], Kevin Fuller[1] 1) University of Oklahoma Health Sciences Center.

Fungal keratitis (FK) is a sight threatening infection of the cornea affecting 1-2 million people annually. Antifungal intervention fails in up to 60% of patients, resulting in long-term vision loss or a need for corneal transplantation. The need for more effective antifungals is therefore clear, but their development requires a better understanding of fungal pathways that are critical for corneal growth and virulence. In other host environments, such as the lung, fungal infection drives the development of hypoxia as a result of tissue inflammation, vascular occlusion, and tissue necrosis. Consequently, fungal hypoxia adaptation represent critical virulence determinants in these contexts. It is unknown, however, whether the cornea- a thin, avascular tissue situated at the ocular surface also becomes hypoxic. To test this, we inoculated corneas of C57BK/6j via intrastromal injection with either *Aspergillus fumigatus* and *Fusarium petroliphilum*, two common agents of FK. At 24 h post-inoculation, animals were injected with pimonidazole (Hypoxyprobe), which forms covalent adducts with proteins in hypoxic environments; corneas were then resected for immunofluorescence staining. Corneas infected with either fungus stained positively for Hypoxyprobe, and this correlated with appreciable fungal burden and inflammation in the tissue. In a topical infection model for *A. fumigatus*, the hypoxia staining was observed as early as 12 h p.i., before recruited inflammatory cells were present. Given these results, we next hypothesized that a key regulator of the hypoxic response in *A. fumigatus*, SrbA, would regulate growth within the infected cornea. Accordingly, we first deleted the *srbA* gene and, consistent with previous reports, found the mutant were unable to grow *in vitro* at oxygen levels below 3%. In contrast to corneas infected with wild-type *A. fumigatus*, those infected with Δ*srbA* failed to develop signs of disease (opacification), displayed normal corneal structure (optical coherence tomography), and did not harbor viable fungus (CFUs). This corresponded with histopathology and flow cytometry, which revealed a lack of inflammatory cells in Δ*srbA*-infected corneas. Taken together, these results support a model in which fungal antigen rapidly drives the development of corneal hypoxia, thereby rendering SrbA essential for fungal growth and virulence. Consequently, proteins essential for fungal hypoxia adaptation could serve as targets for novel FK therapy.

707W The *Aspergillus fumigatus* Spindle Assembly Checkpoint components, *sldA* and *sldB,* play roles in maintenance of triazole susceptibility *Ashley Nywening*[1,2], Adela Martin-Vicente[1], Wenbo Ge[1], Xabier Guruceaga Sierra[1], Jarrod Fortwendel[1] 1) Department of Clinical Pharmacy and Translational Sciences, The University of Tennessee Health Science Center, Memphis, TN, USA; 2) College of Graduate Health Sciences, Integrated Biomedical Sciences Program, The University of Tennessee Health Science Center, Memphis, TN, USA.

Aspergillus fumigatus is the most common cause of invasive mold infections in susceptible human populations. Invasive aspergillosis is characterized by high mortality ranging from 30-90%. The recent rise of antifungal resistance in *A. fumigatus* is of increasing concern as infection with resistant isolates is associated with increased treatment failure. Much remains unknown concerning adaptation to antifungal stress and development of antifungal resistance, threatening the future use of triazole antifungals. Protein kinase activity is involved in mediating many cellular processes in fungi. *A. fumigatus* is predicted to encode 147 protein kinases and the influence of these kinases on triazole susceptibility and adaptation to triazole drugs remains largely unknown. Here, we sought to reveal the impact of each of the predicted protein kinases on susceptibility to medical triazoles. CRISPR/Cas9 gene editing was used to generate a library of 118 protein kinase disruption mutants in a wild type genetic background and voriconazole minimum inhibitory concentration (MIC) for each of disruption strain was determined by broth microdilution assays. Initial screening of the protein kinase disruption library uncovered only two disruption mutants with altered voriconazole susceptibility, both with a 4-fold increase in MIC when compared to the parent strain. One of these mutants possessed a disruption of AFUB_074100, an uncharacterized ortholog of the *Aspergillus nidulans* SldA kinase. This kinase is a vital component of the mitotic Spindle Assembly Checkpoint (SAC). To confirm our screen results, we next deleted the entire gene sequence encoding either *sldA* (Δ*sldA*), or that of the predicted SldA-protein binding partner, *sldB* (Δ*sldB*). These mutants were re-examined for susceptibilities to a panel of triazoles, as well as the spindle poison benomyl. Both Δ*sldA* and Δ*sldB* displayed increased triazole MICs, mimicking the *sldA* disruption mutant. Moreover, both deletion mutants exhibited markedly increased sensitivity to benomyl, a phenotype characteristic of SAC dysfunction. Although loss of *sldA* in *A. nidulans* is associated with moderate growth defects, the *A. fumigatus* Δ*sldA* and Δ*sldB* mutants displayed normal growth. Therefore, loss of *sldA* or *sldB* generate

reduced susceptibility to triazole antifungals and play conserved roles in regulation of the SAC. Future studies will focus on delineating connections between SAC dysfunction and triazole resistance in *A. fumigatus*.

708T Extracellular vesicles and biofilms of the pine tree pathogen *Fusarium circinatum* *Thabiso Motaung*[1], Francinah Ratsoma[2], Quentin Santana[3], Brenda Wingfield [4], Emma Steenkamp [5] 1) University of Pretoria; 2) University of Pretoria; 3) University of Pretoria; 4) University of Pretoria; 5) University of Pretoria.

Background: Extracellular vesicles (EVs) transport bioactive compounds with a complex array of functional effects on target cells. However, functional characterization of EVs has been limited to only a few fungal species, and key filamentous pathogens of trees, such as Fusarium circinatum (*Fc*), have been left behind. All microbes in nature form biofilms, which they use to adapt to harsh environmental conditions. Knowledge gaps in EV biology and biofilm formation will limit our ability to assess the extent to which they contribute to pathogenicity and virulence. As a result, we aim to characterize the role of EVs in the context of a biofilm by isolating these vesicles from *Fc*'s planktonic and biofilm cells.
Methodology: *Fc* vesicles were isolated from biofilms and planktonic cells and characterized following the MISEV2018 guidelines, which included TEM, NTA, and proteomics analyses. A sporulating culture (¼ strength PDA, homogenized (1 min) in filter sterile or autoclaved 1x PBS) was used to form biofilms by incubating the plates at 25 °C for 24-72 hrs under stationary conditions to initiate cell attachment and biofilm formation. Biofilms were then characterized using CLSM and SEM, and their viability analysed using XTT. EV-mediated effects of biofilms on *Fc* growth were analysed using biofilm-derived EVs that were co-incubated with conidia for 30-60 minutes, following which, uptake of vesicles and their impact on fungal morphology was analysed using CLSM and on PDA, respectively.
Result: *Fc* produced EVs in line with what has been reported in other fungal species. A similar pigment was observed in the planktonic EV fraction of *Fc*. TEM analyses indicate that *Fc* planktonic and biofilm cells secrete EVs, and the planktonic EV fraction showed a deep purple pellet while the biofilm EV fraction exhibited a cloudy pellet. TEM further revealed intact particles (50nm–200nm) with a characteristic spherical, rosette-, and cup-shaped morphology. The early biofilm phase (24h) of *Fc* exhibited aggregates of conidia. The maturation phase (48-72h) exhibited pronounced hyphal growth. Both phases demonstrated metabolically active cells. SEM analysis of biofilms displayed a complex aggregated growth of hyphal bundles and layers embedded in a partially visible extracellular polymeric matrix.
Conclusion: *Fc* produced EVs in line with what has been reported in other fungal species. A deep purple EV fraction observed in *Fc* may suggest *Fusarium* phytopathogens produce distinct pigmented EV particles. Biofilm formation was also confirmed in line with structural features reported by previous studies. Our pending data on proteomics and uptake analyses, as well as XTT assay will give us more insights into the biology of EVs and biofilms derived from *Fc*.

709F Identification of a gene cluster encoding at least two effector proteins involved in host-specificity of *Sporisorium reilianum* Lukas Dittiger[1], Shivam Chaudhary[1], *Jan Schirawski*[1] 1) Friedrich-Schiller-Universität Jena, Matthias-Schleiden-Institute / Genetics, Germany.

Sporisorium reilianum is a biotrophic basidiomycete fungus and the causal agent of head smut in sorghum and maize. Two different *formae speciales, S. reilianum* f. sp. *reilianum* (SRS) and *S. reilianum* f. sp. *zeae* (SRZ), can specifically form spores in the inflorescences of sorghum or maize, respectively. Both *formae speciales* were shown to be closely related on the genomic level, however, the characteristic features responsible for their host-adaption remain unknown. In a classical genetics approach, we identified a gene cluster consisting of nine genes, predicted to encode secreted effector proteins, whose presence highly correlated with the capacity to cause disease on sorghum plants. Deletion of the gene cluster in SRS resulted in reduced virulence on sorghum and the accumulation of phytoalexins in the inoculated leave - a characteristic sorghum defence response against SRZ infection. We are currently creating and testing gene deletion strains that lack only individual genes of the cluster. So far, we identified two specific cluster genes that are involved in virulence and phytoalexin induction, respectively. Therefore, the identified gene cluster contains at least two genes important for host-specificity of *S. reilianum*.

710W Chemical interactions between fungi and nematodes *Reinhard Fischer*[1] 1) Karlsruhe Institute of Technology (KIT).

Nematode-trapping fungi, such as *Duddingtonia flagrans*, are fascinating predatory microorganisms (1). In a nutrient-rich environment they live as saprotrophs, but if nutrients are scarce and nematodes are present, they can switch to a predatory lifestyle. The switch is characterized by the formation of adhesive trapping networks. The interaction requires complex interspecies communication involving pheromones, secondary metabolites, and virulence factors.
D. flagrans trap formation is repressed at nutrient-rich conditions by fungal arthrosporols and 6-methyl salicylate. The spatial control of the expression of the genes of the arthrosporol polyketide gene cluster leads to production of 6-MSA at the tip of hyphae and arthrosporols at the rear. Both inhibit trap formation and 6-MSA is an attractant for *C. elegans* (2). If nematodes are present and the nematode population reaches a certain level, nematode-derived ascarosides cause the downregulation of the arthrosporol gene cluster. The decrease of the arthrosporol concentration leads to induction of trap formation.
Trap formation requires intracellular signaling through the STRIPAK signaling complex and a cell-communication system at the tip to allow ring closure (3).
Shortly after *C. elegans* is trapped by *D. flagrans,* hyphae penetrate into the worm body and secrete small proteins as virulence factors to overcome the worm defense and lytic enzymes to digest the organic material. For one of such virulence factors, we showed that it is secreted at a bulbous structure close to the entry point of the hypha (4).

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711T Identification and functional characterization of a putative alternative oxidase (AOX) in the smut fungus *Sporisorium reilianum f. sp. zeae*. *Emma Lamb*[1], Hector Mendoza[1], Caroline Culver[1], Luke Schroeder[1], Michael Perlin[1] 1) University of Louisville.

The mitochondrial electron transport chain of the corn pathogen, *Sporisorium reilianum*, consists of the classical protein complexes (I-IV) involved in oxidative phosphorylation coupled to adenosine triphosphate (ATP) synthesis. This canonical route diverges due to the presence of an antimycin-resistant/ Salicylhydroxamic acid-sensitive alternative oxidase (AOX) that functions like complex IV and can prematurely end the flow of electrons. This alternative respiratory component has been previously identified in a wide range of organisms, including the related species, *Ustilago maydis*, and has been linked to pathogenicity, thermotolerance, osmotic and oxidative stresses, developmental transitions, among others. In this study, a putative AOX was bioinformatically identified in *S. reilianum* based on the previously annotated sequence for the *U. maydis* AOX. Inhibition assays using specific mitochondrial inhibitors confirmed the presence of this alternative respiratory component. A gene knockout approach revealed that AOX is involved in the pathogenic stage of the fungal life cycle, with AOX deletion mutants displaying reduced overall disease severity. Most significantly, gene expression analyses revealed that AOX is upregulated during the diploid teliospore stage of the fungal life cycle, suggesting its relevance during metabolic and morphological permutations.

712F Metabolomic profiling of behaviorally manipulated insects infected by "zombie ant fungus" (*Ophiocordyceps*) *Ian Will*[1,2], Geoff Attardo[3], Charissa de Bekker[1,2] 1) Department of Biology, University of Central Florida, Orlando, USA; 2) Genomics and Bioinformatics Cluster, University of Central Florida, Orlando, USA; 3) Department of Entomology and Nematology, University of California - Davis, Davis, USA.

Ophiocordyceps camponoti-floridani, a species of "zombie ant fungus," infects and modifies the behavior of ants to further its own transmission at a lethal cost to the host. Manipulated ants perform a "death grip" biting and clinging behavior to attach themselves to plants. This behavior is understood as a fungal manipulation that benefits the parasite's growth and transmission. The underlying mechanisms of how these fungi can dysregulate animal behavior in such a coordinated manner has yet to be fully understood. To characterize possible compounds that relate to modified behavioral pathways, we performed liquid chromatography – mass spectrometry (LC-MS) on the head capsule of ants displaying the manipulated "death grip" behavior in the laboratory. By contrasting this against the present compounds in the heads of healthy ants, we aim to characterize candidate lipids (e.g. cell-cell communication and interaction), biogenic amines (e.g. neurotransmitters), and polyphenols/"natural products" (e.g. many toxic metabolites). We additionally link these metabolomics data with our previous transcriptomics study in a multi-omics approach to refine hypotheses about candidate mechanisms and fungal effectors at play.

713W Genes for an extended phenotype: Biosynthesis of volatile sesquiterpenes in a pathogenic fungus is used to entice male flies into fatal mating's with infected female cadavers Andreas Naundrup[1], Björn Bohman[2], Charles A. Kwadha[2], Annette B. Jensen[1], Paul G. Becher[2], *Henrik De Fine Licht*[1] 1) University of Copenhagen, Denmark; 2) Swedish University of Agricultural Sciences, Alnarp, Sweden.

To ensure dispersal, many parasites and pathogens behaviourally manipulate infected hosts. Other pathogens and certain insect-pollinated flowers use sexual mimicry and release deceptive mating signals. However, it is unusual for pathogens to rely on both behavioural host manipulation and sexual mimicry. Here, we show that the host-specific and behaviourally manipulating pathogenic fungus, *Entomophthora muscae*, generates a chemical blend of volatile sesquiterpenes and alters the level of natural host cuticular hydrocarbons in dead infected female house fly (*Musca domestica*) cadavers. We used three different approaches unravel chemical attraction pathways in *E. muscae*. First, we quantified male sexual attraction to fungus-killed cadavers and fungal conidia using behavioural assays. Second, we identified the chemical cues eliciting male mating attraction using chemical analyses (GC-MS) and physiological mechanisms enabling males to detect these cues using electroantennography (GC-EAD). Third, we verified the fungus *E. muscae* as source of the behaviourally active volatile compounds in fungus-killed cadavers using transcriptional profiling (RNAseq) of expressed genes in volatile chemical biosynthesis pathways. We show that healthy male house flies respond to the fungal compounds and are enticed into mating with dead female cadavers. This is advantageous for the fungus as close proximity between host individuals leads to an increased probability of infection. The fungus-emitted volatiles thus represent the evolution of an extended phenotypic trait that exploit male flies' willingness to mate and benefit the fungus by altering the behavioural phenotype of uninfected healthy male host flies.

714T Genetic systems and pH stress in the laurel wilt-Ambrosia beetle symbiotic interaction *Ross Joseph*[1], Kamaldeep Bansal[1], Yonghong Zhou[2], Nemat Keyhani[1] 1) University of Florida; 2) Tibet University.

Raffaelea lauricola is a highly virulent fungal pathogen that causes rapid wilting and death of naïve host trees, resulting in the deaths of hundreds of millions of trees in the United states since its introduction in the early 2000s. One of the major drivers of this epidemic appears to be the dissemination of the pathogen from its invasive ambrosia beetle vector to a number of native ambrosia beetle species, thus expanding the range of *R. lauricola* to include new habitats and host trees. Despite the important role that vector transmission plays in the spread of this disease, however, surprisingly little is known regarding the physiological, molecular, and genetic factors that underlie the establishment and maintenance of fungal-beetle symbioses in any such system, and consequently, essentially no information exists on the genetic and molecular mechanisms that may mediate fungal-beetle symbioses. A broad phenomic screen was performed identifying growth sensitivity above pH 7 which could be rescued by the supplementation of certain amino acids, suggesting that environmental cues such as pH and nutrient availability may be important in *R. lauricola* -host (plant or beetle) interactions. Perceiving environmental pH and responding by differential gene regulation is a critical host-sensing mechanism employed by pathogenic fungi, however its role in symbiotic fungal-host interactions has not been explored to date. Here, we build on previously developed high

efficiency transformation systems established in our lab by developing an efficient and targeted CRISPR Cas9 gene editing system for *R. lauricola*. This system employs a fungal codon-optimized *cas9* gene driven by the *R. lauricola gpdH* promoter and a gRNA expression cassette driven by the *R. lauricola* 5S rRNA gene promoter to generate double stranded breaks in the *R. lauricola* genome. Using these tools, we have targeted the *PacC* gene, a transcription factor which broadly regulates gene expression in response to changes in extracellular pH to explore the role of environmental pH sensing in ambrosia symbioses. By comparing rates and dynamics of beetle colonization between mutant and wild-type strains, we can determine the role that environmental pH sensing plays in the sustained mutualism between these organisms, a crucial step towards characterizing the genetic and molecular determinants of insect symbiosis in a virulent plant pathogen.

715F A shelter from the elements: understanding requirements for fungal chlamydospore formation and bacterial invasion *Isabelle Ludwikoski*[1], Nancy Keller[1] 1) University of Wisconsin - Madison, Madison, WI.

Bacterial-fungal interactions (BFIs) drive microbiome dynamics from environmental to healthcare settings, impacting survival and dispersal of the interacting partners. Previous work from our lab established that the plant pathogen *Ralstonia solanecearum* induces formation of swollen, overwintering spores in *Aspergillus* spp. through production of a cyclic lipopeptide called ralsolamycin (1). With deletion of *rmyA*, the polyketide synthase in the ralsolamycin biosynthetic cluster, chlamydospores are not induced. Further, in co-culture *R. solanecearum* can invade the chlamydospores (1). Recent work in the lab identified survival benefits for bacterial invasion under starvation and cold stress conditions compared to mutants unable to invade chlamydospores (2). Additionally, several Gram-negative bacteria unable to invade chlamydospores independently can invade when co-cultures are supplemented with ralsolamycin (2). Thus far most of the work on this system has been done using WT *Aspergillus flavus* and we have little understanding of mechanistic components required by the fungus and the bacterium to undergo the chlamydospore formation and invasion processes. To dig deeper into the mechanisms underlying this process, we performed an RNA-seq analysis which indicated numerous aberrantly regulated proteins involved in cell wall biosynthesis and secretion. We found *fleA,* a gene encoding a lectin that binds fucosylated structures, to be upregulated in the condition with chlamydospore production. Initial data suggests that the loss of *fleA* leads to changes in chlamydospore density. Additional preliminary data suggests that chlamydospore formation in response to ralsolamycin is dependent on density of the spore inoculum, where more chlamydospores are produced at higher density, which may also impact bacterial invasion dynamics.

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716W From iron to antibiotics: Bacterial-fungal interactions revealed by genome-wide mutational analyses *Emily Pierce*[1], Manon Morin [1], Jessica Little[4], Roland Liu[1], Joanna Tannous[2], Nancy Keller[2], Kit Pogliano [1], Benjamin Wolfe[3], Laura Sanchez[4], Rachel Dutton[1] 1) University of California San Diego, La Jolla, CA, USA; 2) University of Wisconsin-Madison, Madison, Wisconsin, USA; 3) Tufts University, Medford, Massachusetts, USA; 4) University of Illinois at Chicago, Chicago, Illinois, USA.

Intermicrobial interactions are key aspects of the biology of microbiomes. Recently, there has been a shift towards studying interactions in more representative contexts, whether using multispecies model microbial communities or by looking at interactions *in situ*. Cheese rind biofilms have been developed as experimentally tractable systems to study microbiomes. Although many studies of microbiomes focus solely on the bacterial community, previous work in the cheese rind system has revealed the importance of fungi in shaping this microbiome via interactions with bacteria. Leveraging this model system, we identified a diversity of bacterial genes involved in, and the associated fungal contributors to, bacterial-fungal interactions. To achieve this, we combined bacterial cytological profiling, RNA-Seq, metabolomics, and random barcode transposon site sequencing, a high-throughput genetic screen of 120000+ bacterial mutants. We characterized bacterial-fungal interactions across 16 bacterial-fungal pairs made up of 8 cheese-associated fungi and *E. coli* or a cheese-associated *Pseudomonas psychrophila*. We observed broad changes in bacterial mutant fitness in the presence of fungi compared to growth alone. The strongest and most widespread bacterial-fungal interaction that we observed suggests that fungal species can dramatically modulate bacterial access to iron through the provision of fungal hydroxamate siderophores, such as ferrichrome and coprogen. It has long been known that bacteria grown in isolation are able to uptake purified fungal siderophores, but the ecological relevance of this putative interaction had not been demonstrated. Our results demonstrated that this exchange takes place between bacteria and filamentous fungi growing in a biofilm and that this exchange can have impacts on the competitive fitness of bacteria. Due to the importance of iron in bacterial physiology and the prevalence of fungi in microbial ecosystems, we expect that iron-based bacterial-fungal interactions are important in other microbiomes. In addition to filamentous fungi, we showed that the basidiomycete skin yeast *Malassezia pachydermatis* alleviated bacterial iron limitation. Moreover, fermented foods are known to contain fungal siderophores, which could be a source of fungal siderophores in the gut in addition to potential siderophore production by gut-resident species.

717T Using random barcoded transposon-site sequencing (Rb-TnSeq) bacterial libraries to explore the effects of volatiles from *Trichoderma atroviride* *Catharine Adams*[1, 2], Jose Manuel Villalobos Escobedi[1, 2], Mitchell Thompson[1,2], Robin Herbert[1,2], Adam Deutschbauer[1,2], Louise Glass[1,2] 1) UC Berkeley; 2) Lawrence Berkeley National Laboratory.

Plant associated fungi provide their hosts with a number of important health related benefits, and can even protect the plant from invading microbial pathogens. *Trichoderma atroviride* IMI is a plant root-associated filamentous fungus with potent antimicrobial effects, and volatile organic compounds (VOCs) from *T. atroviride* have been shown to discourage growth of a range of pathogenic microbes. However, few studies have explored how these VOCs may impact beneficial root-associated microbes. Here, we used Random Barcode Transposon-site Sequencing (Rb-TnSeq) to investigate the mechanisms of how VOCs from T. atroviride affect the physiology of six beneficial bacterial species selected from across the proteobacteria: *Azospirillum brasilense* Sp245 and *Sinorhizobium meliloti* 1021 (alpha-proteobacteria), *Burkholderia phytofirmans* PsJN and *Herbaspirillum seropedicae* SmR1 (beta), and *Klebsiella michigan-*

ensis M5al and *Pseudomonas simiae* WCS417(gamma). We identified 32 genes across these bacteria that may have fungal volatile-induced phenotypes. In *P. simiae*, we see a physiologically consistent set of genes related to cell division and cell wall modification that show lower fitness when disrupted. Similarly, in *B. phytofirmans*, we see a coherent set of genes related to motility. In *S. meliloti*, we found the bacteria had increased fitness when a gene encoding an outer-membrane lipoprotein was disrupted, and this gene product may therefore be a receptor for one or more fungal VOCs. Furthermore, the overall effect of fungal derived VOCs on *S. meliloti* were similar to that of low pH. Follow up analysis revealed that when *S. meliloti* was grown in the presence of *T. atroviride*, the pH of the bacterial growth environment was lowered, suggesting that fungal VOCs have a role in altering host metabolism. On the plant host, low pH is a critical environmental signal for many plant-associated bacteria. Ongoing work with Gas Chromatography-Mass Spectroscopy (GC-MS) will endeavor to identify the precise fungal VOCs involved in these interactions. By elucidating the system wide effects of fungal derived VOCs in the rhizosphere, we can begin to design microbially driven strategies to enhance beneficial plant relationships, and improve overall plant health.

718F Heterothallic mutants of *Fusarium graminearum* and their use for Genetic Analysis of Fungal Pathogenicity and Toxigenicity *Gabdiel Yulfo-Soto*[1], Franklin Machado[2], Aline Vieira de Barros[1], David Van Sanford[3], Emerson Del Ponte[2], Frances Trail[4] 1) Department of Plant Pathology, University of Kentucky, Lexington KY USA 40546; 2) Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa MG, Brazil; 3) Department of Plant and Soil Sciences, University of Kentucky, Lexington KY USA 40546; 4) Department of Plant Biology, Michigan State University, East Lansing MI USA 48824-1312.

Fusarium Head Blight (FHB), caused by *Fusarium graminearum sensu stricto* and other members of the *F. graminearum* species complex (FGSC), is a plant disease that occurs on cereal crop all over the world, reducing yield of grains and causing accumulation of dangerous trichothecene mycotoxins including deoxynivalenol. There is a high degree of genotypic and phenotypic variation among pathogen species and strains, but current FHB models and treatments do not account for pathogen diversity. Therefore, it is difficult to predict what will happen if a new variant is introduced, or if changes in the environment favor one genotype over another. *Fusarium graminearum* is homothallic, and self-fertility is regulated by the MAT1 locus encoding two idiomorphic compatibility genes called MAT1-1-1 and MAT1-2-1. Previous studies have demonstrated that deletion of MAT1-1-1 or MAT1-2-1 produces obligately heterothallic strains. We produced deletion mutants of the MAT1-1-1 and MAT1-2-1 (MAT) genes and screened them to identify appropriate test maters with normal fitness- and pathogenicity-related phenotypes. These strains could be used in matings with wild type (WT) strains to facilitate genetic analyses of traits of interest. Because the deletion strains engage only in heterothallic mating, this solves the problem of identifying outcrossed perithecia. Many of the deletion strains, especially the MAT1-2-1 deletions, were significantly reduced in pathogenicity or fitness compared with their WT progenitor strain PH-1. Strains also varied in female fertility and levels of interfertility with other strains. Two highly female-fertile MAT1-1-1 strains that did not differ from the WT in pathogenicity, toxigenicity, or fitness were used in test matings with two MAT1-2-1 deletion strains that varied in colony morphology and pathogenicity, and with several WT strains including PH-1, another strain of *F. graminearum ss.* (Gz3639), and *F. meridionale*, another member of the FGSC that can cause FHB. Antibiotic resistance, MAT alleles, and fertility had expected 1-1 segregation patterns in the crosses, while segregation patterns related to colony morphology were more complex and indicated absence of linkage to the MAT deletions. We intend to use these mating tester strains to identify novel genetic markers associated with fitness and pathogenicity that could be incorporated into multi-locus genotyping assays to monitor and predict population shifts.

719W Deletion of the killer kinase *KIL1* abolishes penetration peg formation in the predator yeast *Saccharomycopsis schoenii* *Mareike Rij*[1], Yeseren Kayacan[2], Juergen Juergen[1,2] 1) Hochschule Geisenheim University; 2) Vrije Universiteit Brussel.

Predator yeasts are either homothallic or heterothallic ascomycetes of the genus *Saccharomycopsis*. These yeasts represent a unique genus of necrotrophic mycoparasites that infect a wide range of yeasts and filamentous fungi. Infection can be divided into recognition, adhesion, penetration and killing/nutrient uptake phases. For the penetration of a prey cell a dedicated penetration peg is formed. Penetration may be promoted by multigene families of cell wall degrading enzymes, including chitinases, glucosidases and proteases, which are specifically upregulated during predation. Penetration pegs grow in a polarized manner into the prey cell and are highly enriched in chitin. They do not contain a nucleus and do not grow further or develop into daughter cells. Each penetration peg is thus a one-time investment. We have recently sequenced the genomes of several *Saccharomycopsis* species and determined that in this genus the CTG codon is translated into serine instead of leucine. Predation is promoted by starvation as *Saccharomycopsis* yeasts are natural organic sulphur auxotrophs (i.e. methionine auxotrophs). We also developed a toolbox to initiate the molecular characterization of genes potentially involved in this predacious behavior. Deletion of a map kinase gene, *KIL1*, homologous to the *Magnaporthe grisea PMK1* and the *Saccharomyces cerevisiae KSS1* genes results in avirulent strains as determined by dilution series predation spot assays. *KIL1* is specifically required for penetration peg formation as *kil1* cells are unable to differentiate these structures. We will present *in vivo* time lapse data studying the predation process using GFP-tagged strains and *S. cerevisiae* as model prey cells.

720T Conditional role of a signal peptidase component in the establishment of biotrophy by the maize anthracnose pathogen *Colletotrichum graminicola* *Renata Belisario*[1], Lisa Vaillancourt[1] 1) University of Kentucky.

Colletotrichum graminicola infects maize during several phases of plant growth, resulting in anthracnose leaf blight, stalk rot, and top die-back diseases. This ubiquitous fungus has a significant destructive potential and causes millions of dollars of losses annually in North America. *Colletotrichum graminicola* is hemibiotrophic and initially invades living host cells via biotrophic infection hyphae before switching to necrotrophy and inducing host cell death, cell-wall degradation, and lesion development. An insertion mutation in the 3'UTR region of a gene (*Cpr1*), encoding a homolog of the noncatalytic glycosylated SPC22/23 subunit of the signal peptidase complex, impaired pathogenicity to maize leaves and stalks. The mutation is conditional, in that it has little or no effect on growth and development of the fungus in culture, or in dead maize tissues. The *Cpr1* mutant (MT) is interrupted early in infection, and never shifts to necrotrophy, produces lesions, or sporulates. Although it makes appressoria, penetrates epidermal cells, and produces infection hyphae normally, it fails to move biotrophically beyond the first invaded cell. Interestingly, when the MT is inoculated adjacent to the wild type (WT) strain, it can establish a successful biotrophic infection. This suggests that the WT may be secreting one or more factors that induce susceptibility in surrounding host cells. To test the hypothesis that the MT is deficient in the secretion of proteins that are necessary

for pathogenicity, we are visualizing individual effector proteins and cell wall degrading enzymes (CWDE) as fluorescent fusions in the WT and MT strains, and in the complemented MT strain, in maize leaf sheaths. Additionally, we are quantifying secretion of CWDE *in vitro* for all three strains. Since all fungi have a homolog of the *Cpr1* gene and share the conserved secretion pathway, this work may reveal a novel target for broad-spectrum control of fungal pathogens, including the important *Colletotrichum* species. Furthermore, it may uncover a previously unsuspected role for the SPC22/23 subunit in the regulation of secretory activity.

721F Cytoplasmic effector translocation during early biotrophic invasion by the rice blast fungus *Ely Oliveira-Garcia*[1,2], Jungeun Park[3], Melinda Dalby[1], Magdalena Martin-Urdiroz[4], Clara Rodriguez Herrero[4], Sunghun Park[3], Nicholas J. Talbot[4,5], Barbara Valent[1] 1) Department of Plant Pathology, Kansas State University, Manhattan, KS, USA; 2) Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA, USA; 3) Department of Horticulture and Natural Resources, Kansas State University, Manhattan, KS, USA; 4) School of Biosciences, University of Exeter, Exeter, UK ; 5) The Sainsbury Laboratory, Norwich Research Park, Norwich, UK.

Host-adapted lineages of *Magnaporthe oryzae* (synonym of *Pyricularia oryzae*) cause blast diseases on rice, on millets and, most recently, on wheat. We previously presented evidence that the fungus co-opts the host's clathrin-mediated endocytosis (CME) system for internalization of secreted cytoplasmic effectors inside living host cells. Details of when and how this happens remain unclear. We have shown that the specialized blast biotrophic interfacial complex (BIC) is highly enriched in plant clathrin, based on analysis of transgenic rice lines expressing a translational fusion protein with rice clathrin light chain-1 (CLC1) and green fluorescent protein (GFP), infected by *M. oryzae*. Cytoplasmic effectors in BICs are packaged in effector vesicles bounded by GFP-labeled plant plasma membrane. Additionally, effector vesicles can be visualized in the host cytoplasm surrounding BICs at later stages of BIC development. Live cell imaging with various fluorescently-labeled effectors indicate that effector translocation is highly active before and during growth of the tubular primary hyphae (PH) that first colonize host cells. We analyzed a novel effector, Bas170, which accumulates in effector vesicles beneath the appressorium and in the host nucleus before visible growth of the PH with its associated BIC. Early effector translocation is supported by results in which CME was inhibited using both virus-induced gene silencing (VIGS) and pharmacological approaches. Silencing of two rice CME genes, Adaptor protein complex-2 (AP2) and clathrin heavy chain-1 (CHC1) as well as treatment with CME inhibitors caused a 'swollen BIC phenotype' in BICs associated with PH at about 20% of infection sites. However, at the remaining sites, penetration failed to occur and fluorescent cytoplasmic effectors, such as red fluorescent Pwl2, abnormally accumulate in a cloud of fluorescence under the appressorium. Taken together, our results suggest that extensive cytoplasmic effector translocation happens before and during growth of PH, with some residual translocation activity after hyphal differentiation and BIC relocation beside the first bulbous invasive hyphal cells. The challenge now is to find and characterize blast effectors that are involved in co-opting host endocytosis and achieving effector translocation and host cell targeting.

722W Defining the septin interactome and its role in appressorium-mediated plant infection by the rice blast fungus *Magnaporthe oryzae* *Iris Eisermann*[1], Andrew J. Foster[1], Paul Derbyshire[1], Frank L.H. Menke[1], Nicholas J. Talbot [1] 1) The Sainsbury Laboratory, Norwich, UK.

Rice blast disease is initiated by formation of a specialized infection cell by the blast fungus, called the appressorium. The appressorium generates turgor of up to 8 MPa, enabling the fungus to develop a rigid penetration peg to breach the rice cuticle. *Magnaporthe oryzae* possesses six septin GTPases, which play major roles during plant infection. The four core septins Sep3, Sep4, Sep5 and Sep6 collectively form a hetero-oligomeric ring at the appressorium pore, which is essential for plant infection. Two non-core septins, Sep7 and Sep8, belonging to the class 5 group of septins, which includes AspE from *Aspergillus nidulans*, also form a range of membrane and cytoskeleton-associated structures. To reveal the role of each septin during appressorium-mediated plant-infection we have carried out high throughput yeast two hybrid assays, coupled with *in vivo* immunoprecipitation mass spectrometry (IP-MS) experiments, and phosphoproteomics, to define the septin interactome. We have identified a wide range of interaction partners of each septin during appressorium development, including polarity determinants, cytoskeletal components and a range of regulatory proteins. We observed that Sep7 interacts with Sep3, Sep4, Sep5 and Sep6 specifically during early appressorium formation, 4h after conidial germination, forming a plasma membrane-associated complex. Sep8, which contains a transmembrane helix, also interacts with each septin and may link septins to the plasma membrane. We present a model for how septins organise the appressorium pore and deploy polarity determinants to facilitate cuticle rupture and invasive fungal growth.

723T Genotype and Fusarium head blight selection for microbiomes across barley spikes *Brooke Benz*[1], Abbeah Navasca[1], Diel Velasco[1], Thomas Baldwin[1], Samiran Banerjee[1], Briana Whitaker[2], Barney Geddes[1] 1) North Dakota State University, Department of Plant Pathology, Fargo, ND; 2) USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention & Applied Microbiology, Peoria, IL.

Fusarium head blight (FHB), also known as scab, is a devastating disease of wheat and barley that can affect both yield and quality of small grains. The onset of FHB can be sudden during wheat and barley heading, resulting in contamination with trichothecene mycotoxins (e.g., deoxynivalenol [DON]), and turn otherwise healthy crops into a total loss. Historically, breeding for resistance in barley using traditional methods has been difficult to achieve. While microbiome associations have yet to be fully explored, there is an emerging understanding that plants under biotic stress can recruit beneficial microbes in a "cry for help" strategy. We hypothesized that by analyzing the composition of the barley spike microbiome during FHB disease onset in a breeding population across 4 U.S. locations, we could identify groups of microbes that are both recruited in response to FHB and show differential abundance across barley genotypes. Such microbes could represent targets for selective breeding for microbiome protection from FHB, as well as potential biocontrol agents for application in the field. Here, using 16S amplicon sequencing data, we report our preliminary findings on the composition of the barley spike microbiome across 10 genotypes from a malt barley training population experiencing a range of FHB disease. Our results highlight the importance of testing microbially-based traits for breeding in barley resistance to FHB.

724F Unraveling the role of effectors from *Fusarium* spp. in Fusarium wilt of banana *Carolina Aguilera Galvez*[1], Jelmer Dijkstra[1], Lisanne Kottenhagen[1], Giuliana Nakasato-Tagami[1], Gert HJ Kema[1] 1) Wageningen University and Research.

Fusarium wilt of banana (FWB) is caused by a suite of soil-borne *Fusarium*, which invade the roots and colonize the vascular system eventually leading to plant death. Currently, eleven distinct *Fusarium* species cause FWB in various banana accessions. However, *F. odoratissimum* -also known as Tropical Race 4 (TR4)-, which kills Cavendish bananas, and many others, is the prime global threat for banana cultivation. There are no effective control methods for FWB; there are no functional commercial fungicides, biocontrol is ineffective and there are no commercial banana varieties resistant to TR4. It is crucial to unravel the mechanisms underlying disease development that might aid in efficient disease management strategies. One of these strategies relies on effectors, which are *in planta*-secreted molecules. For instance, SIX proteins (For secreted in xylem) are secreted by pathogenic *Fusarium* spp. and contribute to virulence on banana. Comparative genomic and transcriptomic analyses revealed that *Six* genes are differentially expressed in *F. odoratissimum*(isolate II-5) and *F. phialophorum* (isolate CR1.1A). Both isolates show a differential interaction with Cavendish bananas (compatible with II-5 and incompatible with CR1.1A). Interestingly, *Six6* is only upregulated in the compatible interaction. Cell death responses were observed after agro-infiltrations with *Six6* in *Nicotiana benthamiana* and Cavendish. We hypothesize that *Six6* might have an important role in the virulence of isolate II-5 on banana. Overall, this work aims to understand the interaction between effectors of *Fusarium* spp. and banana, which is crucial for the design of novel management options for FWB.

725W Transcriptome analysis and effector prediction in *Fusarium* spp. causing Fusarium wilt of banana *Jelmer Dijkstra*[1], Carolina Aguilera-Galvez[1], Giuliana Nakasato-Tagami[1], Xiaoqian Shi-Kunne[1], Anouk van Westerhoven[1,2], Fernando Garcia-Bastidas[3], Alexander Wittenberg[3], Gert Kema[1] 1) Laboratory of Phytopathology, Wageningen University and Research, Wageningen, the Netherlands. ; 2) Theoretical Biology & Bioinformatics Group, Department of Biology, Utrecht University, Utrecht, the Netherlands.; 3) Keygene N.V., Wageningen, the Netherlands..

Banana is the fourth most important food crop and is the top global fruit with roughly 155 million tonnes produced annually. Besides being an important product for export to the Western market, several million people in the tropics and subtropics, mainly in eastern and central Africa, subsist on a diet primarily consisting of carbohydrates from banana. The global production is, however, threatened by members of the *Fusarium oxysporum* species complex, most importantly *Fusarium odoratissimum,* which contains the devastating Tropical Race 4 (TR4) that kills Cavendish bananas (>50% of global production; >95% of export trade) and many local varieties destined for domestic markets through progressive colonization of the xylem and subsequent wilting. Despite the immense threat posed by TR4, no effective control methods are available and the pathogen-host interaction is poorly understood at a molecular level. To determine the genes that are important for infection in banana and to predict novel effectors, three high quality full genome assemblies were made of three different isolates (II5, NRRL_36102 and CR1.1A). II5 and 36102, both TR4, represent the compatible interaction on Cavendish, II5 being more virulent than 36102. In contrast, CR1.1A represents the incompatible interaction on Cavendish as a Race 1 isolate, the causal agent of the previous iconic epidemic in Gros Michel banana in Central America a century ago. Subsequently, an extensive RNA-seq dataset was generated with these three different *Fusarium* isolates. RNA was isolated from mycelial material, conidiospores and root and corm samples of infected Cavendish and Gros Michel plants at 4, 8 and 30 days post infection. The RNA-seq data were used for a manual re-annotation of the gene models of the three *Fusarium* isolates. Furthermore, these data indicated the importance of a number of homologues of the Secreted In Xylem (SIX) effectors from *Fusarium oxysporum* f.sp. *lycopersici* for infection in banana, with SIX1 and SIX6 specifically being differentially expressed during various stages of infection. Lastly, a set of novel putative effectors was determined based on common effector characteristics. The transcriptomic analysis and identification of putative effectors is crucial in furthering our understanding of the pathogen-host interaction of this devastating disease.

726T Alternative sulfur scavenging and host colonization by the plant pathogen *Raffaelea lauricola* *Joshua Konkol*[1], Qiang Wang[1,2], Jeffrey Rollins[1] 1) University of Florida, Plant Pathology Department, Gainesville, FL 32611; 2) Northwest A&F University, State Key Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Yangling, Shaanxi, China.

Raffaelea lauricola (Ascomycota, Sordariomycetes, Ophiostomatales), causal agent of laurel wilt disease, is an invasive pathogen to North America where it has decimated populations of native Lauraceae trees in the eastern US and threatens commercial avocado production. Its acute lethality is unusual for an ambrosia beetle nutritional symbiont with the ecological role of sustaining larval development and beetle growth in natal galleries. Pathogenicity is hypothesized to be a trait that co-evolved in its native habitat in Asia where it is reported to cause mild (non-lethal) symptoms on Lauraceae trees. The rapid killing of Lauraceae trees in North America is thought to be the result of an evolutionary mismatch between the fungus and naïve populations of related tree species. We have explored the temporal-spatial dynamics of host colonization using a GFP-labeled *R. lauricola* strain, transcriptomic analysis and gene-specific gene deletion. Transcriptomic analyses of susceptible, infected trees revealed a strong up-regulation of genes encoding sulfur compound uptake and assimilation that included 7 methionine and 3 cysteine permeases, 1 sulfate transporter, 13 alternative sulfur transporters and more than 50 genes for sulfur assimilation. A screen of sulfur compounds determined that *R. lauricola* grows on a large number of organic and inorganic sulfur compounds. By contrast, gene deletion mutants of the *cyc3/metR* homolog (*RlmetR*), a positive regulator of sulfur uptake and assimilation genes, could grow only on growth media supplemented with methionine or cysteine, and, to a lesser extent, glutathione. Genetic complementation of the *RlmetR* mutation restored wild-type utilization of diverse sulfur compounds. Infection studies with the susceptible host *Persea palustris* (swampbay) determined that *RlmetR* is essential for pathogenicity. Its requirement for beetle symbiosis has not yet been established. The myriad factors that have evolved to permit colonization of natal galleries as well as pathogenicity are certain to be intertwined as in the case of sulfur scavenging. The dissection of these processes, and particularly the identification of pathogenicity-specific factors, is being pursued through comparative biology of susceptible and resistant hosts with pathogenic and non-pathogenic *Raffaelea* species and via analysis of gene-specific mutations.

727F Fighting fungi with fungi: the biocontrol potential of *Trichoderma* against *Armillaria* root rot *M Millen*[1, 2], J Drakulic[2], M Cromey[2], AM Bailey [1], GD Foster[1] 1) School of Biological Sciences, University of Bristol, BS8 1TQ, UK; 2) Royal Horticultural Society, RHS Garden Wisley, Woking, GU23 6QB, UK.

Armillaria root rot (ARR) is a major fungal plant pathogen which affects forestry, agriculture and horticulture alike. No chemical controls are currently available and an understanding of how to achieve environmentally friendly, cost effective and long-lasting protection against *Armillaria* is lacking in the scientific literature. *Trichoderma* are soilborne Ascomycete fungi associated with the roots of plants

and are endophytes to certain plant species. One approach we are investigating is whether root endophytic fungi might be able to protect against, or indeed cure infection by *Armillaria*. We previously isolated a collection of 42 root endophytic *Trichoderma* isolates across 12 species and these have been tested in plate-based studies and evaluated for biocontrol potential against ARR *in planta*. Two isolates of *Trichoderma atrobrunneum* have shown strong potential to prevent disease in strawberry and privet plants. Results of pilot studies suggest that biocontrol performance may be linked to both enzyme production and the ability of *Trichoderma* to control environmental pH in the presence of *Armillaria*. The isolate with the highest biocontrol potential is currently being sequenced, and future work will investigate genes potentially important in antagonism against *Armillaria.* This poster will present the current status of our investigations into this candidate biocontrol agent, whether effective control of *Armillaria* is feasible and what we know about how this interaction occurs.

728W Characterisation of novel effectors from the wheat pathogen, *Zymoseptoria tritici* *Eli Thynne*[1], Maja Salman[1], Ana E Bergues[1,2], Janine Haueisen[1], Kyungyong Seong[3], Ksenia V Krasileva[3], Graeme Kettles[4], Eva H Stukenbrock[1,2] 1) Christian-Albrechts University; 2) Max Planck Institute for Evolutionary Biology; 3) University of California, Berkeley, California, USA; 4) University of Birmingham, UK.

Zymoseptoria tritici is the major fungal pathogen of wheat in Europe. Despite its significance, very little is known about how *Z. tritici* is able to induce disease and how it evades host defences during the early stages of infection. Aside from well-characterized LysM domain effectors that mask the fungus' chitin from the host, *Z. tritici's* mechanisms of host immune-suppression are poorly understood. We aim to improve our understanding of these mechanisms by functionally characterizing candidate effectors to uncover their function during the early stages of infection. In particular, we were interested in whether these may have had a role in host-immune suppression or host-microbiome manipulation. We screened a number candidate effectors that are highly expressed during the early stages of infection and identified a number that suppress conserved host-immune responses, when transiently expressed in *Nicotiana benthamiana*. We have screened these effectors for their ability to suppress PAMP-induced ROS burst and BAK1 dependent cell-death. We have also observed that this immune-suppressive activity can enhance the growth of *Pseudomonas syringae* when infecting *N. benthamiana* expressing the effectors. Effectors were also observed with putative antimicrobial activity when expressed *in vitro* and *in planta*. To confirm this observed activity, we have focused on a selection of effectors predicted to belong to the same structural family, of which some display immune-suppression activity and others display putative antimicrobial activity. We are currently performing additional screens on these effectors to confirm their function, and performing structural prediction-guided mutational assays to alter these proteins' activities. This project is part of an ongoing project to dissect the wheat-Z. tritici molecular interactions, with a long-term goal of identifying mechanisms of host-specificity.

729T Fungal Pathogens Utilize Extracellular Vesicles for Transport of Effector Proteins into Plant Host Cells *Claire Whitaker*[1,2], Baoye He[2], Hailing Jin[2] 1) Department of Plant Biology, University of California, Riverside, CA, USA; 2) Department of Microbiology & Plant Pathology, Center for Plant Cell Biology, Institute for Integrative Genome Biology, University of California, Riverside, CA, USA.

Effectors, small, secreted proteins which modulate plant immune response, are critical for successful fungal infection. While the effector proteins of biotrophic and hemibiotrophic fungi have been well characterized, little research has been done on the effector proteins of necrotrophic fungal plant pathogens. Beginning in 2016, research on necrotrophic fungal effectors began to illuminate the vast collection of effector proteins found in the genomes of necrotrophic fungi. Many of these predicted effectors lack a N-terminal signal peptide targeting them for the traditional secretion pathway, so it remains unclear how these effectors are delivered to the apoplastic space, and subsequently, plant cells. Recent discoveries have shown that *Arabidopsis thaliana* utilizes extracellular vesicles (EVs) to deliver sRNAs to its fungal pathogen, *Botrytis cinerea*. This exchange of EVs is potentially bidirectional, with fungal EVs packaging and delivering fungal effectors into plant cells. In fact, EVs have been shown to transport the effectors of human fungal pathogens into human cells, though no such research has been done on necrotrophic fungal plant pathogens. With this in mind, our goal is to examine the EVs of *Botrytis cinerea* to determine if they are trafficking effector proteins. We have identified potential effector proteins and through the generation of mutant *B. cinerea* strains with the effectors knocked out or tagged with YFP we will determine the transport mechanism of the identified effectors. Preliminary data has indicated that our chosen proteins are in fact effector proteins, as the deletion mutants show a decreased ability to infect and kill *A. thaliana* cells. Our research will greatly expand the understanding of the roles of extracellular vesicles in fungal pathogenesis as well as identify a non-conventional secretion pathway of protein effectors.

730F GWAS for identifying genes associated with virulence in *F. circinatum* Benedicta Swalarsk-Parry[1], Magriet van der Nest[1,2], Lieschen De Vos[1], Quentin Santana[1], Brenda Wingfield[1], *Emma Steenkamp*[1] 1) Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa; 2) Biotechnology Platform, Agricultural Research Council, Onderstepoort Campus, Pretoria, South Africa.

Fusarium circinatum, commonly referred to as the pine pitch canker pathogen, is one of the most destructive pathogens on *Pinus* species globally. Despite its economic importance, few studies have attempted to determine the molecular basis of virulence and pathogenicity to its plant hosts. This study therefore aimed to identify genes and/or genomic regions associated with the virulence of *F. circinatum* on *Pinus patula* by making use of genome wide association studies (GWAS). For this purpose, we used a set of 80 *F. circinatum* isolates that are representative of the known diversity of the fungus in South Africa (i.e., based on mating-type, genotype, geographic origin, and *Pinus* host). The genomes for these isolates were sequenced using the Ion Torrent™ sequencing technology, while inoculations studies were used to determine their virulence to *P. patula* seedlings. To identify genes and/or genomic regions that are associated with virulence, correlation between genotypic and phenotypic data was determined using GWAS. Various SNPs were significantly ($P < 0.05$) associated with this phenotype. Some of these were also located in close proximity to genes that are known to be involved in fungal virulence/pathogenicity. For example, genes encoding a putative pathogenicity factor and thioredoxin peroxidase were located downstream of a gene encoding CYB2-lactate dehydrogenase cytochrome b2 that harboured a significant SNP. The identification and association of the set of genes/genomic regions identified in this study would thus contribute to the existing knowledge of *F. circinatum* virulence/pathogenicity. Our future research will seek to functionally characterize these genes, thereby ultimately

improving our understanding of the molecular processes underlying pathogenicity in this important fungus.

731W A new pathosystem to study the plant-fungal interactions underlying Cercospora leaf blight of soybean *Kona Swift*[1], Burt Bluhm[1] 1) University of Arkansas.

Cercospora leaf blight (CLB) is a detrimental soybean disease caused by plant pathogenic fungi in the genus *Cercospora*. Historically, *Cercospora kikuchii* was considered to be the primary causal agent of CLB, but other *Cercospora* spp. have recently been associated with the disease. In particular, *Cercospora* cf. *flagellaris* seems to have displaced *C. kikuchii* as the primary causal agent of CLB in the U.S. *C.* cf. *flagellaris* appears to have a broad host range and is hypothesized to generate genetic diversity via inter- or intra-specific hybridization. However, little is known about the genetic basis of pathogenesis in *C.* cf. *flagellaris*. A key bottleneck for CLB research is that disease symptoms are difficult to reproduce in greenhouse and growth chamber conditions. The goal of this study was to develop a *C.* cf. *flagellaris* - *Arabidopsis thaliana* pathosystem as a proxy for CLB of soybean. We determined that the *C.* cf. *flagellaris* wild type strain ARCK7—which was first isolated from soybean leaves affected by CLB—readily infected *A. thaliana*. Symptoms on *A. thaliana* consistently appeared six to seven days after inoculation, which is consistent with reports of latent infection in soybean. Symptoms included necrotic lesions and premature leaf abscission, although foliar bronzing/purpling was not observed. Approximately ten days after inoculation, ARCK7 produced conidia abundantly from mature lesions on leaves of *A. thaliana*. To determine if the ability to infect *A. thaliana* was strain-specific, eighteen additional strains of *C.* cf. *flagellaris* isolated from soybean were evaluated and confirmed to infect *A. thaliana*. Experimental conditions including spore concentration, inoculation technique, temperature, light, and humidity were optimized for consistent, reliable infection. The ability of *C.* cf. *flagellaris* strains associated with CLB to infect *A. thaliana* suggests a broad host range for the pathogen, which is an important consideration for disease epidemiology and control. Additionally, the utilization of *A. thaliana* as a proxy for soybean has numerous advantages for discovering/engineering novel resistance genes and advancing the fundamental understanding of molecular mechanisms underpinning CLB.

732T Convergent recognition of the *Magnaporthe oryzae* host specificity determinant *PWL2* in divergent grass species *Diana Gómez De La Cruz*[1], Helen Brabham[1], Vincent M Were[1], Inmaculada Hernández-Pinzón[1], Phon Green[1], Motoki Shimizu[2], Ryohei Terauchi[2,3], Nicholas J Talbot[1], Matthew Moscou[1] 1) The Sainsbury Laboratory, University of East Anglia, Norwich, UK; 2) Iwate Biotechnology Research Centre, Kitakami, Japan; 3) Kyoto University, Kyoto, Japan.

The blast fungus *Magnaporthe oryzae* is a devastating pathogen that infects and causes significant yield losses of economically important grass crops such as rice, wheat, barley, oat, and millet. Most *M. oryzae* isolates are host-specific, meaning their pathogenicity is restricted to a few species. However, important host jumps have occurred, such as the relatively recent emergence of wheat blast, which threatens wheat cultivation in South Asia and sub-Saharan Africa. Host range dynamics and species specificity have been shown to be determined by the presence of effectors that are recognised by corresponding plant resistance genes, making individual isolates non-adapted to most grass species. In barley, resistance against *M. oryzae* co-segregates with barley powdery mildew resistance at the *Mla* locus, highlighting a case of multiple pathogen recognition. Using fine mapping and forward genetics, we established that the barley intracellular immune receptor *Mla3* recognises the host-specificity determinant *PWL2* from *M. oryzae. PWL2* belongs to the *PWL* host specificity effector gene family, which exerts a major effect on the ability of the blast fungus to infect weeping lovegrass (*Eragrostis curvula*). Isolates of *M. oryzae* carrying *PWL2* are unable to cause disease on weeping lovegrass, whereas isolates carrying the loss-of-function allele *pwl2-2* are virulent on this host. While barley and weeping lovegrass maintain the same specificity of recognition, no homolog of *Mla* was identified in the latter. Our results show that convergent evolution of *PWL2* recognition has occurred in two divergent grass species, and highlight a complex evolutionary interaction between communities of plant and pathogenic fungi that lead to recognition of distinct pathogens by the same immune receptor.

733F Secreted in xylem (SIX) 6 mediates *Fusarium oxysporum* f. sp. *fragariae* race 1 avirulence to strawberry cultivars with *FW1* resistance *Christine Jade Dilla-Ermita*[1,2], Polly Goldman[1], Amy Anchieta[1], Dominique D.A. Pincot[2], Randi Famula[2], Glenn Cole[2], Steven J. Knapp[2], Steven J. Klosterman[1], Peter M. Henry[1] 1) Crop Improvement and Protection Research, USDA-ARS, Salinas, CA ; 2) Department of Plant Sciences, University of California, Davis, CA .

Fusarium wilt of strawberry is an economically important disease caused by *Fusarium oxysporum* f. sp. *fragariae* (*Fof*). The dominant locus for resistance to Fusarium wilt, *FW1*, is currently deployed in strawberry cultivars and prevents disease caused by *Fof* 'race 1' isolates. However, we previously discovered *Fof* 'race 2' isolates that could overcome *FW1*-mediated resistance and remained highly virulent on cultivars with *FW1*. We hypothesized that *FW1* functioned in a gene-for-gene interaction with an unknown avirulence gene (*AvrFW1*) expressed by *Fof* race 1 and absent in *Fof* race 2. If true, identifying the *AvrFW1* gene would enable surveillance for race 2 isolates and provide crucial insight into the durability of *FW1*-mediated resistance. To test this hypothesis, we identified 21 gene homologs (out of 85,846 total homologs) that were present in 24 *Fof* race 1 genomes and absent in 3 *Fof* race 2 genomes. Only one of these homologs was up-regulated during plant infection. This gene had a secretion signal and was homologous to a known effector, *secreted in xylem 6* (*SIX6*), and shared 100% sequence identity among all race 1 isolates. *SIX6* was considered the only strong candidate for *AvrFW1* and thus was knocked out by homologous recombination in two *Fof* race 1 isolates. All *SIX6* knockout transformants ($n = 6$) gained virulence on *FW1/fw1* cultivars, whereas ectopic transformants and the wildtype strain remained avirulent. Intriguingly, *FW1*/*fw1* strawberry cultivars remained quantitatively less susceptible than *fw1/fw1* cultivars to the knockout strains. To evaluate the relationship between *FW1* and quantitative resistance to the knockout strain, seedlings from a segregating *FW1/fw1* × *fw1/fw1* population were: 1) genotyped for a SNP at the FW1 locus that is highly associated with resistance, and 2) tested for susceptibility to a *SIX6* knockout strain or the wildtype. All *FW1/fw1* seedlings were resistant to the wildtype, whereas 86% of *FW1/fw1* seedlings were susceptible to the knockout transformant. Curiously, 14% of seedlings with the *FW1/fw1* genotype were tolerant to the knockout strain, whereas tolerance was not observed among any *fw1/fw1* seedlings. These results support the conclusion that *SIX6* is an avirulence factor interacting with a resistance gene at *FW1*. Additional experiments are necessary and underway to assess whether additional genes are involved in the resistance phenotype.

734W Pathotypes of *Fusarium oxysporum f. sp. fragariae* express discrete repertoires of accessory genes and induce

distinct host transcriptional responses during root infection. Bradley Jenner[2], *Peter Henry*[1] 1) United States Department of Agriculture; 2) University of California, Davis.

Isolates classified as *Fusarium oxysporum* f. sp. *fragariae* are genetically diverse and cause one of two syndromes on strawberry. One syndrome includes symptoms of wilting and chlorosis and is caused by the "yellows-*fragariae*" pathotype, whereas only wilting symptoms are caused by the "wilt-*fragariae*" pathotype. Past work differentiated these pathotypes by symptoms and comparative genomics, but their effects on host transcription and the genomic organization of wilt-*fragariae* pathogenicity genes remain unexplored. To address these knowledge gaps, we challenged susceptible strawberry (*Fragaria* × *ananassa*) plants to root infection by five fungal isolates: three yellows-*fragariae*, one wilt-*fragariae*, and one that is not pathogenic to strawberry. The host and fungal transcriptomes were characterized at 6- and 13-days post inoculation and contrasted with non-inoculated plants or *in vitro* fungal growth. On average, >6 times more strawberry genes were differentially expressed (DE) in response to yellows-*fragariae* isolates than the other two isolates at each timepoint. Responses to yellows-*fragariae* infection were characterized by early induction of genes related to the jasmonic acid phytohormone pathway and widespread reprogramming of carbohydrate metabolism. The wilt-*fragariae* isolate induced few transcriptional responses at 6-days post-inoculation, when plants remained asymptomatic, but strongly induced ethylene production and response factors by the later timepoint. Pathotypes were not differentiated by conserved, fungal effector gene expression and few pathotype-specific differences were observed in the expression of other conserved fungal genes. By contrast, fungal DE genes on accessory chromosomes were almost entirely distinct between pathotypes. An ~150 kbp 'pathogenicity island' on a wilt-*fragariae* accessory chromosome was enriched with DE genes, many of whose predicted functions were related to plant infection. Sequence conservation suggests this region was horizontally transferred between two wilt-*fragariae* lineages. There were 15 accessory genes expressed by all yellows-*fragariae*isolates during root infection, and only one of these genes was also DE by the wilt-*fragariae* isolate. These results support the conclusion that wilt- and yellows-*fragariae* cause physiologically distinct syndromes by the expression of discrete repertoires of genes on accessory chromosomes. Implications for our understanding of classification by '*forma specialis'* will be discussed.

735T Differences in fungal aggressiveness and host susceptibility revealed key drivers of Fusarium Head Blight infection in wheat *Florian Rocher*[1], Marie-Laure Martin-Magniette[2], Philippe Label[3], Thierry Langin[1], Ludovic Bonhomme[1] 1) UCA/INRAE, UMR GDEC, Clermont-Ferrand, France; 2) AgroParisTech, INRAE, Université Paris Saclay, UMR Mia-Paris, Paris, France; 3) UCA/INRAE, UMR PIAF, Clermont-Ferrand, France.

Fusarium graminearum, the main causal agent of Fusarium Head Blight (FHB), is one of the most damaging pathogens in small grain cereals such as wheat. Because of the weak effects of the multiple genetic FHB resistances, elucidating the mechanisms regulating plant susceptibility and identifying their main drivers, the so-called pathogen's effectors, appears as a promising alternative to control epidemics in bread wheat. Although the *F. graminearum* catalog of effectors has been well characterized at the genome scale (Brown et al., 2012; Wang et al., 2017, Laurent et al. 2017; Alouane et al., 2021), *in planta* studies are still necessary to identify their effective expression and their exact role in the infection process (Fabre et al., 2019a, 2019b). Taking advantage of the genetic variability from both partners of this interaction, a RNAseq-based profiling of the secretome coding gene expression was performed during a time course infection in a single aggressive strain facing five wheat cultivars of contrasting susceptibility as well as in three strains of contrasting aggressiveness infecting a single susceptible host. Genes coding for secreted proteins and exhibiting significant expression changes along infection progress were selected to refine the secretome gene sets and to identify the most reliable effector gene candidates. During the interaction with different cultivars, 476 reliable effector-encoding genes were expressed by the aggressive fungal strain, among which 91% were regulated in all the infected hosts and constituted a host-driven core-effectome gene set. In the different strains facing a single cultivar, a total of 761 reliable effector genes were identified, among which 90% were systematically expressed in the three strains and constituted a strain-driven core-effectome gene set. The intersection of both core-effectome gene sets revealed a robust *F. graminearum* infection signature of 357 genes that proved to be highly relevant in discriminating the infection stages. Several wheat compartments were predicted to be targeted by these *F. graminearum* putative effectors along the infection progress including apoplast, nucleus, chloroplast and mitochondria. Taken together, our results lead to the identification of reliable key fungal genes involved in the wheat susceptibility to *F. graminearum* and brought valuable information on their putative targets.

736F Analysis of Superoxide Dismutase Activity in *Fusarium oxysporum* Rafael Palos Fernández[1], Antonio Di Pietro[1], *Manuel Sánchez López-Berges*[1] 1) Universidad de Córdoba.

Fusarium oxysporum causes vascular wilt disease on more than one hundred plant species. The production of Reactive Oxygen Species (ROS) plays a central role in plant defense against several pathogens including fungi. These molecules are highly toxic due to their reaction with macromolecules producing DNA mutations, lipid peroxidation, and protein oxidations, generating cellular dysfunction and ultimately cell death. To deal with the effect of ROS in cells, all organisms have developed complex ROS scavenging systems, enzymatic and nonenzymatic. Superoxide dismutases (SODs) protect against superoxide radicals both intra- and extracellularly establishing a frontline defense against ROS. However, the role of SODs is poorly understood in *F. oxysporum* pathogenicity. Depending on the cofactor used, SODs are divided into Cu/Zn SODs and Fe/Mn SODs and *Fusarium* encodes five different. We are mainly interested in the study of copper-related SODs including the Cu/Zn Sod1 and Sod5, and the Fe/Mn Sod3, particularly relevant under copper-limiting conditions (-Cu). Sod1 is cytosolic while Sod5 contains an N-terminal secretion signal and Sod3 is mitochondrial. Ingen analysis of SOD activity reveals that Sod1 is the most active in *F. oxysporum* and that the activity of two additional SODs is solely induced in the presence of the ROS generator menadione (MD). Importantly, copper is required for the full activity of Sod1 and one of the MD-induced SODs, most likely Sod5. On the other hand, Sod3 is exclusively active under copper-limiting conditions (-Cu). Targeted deletion of *F. oxysporum sod1*, but not of *sod3*, caused markedly increased sensitivity to MD and a delay in the induction of vascular wilt symptoms on tomato plants. We are currently characterizing Sod5 in *F. oxysporum* and our results suggest that SOD activity is relevant for *F. oxysporum* pathogenicity on tomato plants.

737W Effector proteins of *Botrytis elliptica* as tools for resistance breeding in lily against fire blight disease *Michele Malvestiti*[1], Jan van Kan[1], Richard Immink[1], Paul Arens[1] 1) Wageningen University and Research.

Fire blight represents a widespread disease in lily and is caused by the necrotrophic Ascomycete *Botrytis elliptica*. Pathogenicity in lily is conferred by secreted effector proteins which induce programmed cell death. We hypothesise that susceptibility to fire blight in a lily genotype correlates to effector sensitivity in a quantitative manner. This research aims to identify and characterize *B. elliptica* effectors and use them to screen the lily germplasm to select for plants which display increased fire blight resistance. To quantify differences in fire blight susceptibility between plant genotypes and differences in virulence between fungal isolates, we inoculated in four biological replicates over two years, a set of 12 *B. elliptica* isolates on a panel of 18 lily genotypes representing seven *Lilium* hybrid groups. A wide spectrum of variation in symptom severity was observed in different isolate-genotype combinations. There was a good correlation between the lesion diameters on leaves and flowers of the *Lilium* genotypes, although the flowers generally showed faster expanding lesions. We selected two aggressive isolates and one mild isolate and collected culture filtrate samples to compare the cell death inducing activity of their secreted compounds in lily. After leaf infiltration with the crude culture filtrates samples and with purified proteins fractions, variation was observed in cell death responses between the diverse lilies. The severity of cell death response upon infiltration of fungal secreted compounds observed among the diverse *Lilium* hybrid groups correlated well to their fire blight susceptibility. At this point, the protein profile of active fractions was determined and integrated with genomic and transcriptomic data to obtain a list of candidate effector genes. Candidate genes will be expressed in yeast to obtain purified effector proteins. We propose that the sensitivity of lily genotypes to *B. elliptica* effector proteins that induce PCD is predictive of the susceptibility of that individual plant to a fungal isolate that produces such effector(s).

738T Extracellular vesicles of and biofilm formation in a maize fungal pathogen *Fusarium verticillioides* *Thabiso Motaung*[1], Chizne Peremore[2], Quentin Santana[3], Brenda Wingfield[4], Emma Steenkamp[5] 1) University of Pretoria; 2) University of Pretoria; 3) University of Pretoria; 4) University of Pretoria; 5) University of Pretoria.

Background: Extracellular vesicles (EVs) mediate induce significant functional consequences in fungi as they internalize bioactive molecules including proteins, nucleic acids, and secondary metabolites from a source cell. These luminal contents can be launched into the extracellular environment where they can persist or be taken up by recipient cells. In fungi, EVs contribute to the production of virulence factors including biofilms, the matrixed and architecturally complex community that takes advantage of new environmental opportunities. Biofilms also generate EVs, however, both the importance of vesicles and biofilm formation in *Fusarium verticillioides* (an important maize fungal pathogen), remains poorly established. For this reason, this study aimed to characterize biofilm formation and extracellular vesicles derived from *F. Verticillioides* (*Fv*).
Fv vesicles were isolated from biofilms and planktonic cells and characterized following the MISEV2018 guidelines, which included TEM, NTA, and proteomics analyses. A sporulating culture (¼ strength PDA, homogenized (1 min) in filter sterile or autoclaved 1x PBS) was used to form biofilms by incubating the plates at 25 °C for 24-72 hrs under stationary conditions to initiate cell attachment and biofilm formation. Biofilms were then characterized using CLSM and SEM, and their viability analysed using the XTT. EV-mediated effects of biofilms on *Fv* growth were analysed using biofilm-derived EVs that were co-incubated with conidia for 30-60 minutes, following which, uptake of vesicles and their impact on fungal morphology was analysed using CLSM and on PDA, respectively.
Result: *Fv* forms biofilms following a typical model previously reported in other filamentous fungi. A mature biofilm comprise of hyphae intertwined in a visible extracellular polymeric substance. Under TEM analysis confirms the formation of EVs from planktonic and biofilms cells, which show a typical cup-like shape of EVs seen in fungi. According to NTA, the average number and size of *Fv* EVs is 1,5x 10^9, mean diameter 189.33 +/- 14.1 nm (planktonic) and 7,13x 10^8 , mean diameter 185.95 +/- 12,9 nm (biofilm). Planktonic and biofilm-derived vesicles are identical in size but significantly differed in concentration, with biofilms containing far fewer EVs when visualized under TEM.
Conclusion: The maize pathogen Fv forms biofilms following a typical model, which includes attachment, colonization, growth (including EPS production), maturation, and detachment from EPS. TEM analysis showed EVs with the usual shape, while the NTA reveals the size distribution and but significant concentration differences. Our pending data on proteomics and uptake analyses, as well as XTT assay will give us more insights into the biology of EVs and biofilms derived from *Fv*.

739F A GPI-anchored protein gene from the chestnut blight fungus *Cryphonectria parasitica* is a hypovirus-specific virulence factor and a tolerance factor against hypovirus infection Jeesun Chun[1], Yo-Han Ko[1], Kum-Kang So[1], Su-Hwan Cho[1], *Dae-Hyuk Kim*[1] 1) Department of Molecular Biology, Department of Bioactive Material Sciences, Institute for Molecular Biology and Genetics, Jeonbuk National University, Jeonju, Jeonbuk, Korea.

The chestnut blight fungus, *Cryphonectria parasitica*, and its interaction with hypovirus, Cryphonectria hypovirus 1 (CHV1), is a model to study the fungus-virus interaction. Our previous transcriptomic analysis identified a transcript that encodes a glycosylphosphatidylinositol (GPI)-anchored protein (GPI-AP) was differentially expressed by sectorization and CHV1 infection. Sequence analysis of a deduced amino acid of the cloned gene (*CpGap1*) showed a high similarity to and phylogenic clustering with known fungal GPI-APs with canonical N-terminal leader peptide and C-terminal GPI-anchoring signal. Functional analysis comparing the *CpGap1*-null mutant with the wild type resulted in no observed phenotypic changes in growth rate, sporulation, and pigmentation. The mutant showed no changes in colonial growth in response to osmotic and temperature stresses observed. However, the *CpGap1*-null mutant showed an increased sensitivity to the cell-wall disturbing agent SDS, but not CR and CFW. Hypersensitivity of the *CpGap1*-null mutant was observed in response to ROS. *In silico* analysis of a matured peptide of the protein product of the *CpGap1* gene (CpGAP1) suggested five motifs with antioxidizing properties, and three of these five synthesized peptides showed a strong radical scavenging capacity. Interestingly, virulence was significantly reduced in the *CpGap1*-null mutant. Phytotoxic activity, as measured by leaf discs assay using synthetic peptides, was observed in specific peptides. These results suggest that CpGAP1 functions as a protective barrier against host defenses such as ROS, even as it acts as a virulence factor that places stress on host cells. Moreover, when the CHV1 was transferred to the *CpGap1*-null mutant, severely retarded colonial growth was observed. In addition, virus-titer in the mycelia of CHV1-infected *CpGap1*-null mutant was significantly higher than those in the CHV1-infected isogenic strain (UEP1). These results indicate that CpGAP1 functions as a protective barrier against host defenses such as ROS, but also act as a virulence factor that stress host cells. Moreover, our study demonstrates that the *CpGap1* gene is a host-tolerating antiviral factor that helps maintain fungal growth and suppress viral titer after infection of *C. parasitica* with CHV1.

740W Functional analysis of Heat Shock Protein 90 co-Chaperon p23, *CpCo23*, of chestnut blight fungus *Cryphonectria parasitica* in variety of stress *Yo-Han Ko*[1], Jeesun Chun[1], Kum-Kang So[1], Jung-Mi Kim[2], Dae-Hyuk Kim[1] 1) Department of Molecular Biology, Department of Bioactive Material Sciences, Institute for Molecular Biology and Genetics, Jeonbuk National University, Jeonju, Chonbuk, Korea; 2) Department of Bio-Environmental Chemistry, Institute of Life Science and Natural Resources, Wonkwang University, Iksan, Chonbuk, Korea .

Co-chaperon p23 of Heat shock protein 90 (Hsp90) up-regulated by Cryphonectria hypovirus 1 (CHV1) and/or tannic acid (TA) was identified through proteomic analysis. The amino acid of CpCo23 showed the highest sequence homology to *Neurospora crassa* Hsp90 co-chaperone p23, and was found to be *Saccharomyces cerevisiae* Sba1 ortholog protein. The transcription of *CpCo23* was peaked at 24 hours in CHV1 and/or TA treatment or in both treatment condition. Particularly, the accumulation of *CpCo23* transcript in response to CHV1 and TA both supplementations was rapidly decreased. For the functional analysis of *CpCo23*, a *CpCo23*-null mutant in which the *CpCo23* gene was deleted was obtained. After single-sporing of *CpCo23*-null mutant, *CpCo23* deletion was reconfirmed through Southern blot analysis. Compared to the wild-type strain (EP155/2) on PDAmb, retarded growth with less aerial mycelia and strong pigmentation was observed in *CpCo23*-null mutant. To study the function of *CpCo23* against stress, *CpCo23*-null mutant were cultured on various stress media. Compared to the wild-type strain, temperature, ROS (Reactive oxygen species), and cell wall disturbing agents were not significantly affected, but the growth of *CpCo23*-null mutant was recovered on 1M osmotic stress (sucrose, sorbitol, manitol) media. The phenotype of *CpCo23*-null mutant infected with CHV1 was observed to be similar to that of UEP1, wild-type strain infected CHV1. The effect of the antifungal drugs, particularly Geldanamycin, inhibited the growth of both *CpCo23*-null mutant and *CpCo23*-null mutant infected with CHV1.

741T Unconventional suppression of plant defence responses by the signal peptide peptidase Spp1 in the *Ustilago maydis* - maize interaction Nora Kuehne[1], Niko Pinter[1], Anja Poehlein[1], Rolf Daniel[1], *Kai Heimel*[1] 1) Georg-August-University.

Secreted effector proteins are central for communication between biotrophic fungi and their host plants. The interaction between the biotrophic plant pathogen *Ustilago maydis* and its host plant maize requires an optimised intracellular infrastructure for correct folding, efficient processing and secretion of effectors. During pathogenic growth, activation of the unfolded protein response (UPR) is crucial for accommodating the huge diversity and quantity of secreted effectors and for maintaining homeostasis of the endoplasmic reticulum (ER). Consequently, a functional UPR is essential for virulence in fungal plant pathogens. However, it is only poorly understood how individual UPR regulated factors contribute to fungal virulence. To address this question, we used a combined RNAseq/ChIPseq approach to identify direct UPR targets. Screening of more than 40 deletion strains for altered ER stress resistance and virulence, identified the signal peptide peptidase Spp1 as a central virulence factor, which is essential for pathogenicity. SPPs are ER-membrane localised aspartic proteases, cleaving type II oriented transmembrane domains, including remnant signal peptides that were previously processed by the signal peptidase complex. Spp1 is dispensable for vegetative growth, filament formation and ER stress resistance. Although, the virulence function depends on the conserved catalytic activity we were not able to attribute it to any of the known physiological roles of SPPs, such as ER-associated degradation (ERAD) or hypoxia adaptation. Moreover, *spp1* deletion mutants elicit massive plant defence responses upon infection of its host plant maize, but are not affected in effector secretion or ER homeostasis. In essence, our data suggests that suppression of plant defence responses by Spp1 involves a previously unknown mechanism of fungal plant communication.

742F Identification and characterisation of an expanded family of effector from Asian soybean rust, *Phakopsora pachyrhizi* *Kelly Robinson*[1, 2], Yogesh Gupta[1, 2], Peter van Esse[1, 2] 1) The Sainsbury Lab, Norwich, UK; 2) The 2Blades Foundation, Evanston, Illinois, USA.

The Rust fungi (*Pucciniales*) are an order containing ~7,800 species of obligate biotrophic plant pathogenic fungi, some of which cause disease on key crops such as wheat, barley, oat, soybean, coffee, flax, and poplar[1]. Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi,* is a major fungal disease of soybean. This highly adaptive pathogen causes yield losses of up to 90% and is gradually building resilience against known fungicides and rapidly overcoming individually deployed disease resistance genes in soybean[2]. Recently, the Soybean Rust Consortium released three high-quality genome assemblies to provide a rich resource to study critical virulence factors of *P. pachyrhizi*. These include pathogen-secreted molecules, effectors, that modulate host physiology by targeting pathways involved in host defence and metabolism to facilitate host colonisation[3]. Despite the economic impact of *P. pachyrhizi*, little is known about the virulence functions of its effectors. This information is critical to understand the evolution of virulence in this highly adaptive pathogen and can furthermore inform disease resistance strategies. To identify effectors of *P. pachyrhizi*, an extensive RNAseq time-course was performed during key stages of infection on soybean. Rust genomes are marked by expansions in lineage-specific effector gene families which are hypothesized to reflect host adaptation[4]. To identify putative effector families, Markov Cluster (MCL) Analysis was performed using secretomes of *P. pachyrhizi* and ten other rust fungal genomes. From this robust dataset, a single family, tribe_36, was identified as a conserved, rust-specific effector tribe. Tribe_36 contains five novel homologues of *P. pachyrhizi* effectors previously reported to suppress immunity[5,6]. To characterise role of this effector family in the interaction with the host, potential host targets were identified by yeast-two hybrid screening of a *Medicago truncatula* cDNA library. Interactions between effector and candidate host targets will be confirmed *in planta* using co-immunoprecipitation in *N. benthamiana*. Genetic approaches will be used to functionally characterise the role of this and any further confirmed host targets during *P. pachyrhizi* infection.

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743W Fungal alcohol oxidase (AOX): a broadly conserved protein facilitating ascomycete invasion of plants *Nathaniel We-*

strick[1], Mehdi Kabage[1] 1) University of Wisconsin - Madison.

Sclerotinia sclerotiorum is a broad-host range necrotrophic pathogen capable of causing devastating disease in a range of economically important crops. Through a time-course transcriptome analysis of S. sclerotiorum infection in soybean, an alcohol oxidase (*SsAOX*) was identified as one of the most highly expressed genes during infection. Orthologues of *SsAOX* are highly conserved throughout the phylum Ascomycota and appear largely associated with the genomes of plant associated fungi. These orthologues are maintained in 92.5% of assessed plant pathogen genomes and lost almost exclusively in fungi which have transitioned away from plant material as a primary carbon source. Additionally, an analysis of existing transcriptome datasets highlights its use by a broad range of fungal pathogens during pathogenesis. A knockout mutant of *SsAOX* (*ΔSsAOX*) shows significantly reduced virulence on inoculated soybeans as well as a drastically reduced mortality rate (~10%) when compared to the WT strain (~90%), likely due to its inability to properly colonize soybean stem tissue. Chemical genomics suggest that SsAOX may function as an aromatic alcohol oxidase, and growth induction/repression assays of the WT and mutant demonstrate the mutant is incapable of properly utilizing plant extract as a carbon source. Profiling of known plant metabolites point towards the monolignol coniferyl alcohol (CA) as a likely substrate for SsAOX and oxidation of CA by SsAOX appears to facilitate both the detoxification and metabolism of this compound. As CA and other monolignols are ubiquitous among land plants, the presence/utilization of highly conserved *AOX* orthologues throughout Ascomycota imply that this is a broadly conserved protein used by plant pathogenic fungi when interacting with lignified plant material.

744T Extracellular vesicle-mediated transfer of plant mRNA into fungal cells to suppress pathogenicity *shumei wang*[1] 1) university of california riverside.

Extracellular vesicles (EVs) are implicated in cell-to-cell RNA transport in animals, and mobile small RNAs can be transferred across kingdoms to regulate gene expression in interacting organisms was elucidated by Cai *et al* in 2018. However, whether mRNAs are transferred between hosts and microbes is unknown. Here, RNA-seq was carried out using Arabidopsis-derived EVs isolated from *B. cinerea* infection or from uninfected Arabidopsis leaves. Gene ontology analysis revealed that there was a clear enrichment of defence-associated genes represented in the host transcripts identified in Arabidopsis EVs. A nuclease protection assay showed that EV-associated mRNAs were protected from nuclease digestion, demonstrating that the mRNAs are indeed contained within the vesicles rather than bound to the outer surface. Moreover, host transcripts were detected in interacting fungal cells by RT-PCR, we assume that these transferred mRNAs can be translated into protein to suppress pathogenicity. This fundamental research reveals a novel means by which hosts defend against pathogen invasion.

745F Functional analyses of genes involved in disease development in rice caused by both pathotypes of *Fusarium fujikuroi* *Sang-Won Lee*[1], Da-Woon Kim[1], Hee-Kyoung Kim[1], Sung-Hwan Yun[1] 1) Soonchunhyang Univ.

Fusarium fujikuroi is known to cause rice bakanae disease by producing gibberellic acids (GA) within rice. In addition to this prominent disease symptom, the other symptom showing a stem blight with a root rot in rice is known to be cuased by the phylogenetically distinct pathotype (called stunting or blight-type) of *F. fujikuroi*. Previous studies have demonstrated that biosynthesis of fumonisins in blight type strains is responsible for the development of the blight symptom. To identify additional genes involved in the symptom development in *F. fujikuroi* B14, the representative strain of blight pathotype, we selected a total of 63 genes from B14, which were specifically expressed during pathogenesis in rice compared to the case of the other pathotype strain (B20) causing bakanae symptom. Targeted deletion analysis, however, revealed that single pathogenesis-specific genes were not essential for the blight development. Subsequently, we have performed to silence several target genes simultaneously by expression of inverted repeat DNA constructs derived from each gene, and found that the silencing of a gene combination consisting of two cell wall degrading enzyme genes led to a significant reduction in blight development in rice. Meanwhile, we also determined the functional requirement of 14 genes encoding global regulators such as velvet complex proteins or sporulation-related transcription factors in both B14 and B20 strains during pathogenesis on rice. The trasnsgenic B14 strains sustaining Δ*veA*, Δ*velB*, or Δ*wetA* showed a dramatic reduction in the blight symptom development; *FUM1* responsible for fumonisin biosynthesis was down-regulated in these deletion strains. In B20, *veA* and *brlA* orthologues, which controled the expression of a GA biosynthetic gene (*CPS/KS*), were required for bakanae symptom development. Interestingly, The Δ*laeA* strain of B20 showed the blight symptom rather than typical bakanae symptom in rice seedling by increasing the *FUM1* transcript levels, indicating that *laeA* may play an important role in determinng the pathotypes of *F. fujikuroi* isolates.

746W The monothiol glutaredoxin Grx4 is a key regulator of secondary metabolism, iron homeostasis, nitrogen sensing and virulence in *Ustilago maydis* *Sean McCotter*[1, 2], Kai Heimel[3], James Kronstad[1, 2] 1) Michael Smith Laboratories, The University of British Columbia, Vancouver, BC, Canada; 2) Dept. of Microbiology and Immunology, The University of British Columbia, Vancouver, BC, Canada; 3) Dept. of Molecular Microbiology and Genetics, Georg-August-Universität, Göttingen, Germany.

The corn smut fungus, *Ustilago maydis*, is the premier basidiomycete model for the study of biotrophic plant-pathogen interactions. In fungi, monothiol glutaredoxins are central regulators of key cellular functions such as iron homeostasis, cell wall integrity and redox status, acting mainly via their roles in iron-sulfur cluster/glutathione coordination, trafficking, and delivery. In this study we characterized the novel roles of the monothiol glutaredoxin Grx4 in the biology of *U. maydis*. In addition to its roles identified in other fungi, Grx4 is necessary for pathogenesis by *U. maydis* on its plant host, *Zea mays*. Mutants expressing a conditional allele of *grx4* under the control of the arabinose-induced/glucose-repressed promoter P_{crg}, exhibited decreased virulence on maize which correlated with *grx4* transcript levels at the time of infection. Additionally, perturbations were detected in homeostasis and perception of the essential nutrients iron and nitrogen, following *grx4* repression in glucose-containing media. Furthermore, *grx4* repression strongly altered secondary metabolism, leading to induction of melanin and itaconic acid biosynthetic genes and accumulation of these metabolites *in vitro*. Together, these data suggest that glutaredoxins could play important roles in the virulence of plant pathogenic fungi in addition to their established roles as key regulators of fundamental cellular processes. The involvement of Grx4 in the production by *U. maydis* of industrially relevant secondary metabolites (e.g., itaconic acid) also renders these findings of biotechnological interest.

747T Co-transcriptomic time course analysis for mechanistic understanding of the *Arabidopsis-Botrytis* pathosystem *Anna*

Muhich[1], Celine Caseys[1], Daniel Kliebenstein[1] 1) University of California, Davis.

The generalist fungal pathogen *Botrytis cinerea* infects a wide range of plants, including the model plant *Arabidopsis thaliana*. Upon infection, nearly all major transcriptional responses occur in *Arabidopsis* leaf within 24 hours of inoculation and before visible lesion development. *Arabidopsis-Botrytis* interactions are affected by many categories of factors, including biochemical defenses such as glucosinolates in *Arabidopsis*, tissue differences such as structurally distinct leaf surfaces (abaxial or adaxial), and genetic diversity. While individual effects of these factors on disease outcome is often studied, their factorial effects on transcriptional responses in host and pathogen have not yet been evaluated. To better understand factorial mechanics of *Botrytis* disease progression, we are generating a large RNA-seq dataset including both *Botrytis* and *Arabidopsis* transcriptomes during infection. To account for genetic diversity, we selected four *Botrytis* isolates based on their ability to elicit diverse transcriptional responses. To determine whether various chemical arsenals and leaf surfaces alter gene expression, we infected three *Arabidopsis* genotypes with differing glucosinolate levels on either the adaxial or abaxial surface of the leaf. Finally, to observe the dynamic shifts in transcription during pathogen attack and plant response, we collected six timepoints throughout initial infection (0-26 HAI). The resulting co-transcriptomic networks will provide valuable and detailed insight into the interplay of metabolism, physiology and pathogenicity to the underlying mechanics of generalist fungal pathogen virulence and plant host defense strategies.

748F *Botrytis cinerea* secretes small RNA containing extracellular vesicles that enter plant cells through clathrin-dependent endocytosis *Baoye He*[1], Qiang Cai[2], Hailing Jin[1] 1) University of California, Riverside; 2) Wuhan University.

Extracellular vesicles (EVs) are membranous structures that are involved in the release of hundreds of different molecules to the cellular outer space by diverse cells from all life domains, including fungi. Fungal EV cargoes contain many proteins, nucleic acids neutral lipids, glycans, and pigments. Many of these cargos can be recognized and accepted by specific “recipient” cells and modulate normal physiological processes as well as pathological progression. Here we demonstrate that the necrotrophic fungus Botrytis cinerea can also secret EVs during its infection period. Many fungal sRNA effectors are detected in Botrytis EVs, and these EVs can partially restore the virulence of Botrytis *dcl1/dcl2* mutant, which lost its ability to produce most of the Botrytis small RNAs (sRNAs). We further find that these vesicle-delivered small RNA effectors are taken up by plant host cells through clathrin-mediated endocytosis. After getting into plant cells, the Botrytis EVs cargo sRNAs can suppress host target gene expression. These results reveal that EVs can be used by Botrytis cinerea to deliver virulence factors into host plant cells.

749W Characterising an effector from the fungal pathogen of wheat, *Zymoseptoria tritici* *Nikolaos Mastrodimos*[1,2], Sujit J. Karki[1,2], Paola Pilo[1,2], Eoghan Curran[1,2], Angela Feechan[1,2] 1) School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland ; 2) Earth Institute, University College Dublin, Belfield, Dublin 4, Ireland.

Septoria tritici blotch (STB) is a severe wheat disease caused by the filamentous ascomycete *Zymoseptoria tritici*. During outbreaks, wheat yields can be reduced up to 40% (Eyal *et al.*, 1987), while no wheat varieties are fully resistant to the pathogen (Torriani *et al.*, 2015). The *Z. tritici* genome includes around 250 genes which encode small, secreted proteins (ZtSSPs) (do Amaral et al., 2012). These effectors/ZtSSPs have a potential role in pathogenicity and virulence as they can modulate the host’s defense and facilitate infection (Karki *et al.*, 2021; Macho & Zipfel, 2015). However, only a limited number of such ZtSSPs have been characterised from *Z. tritici*. Previously, ZtSSP2; a small, conserved, cysteine-rich secreted effector from *Z. tritici*, was found to interact with a wheat ubiquitin ligase which plays a role in defense against STB (Karki *et al.*, 2021). In this study, we further explore the role of *ZtSSP2* in *Z. tritici* pathogenicity and virulence by generating gene replacement fungal knock-out strains (Δ*ZtSSP2_A;* Δ*ZtSSP2_B;* Δ*ZtSSP2_C*). These *ZtSSP2* knock-out mutants had significantly reduced disease symptoms and produced fewer fruiting bodies in wheat, compared to wildtype *Z. tritici*. Analysis of ZtSSP2 haplotypes from 175 *Z. tritici* isolates taken from four sites across the UK over three years show high levels of conservation. Yeast two-hybrid (Y2H) assays are ongoing to elucidate further potential interactions of ZtSSP2 with host proteins from wheat. In summary, our findings suggest that ZtSSP2 is key for STB.

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750T Unexpected fitness advantage from hydrophobin loss in *Penicillium expansum* upon apple co-inoculation *Dianiris Luciano-Rosario*[1], Justin Eagan[2], Nancy P. Keller[2,3] 1) Department of Plant Pathology,University of Wisconsin, Madison,WI, USA; 2) Department of Medical Microbiology and Immunology, University of Wisconsin, Madison,WI, USA; 3) Department of Bacteriology, University of Wisconsin, Madison, WI, USA.

Blue mold disease is mainly caused by the ascomycete *Penicillium expansum*. This pome fruit post-harvest pathogen is also a mycotoxigenic fungus that produces patulin and citrinin, two main toxins that threatens both economical and food safety aspects of the apple industry. Regulatory agencies around the world, have imposed threshold levels of patulin in food products. There is a gap of knowledge on exploring the interactions at the interphase of the *P. expansum* and host pathosystem. Hydrophobins are small fungal-specific secreted proteins that mediate different aspects of fungal biology and physiology including hydrophobicity, dispersal, attachment, virulence, and interaction between the air-water interphase in fungal cells. Thus, we are interested in studying their role in fungal biology and the disease cycle of *P. expansum*. We generated single deletion mutants and a septuple deletion mutant of the seven hydrophobin-encoding genes in the *P. expansum* genome. Unexpectedly, our data suggests that the absence of hydrophobins provide

a fitness advantage in the apple infection model. We conducted a passage study in which upon co-inoculation of apples with the WT and septuple deletion mutant, found that the latter was more abundant than the WT strain by the end of five passages. Our data also suggests that hydrophobins in *P. expansum* may be important in survival in long term storage conditions such at room temperature, -80°C, and desiccation.

751F The *Zymoseptoria tritici* effector *Zt-11* is involved in the switch to necrotrophy and contributes to virulence in wheat Sujit Karki[1], *Paola Pilo*[1], Colleen Lawless[1,2], Eoghan Curran[1], Anna Tiley[1,3], Hesham Gibriel[1,4], James Burke[1], Angela Feechan[1] 1) School of Agriculture and Food Science and UCD Earth Institute, University College Dublin, Belfield, Dublin 4, Ireland; 2) School of Biology and Environmental Science and UCD Earth Institute, University College Dublin, Belfield, Dublin 4, Ireland; 3) Agri-Food and Biosciences Institute, Belfast BT9 5PX, United Kingdom, Northern Ireland; 4) Royal College of Surgeons in Ireland, Dublin 2, Ireland.

Wheat fungal pathogens every year cause around 15-20% of yield losses. *Zymoseptoria tritici* is a hemibiotrophic ascomycete fungus and the causal agent of Septoria tritici leaf Blotch (STB) in wheat. Recent studies have shown that *Zymoseptoria tritici* secretes an array of effector proteins (SSPs) that are likely to facilitate host infection and colonisation. However, to date only a handful of them have been functionally characterized for their role in virulence.
In this study we demonstrate a role for Zt-11 protein as an effector important for disease progression. Zt-11 is a small, cysteine-rich, secreted protein, which we found to be upregulated during the transition of the pathogen from the biotrophic to the necrotrophic phase of infection. Data from RT-qPCR also shows no expression of *Zt-11* from *in-vitro* conditions. On the other hand, three independent knock out mutants of *Zt-11* showed delayed disease development in wheat, with a deferred appearance of the necrosis symptoms. The *Zt-11* mutants had lower pycnidia numbers and sizes compared to the wild type. This results suggests that Zt-11 protein has an essential role in the transition phase of the fungus from the biotrophic to necrotrophic phase of wheat infection. We also observed that Zt-11 contains no known homologues in other fungi, suggesting it to be specific to *Zymoseptoria tritici*.
Furthermore, population analysis on 49 isolates from 4 UK field sites, demonstrates that Zt-11 is polymorphic and under positive selection. Ongoing effector enrichment analyses of *Z. tritici* DNA from spore traps from the same fields could confirm the polymorphic nature of Zt-11 and its positive selection.
Overall, these data suggest that the *Zt-11* effector plays an important role in *Z. tritici* virulence and disease progression in wheat.

752W RNA interference affects fungus-fungus interactions in the biocontrol agent *Clonostachys rosea* *Edoardo Piombo*[1], Ramesh Raju Vetukuri[2], Anders Broberg[3], Pruthvi Balachandra Kalyandurg[2], Sandeep Kushwaha[2,4], Dan Funck Jensen[1], Magnus Karlsson[1], Mukesh Dubey[1] 1) Department of Forest Mycology and Plant Pathology, Uppsala Biocenter, Swedish University of Agricultural Sciences, Uppsala, Sweden; 2) Department of Plant Breeding, Horticum, Swedish University of Agricultural Sciences, Lomma, Sweden; 3) Department of Molecular Sciences, Uppsala BioCenter, Swedish University of Agricultural Sciences, Uppsala, Sweden; 4) National Institute of Animal Biotechnology, Hyderabad, Telangana, India.

Clonostachys rosea is an antagonistic fungus with a proven role in the biocontrol of numerous insects, nematodes and fungi with a plant pathogenic activity. The antagonistic action of *C. rosea* is carried out through the production of toxic metabolites and reactive oxygen species, the induction of defence reactions on plant hosts, and the degradation of the target's cell wall with hydrolytic enzymes, such as chitinases. While it is proven that *C. rosea* reacts to different mycohosts by regulating the expression of precise host-dependent groups of genes, little is known regarding the role of RNA interference (RNAi) in regulating the biocontrol activity of this fungus. Since RNA interference is usually mediated by miRNA-like RNAs (milRNAs) produced through the cleaving of double strand RNAs by enzymes called "Dicers", we produced *C. rosea* deletion mutants for the two Dicer-like (dcl) enzymes identified in this fungus, and we verified that the Δdcl2 mutant has a diminished capacity of controlling the plant pathogens *B. cinerea* and *F. graminearum*. Afterwards, we sequenced small and messenger RNAs from *C. rosea* Wild Type and mutants interacting with the fungal plant pathogens *Botrytis cinerea* and *Fusarium graminearum.* The results show how RNA interference is involved in *C. rosea* antagonistic action at multiple levels, both directly and indirectly. The Δ*dcl2* mutant had a lower expression of hydrolytic enzymes, secondary metabolite gene clusters and transporters involved in the removal of harmful compounds. Moreover, eleven milRNAs were identified in *C. rosea* WT but not in the Δ*dcl2* mutant, and they were predicted to target nine endogenous regulatory enzymes. Additionally, four proven virulence factors of *B. cinerea* and three of *F. graminearum* were predicted to be targeted by the dcl2-dependent milRNAs. All of them showed higher expression in the situations in which the targeting milRNAs were not produced, suggesting a putative role of fungal-fungal crossregulation in the biocontrol action of *C. rosea*.
These results improve our understanding of the complex interactions occurring between antagonistic fungi and plant pathogens, and they pose the base for future studies focusing on the role of cross-species RNAi-regulated mycoparasitic interactions.

753T Mycangial colonization in the laurel wilt (*Raffealea lauricola*)-Ambrosia beetle symbiosis *Ross Joseph*[1], Kamaldeep Bansal[1], Nemat Keyhani[1] 1) University of Florida.

Ambrosia symbioses are reciprocally obligate mutualisms between a group of specialized wood-boring weevils (*Curculionidae*: *Scolytinae* and *Platypodinae*) and their associated fungal symbionts. Beetles rely on fungal symbionts as their sole food source and the fungi rely on their beetle hosts for dispersal and cultivar maintenance. These beetles are unique among fungus-farming insects in their development of specialized fungal transport organs called mycangia**,** which promote the growth of their fungal partners and dispersal to new environments, allowing for vertical symbiont transmission across beetle generations. Mycangia vary in size, shape, complexity, and location, and the more complex of these organs house glandular cells and display morphological plasticity upon symbiont recognition, suggesting that sustained chemical crosstalk occurs between microbe and host. Despite the critical role that mycangia play in these sustained, high-fidelity, interactions, very little is known regarding the factors contributing to the establishment and maintenance of mycangial symbioses. Here, we report a model preoral mycangia colonization system between the laurel wilt pathogen, *Raffaelea lauricola*, and its symbiotic beetle vectors in the *Xyleborus* group. Aposymbiotic beetles were reared in the presence of specific reporter strains of *R. lauricola*, and mycangia were colonized through beetle feeding. Aspects of mycangial colonization including rate, dynamics, fungal morphology, and physical interactions between the beetle host and the fungal microbe were subsequently examined by electron microscopy, cryosectioning and fluorescence microscopy, and counting of colony forming units. Specific hypotheses regarding

mycangial symbiosis were tested to address questions concerning the stability of colonization over time, the dynamics of mycangial change, and competition between different fungal species during mycangial colonization. Our data provides new insights into the nature of ambrosia symbioses that are important in light of recent ambrosia fungi emerging as devastating agriculture and forest pathogens. These data also establish a framework for genetically probing insect-fungal symbioses, ubiquitous but understudied natural systems.

754F The fungal root endophyte *Serendipita vermifera* displays inter-kingdom synergistic beneficial effects with the microbiota in *Arabidopsis thaliana* and barley *Gregor Langen*[1], Lisa Mahdi[1], Shingo Miyauchi[2,1], Ruben Eichfeld[1,3], Alga Zuccaro[1,3] 1) University of Cologne, Germany; 2) Max Planck Institute for Plant Breeding Research, Cologne, Germany; 3) Cluster of Excellence on Plant Sciences (CEPLAS), Cologne, Germany.

Plant root-associated bacteria can confer protection against pathogen infection. By contrast, the beneficial effects of root endophytic fungi and their synergistic interactions with bacteria remain poorly defined. We previously demonstrated that both local and systemic root colonisation by the Sebacinales endophyte *Serendipita vermifera* (syn. *Sebacina vermifera*, hereafter *Sv*) afford protection against infection with the soil-borne plant pathogen *Bipolaris sorokiniana* (syn. *Cochliobolus sativus*, hereafter *Bs*) in *Hordeum vulgare* (barley). Here we explore how *Sv* and *Bs* colonisation capacities in two plant species, barley and Arabidopsis, are modulated by the presence of individual members of the core bacterial microbiota or synthetic communities (SynComs) isolated from the barley rhizosphere or Arabidopsis roots. The finding that *Bs* also infects and causes disease symptoms in Arabidopsis roots motivated us to develop a set of physiological measurements to characterize disease severity and plant growth in Arabidopsis under different microbe treatment regimes. These measurements include ion leakage (quantified via electric conductivity) and photosynthetic activity (measured using pulse amplitude modulation fluorometry) as readouts for host cell death progression and biotic stress during the host-microbe interaction. Analyses of inter-kingdom activities in barley and Arabidopsis revealed that *Sv* can functionally replace root-associated bacteria by mitigating pathogen infection and disease symptoms in both hosts. Additionally, we show that cooperation between bacteria and beneficial fungi leads to inter-kingdom synergistic beneficial effects. We found that inter-kingdom protective benefits are largely independent of the host while synergism leading to early growth promotion is driven by host species and microbiota composition. Using RNA-sequencing (JGI proposal ID 505829), we additionally analyzed transcriptional responses of *S. vermifera* and the closely related *S. indica* to *A. thaliana*, barley, *Brachypodium distachyon,* bacterial Syncoms and to the fungal plant pathogen *Bipolaris sorokiniana* at 4 different time points providing insights to how microbes respond to plants and to other microorganisms. We conclude that plants have evolved to preferentially accommodate communities that support their health and that root-associated prokaryotic and eukaryotic microbes can act synergistically with the plant host in limiting fungal disease.

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755W The combined activity of two secreted fungal enzymes is implicated in fungal accommodation in the roots and triggers cell death in different host species Nick Dunken[1], Patrizia Zecuara[1], Pia Saake[1], *Alga Zuccaro*[1,2] 1) University of Cologne, Germany; 2) Cluster of Excellence on Plant Sciences (CEPLAS), Cologne, Germany.

Intracellular colonization of plant roots by the beneficial fungal endophyte *Serendipita indica* (syn. *Piriformospora indica*) follows a biphasic strategy. After an early biotrophic phase the interaction switches to a host cell death phase restricted to the root epidermis and cortex layer. This host cell death is required for fungal accommodation and the establishment of a long-lasting beneficial interaction in barley and *Arabidopsis thaliana*. However, how this cell death is activated and regulated is largely unknown. Here we show that two fungal enzymes, the ecto-5'-nucleotidase *Si*E5NT and the nuclease *Si*NucA act synergistically in the apoplast at the onset of cell death to produce deoxyadenosine (dAdo), a potent cell death inducer in animal systems. Uptake of extracellular dAdo, but not of the structurally related adenosine (Ado), activates a previously undescribed cell death mechanism in *A. thaliana* as well as in the liverwort *Marchantia polymorpha*, suggesting that a conserved cell death response to dAdo exists across plant lineages. Mutation of the root-expressed *A. thaliana* equilibrative nucleoside transporter *ENT3* confers resistance to extracellular dAdo-induced cell death and leads to decreased fungal-mediated cell death during root colonization. Additionally, in an attempt to identify downstream components mediating dAdo cell death, we performed a mutant screening of 6800 Arabidopsis T-DNA insertion lines. A gene encoding a nucleotide-binding leucine-rich repeat protein (NLR) was identified and proven to be implicated in dAdo-mediated cell death. Taken together our data show that the combined activity of two secreted fungal enzymes leads to the production of a metabolite, which is sufficient to trigger regulated cell death in different host species.

756T Decoding the nuances of fungal symbiosis using ambrosia beetles-*Raffaelea lauricola* as a model system *Kamaldeep Bansal*[1], Qiang Wang Wang[2,3], Jeffrey Rollins[3], Nemat Keyhani[1] 1) Department of Microbiology and Cell Science, University of Florida, Gainesville, FL; 2) Northwest A&F University, State Key Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Yangling, Shaanxi, China; 3) Department of Plant Pathology, University of Florida, Gainesville, FL.

Raffaelea lauricola, belonging to the Ophiostomataceae family, is a vascular pathogen which causes laurel wilt on hosts belonging to the Lauraceae family. *R. lauricola* is vectored by the redbay ambrosia beetle *Xyleborus glabratus,* native to Asia, while invasive in North America. *R. lauricola* exists in a symbiotic association with its vector ambrosia beetle, where it provides nutrition to the beetle colonies. In return, host beetles help disperse fungus to new plant hosts. Ambrosia beetles have developed highly specialized structures called mycangia to carry fungal cells. While many fungal partners of beetles are benign to the host plant, economic losses caused by laurel wilt threaten a number of important agricultural crops including avocado. To explore the intricate symbiotic association between ambrosia fungi and its vectors, we are developing physiological and molecular assays to characterize key genes involved in symbiosis. The *metR* gene encodes a bZip transcription factor that is a positive regulator of genes encoding transporters and enzymes involved in the uptake and utilization of sulfur sources. We have investigated a loss-of-function *metR* mutant for its possible impact on beetle-fungal symbiosis. Additionally, to understand and observe the ambrosial fungal interactions within the beetle mycangia, we have developed a protoplast-mediated genetic transformation system. This system will be utilized to produce fluorescently tagged strains of different *Raffaelea* species. These strains will be utilized for beetle feeding assays to characterize colony forming unit counts from mycangia of

ambrosia beetles. These strains will also assist in tracking fungal growth within the beetle mycangia.

757F ***Systems Biology of the Symbiosis of Arbuscular Mycorrhizal Fungi (AMF) in Sorghum*** *Shufan Zhang*[1], Michael Skaro[1], Amanda Bouffier-Lantrum[1], Isaac Torres[1], Yue Wu[1], Yinping Guo[1], Lauren Stupp[1], Camryn Felt[1], Brooke Lincoln[1], Sedonna Spann[2], Nancy Johnson[2] 1) University of Georgia, Athens, GA; 2) Northern Arizona University, Flagstaff, AZ.

Arbuscular Mycorrhizal Fungi (AMF) are obligate root symbionts, associating with at least 80% of terrestrial plant families and about which little is known. In return for plant assimilated carbon, these fungi provide nutrients, such as nitrogen and phosphorus, to their host plants. A systems biology approach is being used to understand this symbiosis in sorghum. To identify sorghum genotypes and genes that influence AMF abundance and plant biomass performance, a multi-year Genome Wide Association Study (GWAS) of AMF in sorghum on Wellbrook Farm in Watkinsville, GA is underway. Year 1 of the GWAS involves 79 accessions from the Bioenergy Accession Panel (BAP). The field was planted in early June, 2021 in 3 blocks with 79 rows and 9 seedlings of the same accession per row for a total of 2200 seedlings. 3 plants were harvested per row before and after flowering at three time points and measured for dry canopy weight and fresh and dry root weight. A high-throughput protocol for imaging symbiotic structures was developed using ink and vinegar staining. A total of 576 images of root intersections are being captured per plant for a total of 800 plants (88+288+424). Machine learning methods are being used to segment and classify AM fungal structures from the images for GWAS mapping to the sorghum genetic map. The results from GWAS, host biomass, microbiome, morphological, microscopic and transcriptome studies will feed into systems models, including structural equation models to predict sorghum genotypes that will maximize production (*i.e.*, plant health) knowing indigenous AMF in the field under different field conditions. **DOE DE-SC0021386.**

758W **Testing the role of the transcription factor TvSom1 in adhesion of *Trichoderma virens* germlings** Ariella Alperovitch-Lavy[1], Tri-Thuc Bui[2,3], Harting Rebekka[2], Braus Gerhard[2], *Benjamin Horwitz*[1] 1) Technion - IIT, Haifa, Israel; 2) Institute of Microbiology and Genetics, University of Göttingen, Germany; 3) College of Agriculture and Forestry, Thai Nguyen University , Vietnam.

Trichoderma-root interactions prime the plant immune response, attenuating disease upon later challenge with a pathogen. Relatively little is known about the molecular details of this opportunistic fungal-plant symbiosis. Attachment of hyphae or germlings to the root, however, is likely to be a critical early step. Like other microorganisms, Trichoderma may adhere to the plant host with adhesive molecules found on the hyphal surface. In the soilborne pathogen *Verticillium dahliae*, three transcription factors controlling the network underlying adhesion have been isolated by a yeast expression strategy [1]. TvSom1 is a candidate *T. virens* ortholog of one of these, Som1. The 2668 bp predicted coding region of TvSom1 in the *T. virens* database (Joint Genome Institute [2]) is interrupted by 4 introns and encodes a 795 amino acid protein. Alignment of TvSom1 with *V. dahliae* Som1 gives 63.9% identity and 82.4% similarity. The sequences of the LisH domain, nuclear localization signal (NLS) in the N-terminal half of the protein, and SSDP domain are well-conserved in the alignment, while the SnAPC domain is less so with disconnected regions of identity, and the NLS in the C-terminal region differs at 3 residues. TvSom1 is expressed in germinating conidia. In the most abundant transcript, the first two introns are spliced. Transcripts in which either the first or the second intron is spliced were detected at the order of 1%, and we are testing whether their expression is developmentally regulated. Adhesion-related genes can eventually be targeted for both understanding of, and agriculturally-relevant manipulation of, the Trichoderma-root interaction. New insights can be obtained into biocontrol of fungal pathogens in the soil, and the trade-off between fungal-fungal and fungal-root interactions in the rhizosphere. This balance is agriculturally relevant.
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759V **The puzzle of mini-chromosomes and Fumonisins in *Fusarium verticillioide*** *Luigi Faino*[1], Alessandro Grottoli[1], Marzia Beccaccioli[1], Valeria Scala[2], Maria Aragona[2], Rosita Silvana Fratini[1], Massimo Reverberi[1] 1) Department of Environmental Biology, University of Rome «Sapienza», P.le Aldo Moro 5 , 00185 Rome, Italy; 2) Research Centre for Plant Protection and Certification, Council for Agricultural Research and Economics (CREA), Via C.G. Bertero 22, 00156 Rome, Italy.

The maize pathogen *Fusarium verticillioides* (*Fv*) causes huge economic losses per year due to the production of fumonisins (FBs). To clarify the role of FBs in the interaction maize-*Fv*, we infected maize kernels and stalk and we quantified their amount. The results show the ability of *Fv* infecting seeds to produce FBs while no production in maize infected on the stalk occur. These results were corroborated by the lack of expression of the *Fum* cluster in the stalk assay while it is highly expressed in the kernel assay. Independent stalk assay with *Fv Fum1* deletion mutant showed no difference between wild type and *Fum1-Fv* mutant on necrosis development whilst these differences emerged in the roots of germinating kernels. FBs content was analyzed during the kernel assay using two *Fv* strains *Fusarium verticillioides* 7600 and ITEM 10027 (*Fv*10027) which is an Italian isolate. Difference in FB production was observed and we start to investigate the reasons behind this difference. Although the difference in fumonisins production, the pathogenicity tests on maize stalk did not show any difference in infection levels. To better understand *Fv* virulence, the genome of *Fv*10027 was sequenced and assembled. Comparative genomics between *Fv*7600 and *Fv*10027 showed a difference in genome size of about 1.4 Mb despite 99% nucleotide identity. Genome assembly of *Fv*10027 showed that two mini-chromosomes of about 1 Mb and 750 Kb were unique to *Fv*10027 while a mini-chromosome of about 400Kb to *Fv*7600. To determine the presence of these mini chromosomes in the Italian *Fv* population, 24 strains, sampled in the Po valley, were sequenced; presence/absence analysis showed that only three *Fv* strains had those chromosomes. The analysis of *Fv*10027 dispensable chromosomes showed an enrichment of secreted proteins and a higher level of repetitive elements. Intriguingly, BLAST analysis on the *Fv*10027 proteome showed that proteins codified on mini chromosomes have the best identity to *F. oxysporum* proteins located at dispensable chr3 and chr6. Moreover, synonyms substitution analysis suggests that mini chromosomes of *F. verticillioides* were not probably acquired through a horizontal chromosomal transfer from *F. oxysporum* but rather originated before the split of the two species.

760V Isolate specific effects of *Botrytis cinerea* on the expression of biosynthetic enzymes and stimulation of Jasmonic and Salicylic acid signaling in *Arabidopsis thaliana*. *Jordan Dowell*[1], Celine Caseys[1], Daniel Kliebenstein [1] 1) University of California, Davis.

Botrytis cinerea is a common necrotrophic fungal pathogen capable of infecting over a thousand plant-host species. Earlier studies exploring the co-transcriptomic responses of 98 isolates of *B. cinerea* infecting *Arabidopsis thaliana* wild-type Col-0 and the JA- and SA-signaling mutants, coi1-1, and npr1-1, revealed that Jasmonic and Salicylic acid signaling pathways function to constrain and canalize the range of virulence in the *B. cinerea* population with variable efficacy. However, a detailed examination of how individual isolates vary in their inducive effects on metabolic pathways has not been explored. To address this, we deconstruct earlier modeling efforts in a Bayesian framework to describe isolate-specific effects in the context of JA- and SA- signaling on biosynthetic enzymes in *A. thaliana* wild-type Col-0 and the JA- and SA-signaling mutants, coi1-1, and npr1-1. Isolate specific effects vary among the *B. cinerea* population with differential inducive effects on plant primary and specialized metabolic pathways. Further clustering of isolate specific effects and variation in virulence buffering of JA- and SA- signaling pathways implicates a potential range of inducible metabolic phenotypes in *A. thaliana* when infected with diverse *B. cinerea* isolates. Finally, we examine the genetic architecture of induction of *A. thaliana* JA- and SA- signaling by *B. cinerea* with genome-wide association (GWA) in *B. cinerea*, leveraging the change in expression of several plant specialized metabolite biosynthetic enzymes and defense signaling genes between *A. thaliana* wild type Col-0 and the JA- and SA-signaling mutants, coi1-1, and npr1-1 during infection with *B. cinerea*.

761V Transcriptomic approach to unveil the interaction between a biocontrol yeast and a postharvest fungal pathogen on the host fruit *Giuseppe Ianiri*[1], Giuseppe Barone[1], Davide Palmieri[1], Filippo De Curtis[1], Giuseppe Lima[1], Raffaello Castoria[1] 1) University of Molise.

Biocontrol strategies are a promising alternative to achieve food safety and food security. The aim of this study was to decipher the molecular interactions involving the biocontrol agent (BCA) yeast *Papiliotrema terrestris* strain LS28, the postharvest pathogen *Penicillium expansum*, and *Malus domestica*. RNAseq analysis was performed during both their dual and tritrophic interactions to identify the differentially expressed genes of BCA, pathogen, and host. Analysis of transcriptome changes in the BCA revealed that, regardless of the presence of the pathogen, there was overexpression of genes involved nitrogen catabolite repression and oxidative stress response, suggesting that these pathways are crucial for the BCA to rapidly colonize the ecological niche (fruit wounds) and outcompete the pathogen. In the absence of *P. expansum*, BCA genes involved in metabolism and transport of carbohydrates and branched-chain amino acids were highly represented, suggesting a different nutritional requirement of *P. terrestris* when it is not competing with the pathogen. To confirm transcriptomic data at phenotypic level, for the BCA targeted mutants were generated for several overexpressed genes, and the *in vitro* and *in vivo* phenotypic characterization revealed a crucial role of a putative aminoacid transporter in the biocontrol activity of *P. terrestris* against *P. expansum*. For transcriptomic analysis of *P. expansum*, genes involved in transcription, oxidation reduction process, transmembrane transport, and amine and peptide metabolism were the most represented GO categories, regardless of the presence of the BCA. While in the absence of the BCA there was only enrichment of oxidation reduction process, in the presence of the BCA metabolic processes of polysaccharides, aminoglycan and glucosamine-containing compounds were strongly enriched, suggesting a substantial nutritional rewiring of the pathogen to directly outcompete the BCA. Analysis of the transcriptomic changes of the host *M. domestica* revealed overexpression of genes involved in host defense signaling pathways both in the presence of the BCA and the pathogen, and a prevalence of PTI and ETI host genes overexpressed only during interaction with *P. expansum.* This comprehensive analysis contributes to advance the knowledge on the molecular mechanisms that underlie biocontrol activity and the tritrophic interaction with the pathogen and/or the host.

762V Accessory chromosome loss contributes to increased symbiotic effectiveness of a tree root fungus Huanshen Wei [1,2], Zhongfeng Li[1,2], Long Peng [1,2], Xinghua He [1,2], Yuzhan Yang [1,2], *Zhilin Yuan*[1,2] 1) Research Institute of Subtropical Forestry, Chinese Academy of Forestry.; 2) State Key Laboratory of Tree Genetics and Breeding, Chinese Academy of Forestry..

Intraspecific genetic variation reflects the potentials for evolutionary adaptation and speciation. Although ubiquitous across all forms of life, the degree of phenotypic plasticity, ecological consequences, and underlying mechanisms of symbiotic fungi remain elusive. To fill this gap, we combined phenotypic, multi-omic, and forward genetic to investigate the unique genetic divergence in two co-existing isolates (16B and 16W) of a fungus (*Stagonosporopsis rhizophilae* sp. nov) an endemic endophyte in natural poplar roots, which vary considerably in colony appearance, metabolic repertoires, and capability in affecting plant growth. Comparative genomic and transcriptomic analyses suggest that the two isolates exhibited subtle differences in genomics sequences, while nearly *one-third* of genes are differentially expressed due to a contrasting *Cis*-regulatory pattern as evidenced by distinctive chromatin accessibility profiles. Intriguingly, we identified an accessory chromosome (AC) in 16W with ~0.6 Mb in length. The 16W only colonizes on root surface, and marginally inhibits plant growth without causing visible symptoms. Intriguingly, the AC deletion mutant (D16W) resembles 16B in many aspects, particularly in behaving as a dark septate endophyte, significantly promoting root development and salinity tolerance. Together, these results suggests that the intraspecies transcriptional dynamics mediated through differentiation in chromatin accessibility are sufficient to favor the divergence of the conspecific isolates. This discovery challenges our understanding of role of AC in mediating plant-fungal beneficial interactions and suggests frequent transcriptional regulation of AC on core chromosomes drives mutualism breakdown.

763V Comparative Genomics of Four Mollicutes-Related Endobacteria from the Mortierellaceae *Reid Longley*[1], Aaron Robinson[2], Robert Riley[3], Kerrie Barry[3], Igor V. Grigoriev[3], Kurt LaButti[3], Alessandro Desiro[1], Julian Liber[4], Patrick Chain[2], Gregory Bonito[1] 1) Michigan State University, East Lansing, MI; 2) Los Alamos National Laboratory, Los Alamos, NM; 3) Joint Genome Institute, Berkeley, CA; 4) Duke University, Durham, NC.

Diverse members of the early-diverging Mucoromycota including arbuscular mycorrhizal fungi and soil fungi in the Mortierellaceae are capable of harboring both Gram-positive Mollicutes-related endobacteria (MRE) and Gram-negative Burkholderia related endobacteria (BRE). Previous work has shown that MRE are dependent on their fungal host and their genomes are thought to be severely reduced in size. Given this, it has been hypothesized that MRE bacterial endosymbionts were acquired early, prior to the diversification

of Mucoromycota. Alternatively, these myco-symbionts could have been acquired after the divergence of these lineages and spread horizontally between lineages. To address these hypotheses, we obtained four complete MRE genomes from two genera in the Mortierellaceae: *Linnemannia* (LMRE) and *Benniella* (BMRE). The size of these genomes ranged from 326 to 615 Kbp and includes the smallest known complete bacterial genomes of myco-symbionts. Comparative analyses of these genomes revealed unique content and organization with respect to each MRE lineage and provides insight as to how bacterial genomes may adapt to a particular fungal host. Homology based comparisons of predicted proteins revealed differences in genome reduction as a result of the endosymbiosis. Additionally, MRE protein lengths were significantly shorter on average compared to closely related *Mycoplasma* and *Sprioplasma* relatives. Multigene phylogenetic analysis also indicated that the MRE genomes within *Benniella* were more closely related to MRE from Glomeromycotina compared to MRE in more closely related *Linnemannia*. These results indicate that *Linnemannia* and *Benniella* isolates may have acquired their MRE after divergence from a common ancestor. The outcomes of this work expand upon foundational knowledge of the evolutionary impacts of bacterial-fungal interactions, towards the goal of continued investigations of evolution and impacts of these interactions on host and endosymbiont.

764V Investigating microbial reservoirs for antivirulence compounds that attenuates dimorphism in the fungal pathogen *Candida albicans*. *Jehoshua Sharma*[1], Rebecca Shapiro[1] 1) Department of Molecular and Cellular Biology, University of Guelph, Ontario, Canada.

Fungal pathogens are estimated to infect over 1 billion people and kill over 1.5 million people a year, establishing themselves as a global contemporary threat. *Candida albicans*, a prominent opportunistic fungal pathogen, undergoes a dimorphic switch from ovoid yeast to filamentous hyphae; *C. albicans*' dimorphism enables severe and invasive bloodstream infections, with mortality rates ranging from ~30-70%. In the clinic, these infections have been treated with antifungals that target *C. albicans* and disrupt its cell integrity to induce lysis. However, a predictable consequence of the extensive use of antifungals is the subsequent rise in antifungal drug resistance. These new drug resistant isolates proliferate unchecked, causing further harm to the infected host and are spreading globally. Fundamentally, the use of antimicrobials are problematic as they possess a high selection pressure they impose on microbial pathogens to evolve resistance. A promising alternative, and the focus of this research, is to find compounds that target and inhibit *Candida* virulence factors, such as dimorphism, rather than attempting to eliminate the pathogen altogether. Previous compounds that have been investigated to inhibit dimorphism provide limited uses, having off-target or cytotoxic effects, demonstrating a need for new compounds that are both potent and safe.
Given that *C. albicans* is a human commensal and commonly found in the gut, I hypothesize that screening the supernatant of other gut commensals will yield antivirulence compounds that are noncytotoxic and can selectively inhibit *C. albicans*' dimorphism and hyphal formation. The screen is being conducted using high-throughput whole well imaging of a dual reporter strain that has hyphal formation tagged to GFP expression. When this strain is observed growing in the supernatants of other gut commensal microbes, fluorescence intensity quantifications can be used as a direct measure of hyphal formation inhibition. My presentation will describe the initial validation and optimization of this platform against a library of over 200 human gut commensals. Overall, this research will create a high level screen that can monitor the dimorphic switch of *C. albicans*. This reporter system can then be used to not only study dimorphism but also discover compounds that prevent this pathogenic metabolic process.

765V Identification of novel effector proteins in *Cercospora beticola* *Olivia Hamilton*[1,3], Lorena Rangel[1], Ronnie de Jonge[2], Melvin Bolton[1] 1) United States Department of Agriculture, Fargo, ND; 2) Department of Plant-Microbe Interactions, Utrecht University, Utrecht, The Netherlands; 3) North Dakota State University, Fargo, ND.

Cercospora beticola is a hemibiotrophic fungus responsible for Cercospora leaf spot disease of sugar beet (Beta vulgaris). Plant pathogens such as *C. beticola* utilize "effector" molecules to aid in disease establishment. Effectors are generally characterized as small, secreted molecules that contribute to pathogen virulence. A culture filtrate infiltration study was conducted to identify potential effector molecules secreted by *C. beticola*. A variety of fungal growth conditions were pursued, one of which resulted in a necrotic phenotype when the culture filtrate was infiltrated into sugar beet leaves. The culture filtrate was fractioned using ion-exchange chromatography, and fractions were then infiltrated into sugar beet leaves to identify the protein responsible for necrosis. Three culture filtrate fractions were sent for mass spectrometry analysis, identifying five candidate necrosis-inducing effector proteins. Targeted gene disruption of these candidates and subsequent virulence assays displayed an increase in virulence for Δ05663 strains demonstrated by higher levels of disease severity on inoculated sugar beet when compared to the wild-type strain. Full characterization of this candidate effector will shed light on the *C. beticola*-sugar beet interaction.

766V Seasonal dynamics in the bacterial microbiome of field grown CLS-resistant and -susceptible sugar beet varieties *Lorena Rangel*[1], Mari Natwick[1], Nathan Wyatt[1], Melvin Bolton[1] 1) United States Department of Agriculture, Fargo, ND .

Cercospora leaf spot (CLS), caused by the fungal pathogen Cercospora beticola, is the most destructive phyllosphere pathogen of field-grown sugar beets (Beta vulgaris). Located on the Minnesota and North Dakota border, the Red River Valley (RRV) is the top growing region for sugar beets in the United States and is responsible for over 20% of all domestically produced sugar. The sugar losses due to CLS in the RRV can be devastating as a result of phyllosphere destruction by this fungus and subsequent biomass loss of the sugar beet root. Here, we sampled leaves of CLS-resistant and CLS-susceptible cultivars grown in a single field in the RRV for the purpose of monitoring changes in the phyllosphere microbiome over the growing season. Field-grown sugar beets were allowed to naturally acquire CLS although they did undergo a typical fungicide regime. Fifty leaves from CLS-resistant or -susceptible sugar beets were harvested every three weeks starting when leaves were fully expanded by the beginning of June and ending during harvest in late September 2021. We harvested a total of six timepoints, ensuring that we sampled before and after CLS symptoms were observable. Additionally, we surveyed adjacent fields growing corn and soybean to assess the effect of locality on the given microbiomes. Leaves were sonicated in potassium phosphate buffer and leaf wash was filtered through a 0.45μm filter and concentrated into a pellet. Total DNA was extracted from this pellet and the full bacterial 16S rRNA gene and fungal ITS gene regions were amplified, although here we will strictly discuss bacterial community results. The Oxford Nanopore MinION platform was used to sequence the V1-V9 16S rRNA gene region for each sample from each time point. Bacterial microbiome communities were profiled and compared between CLS-re-

sistant and -susceptible sugar beet varieties. Temporal changes within sugar beet varieties were also monitored in efforts to note major bacterial assembly shifts upon CLS introduction and/or symptom development. This research uncovers key taxa indicative of healthy or CLS-diseased sugar beet plants and can be translated into the management of ecosystem health and preventative measures against CLS.

767V Impact of the mycoparasite *Pythium oligandrum* on mutualistic interactions and disease resistance as well as growth induction in *Medicago truncatula* *Maryam Hashemi*[1], Jean-Malo Couzigou [1], Elodie Gaulin[1], Remi Pendaries[1,2], sebastien Roy[1,3], Thomas Rey[1,2], Bernard Dumas[1] 1) Laboratoire de Recherche en Sciences Végétales, Université de Toulouse, CNRS, Université Toulouse II; 2) DE SANGOSSE, Bonnel, 47480, Pont-Du-Casse, France; 3) AGRONUTRITION, 31390, Carbonne, France.

Pythium oligandrum is a soil-borne oomycete associated with rhizosphere and root tissue and has been used in different plant systems to control various drastic soil-borne pathogens. Although numerous studies have shown the effect *P. oligandrum* on growth promotion and protection, its impacts on mutualistic interactions and more generally on root microbial community is yet unclear. Our research aims to understand interactions between *P. oligandrum* and mutualistic organisms as well as the overall root microbiota. To do so, we devised a model system using the model legume *Medicago truncatula* and *P. oligandrum* strain M1*,* a commercially available strain. Our results showed that *P. oligandrum* increased *M. truncatula* biomass and yield and a similar observation was drawn for *P.sativum*. Likewise, *P.oligandrum* soil inoculation induced multiple defense pathways in *M. truncatula*, as seen by RNAseq experiments and promoted resistance to the root rot caused by *Aphanomyces euteiches* in *M. truncatula* and *P.sativum*. In terms of mutualistic interactions, *P. oligandrum* promoted the multiplication of a nitrogen-fixing endobacteria *Ensifer meliloti* around *M. truncatula* roots in early stage of growth, while, *P.oligandrum* soil inoculation resulted in the development of large and multilobed nodules. In addition, *P. oligandrum* did not negatively affect the formation of arbuscular mycorrhizal symbiosis in *M. truncatula*. Metagenomic analysis showed that application of *P.oligandrum* mycelium in soil changes the structure of microbial community (bacteria and fungi) by reducing the relative abundance of fungal taxa related to phytopathogens such as *Fusarium sp.*, or by promoting the relative abundance of other mycoparasite fungal genera like *Trichoderma, Clitopilus* and *Alatospora*. Together, our results opens new horizons toward understanding the benefits of *P. oligandrum* on microbial and mutualistic interactions of plants roots.
keywords: *Pythium oligandrum,* Microbiota, symbiotic interaction, plant protection and development, legumes

768V Breakdown and maintenance of tree-fungal mutualism: why the nitrogen form matters? *Long Peng*[1], Yan Zhang[3], Zhi-yong Zhu [2], Irina S. Druzhinina[4], Francis Martin[5], Zhilin Yuan [1] 1) State Key Laboratory of Tree Genetics and Breeding, Chinese Academy of Forestry, Beijing 10091, China; 2) Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Hangzhou 311400, China; 3) Liaoning Provincial Institute of Poplar, Gaizhou 115213, China.; 4) Fungal Genomics Laboratory (FungiG), College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China; 5) INRA, UMR 1136 INRA-Université de Lorraine 'Interactions Arbres/Microorganismes', Laboratoire d›Excellence ARBRE, Centre INRA-Lorraine, Champenoux, France.

External nutrient supply often drives the establishment or breakdown of symbiotic associations. Although intuitive, this perspective has not often been stated explicitly. Here, we take advantage of the agaric fungus Clitopilus hobsonii and poplar association as a tractable model system to decipher the molecular mechanism underlying such symbiosis plasticity. Parallel assays of whole plant, detached leaves, and suspension cells exposed to either the living fungus or its crude extracts indicated that the nitrogen form could trigger on/off status of a mutualism. Specifically, an active functional symbiosis between C. hobsonii and poplar under organic nitrogen conditions is proposed, featured by the development of ectomycorrhizal (ECM)-like structures. Isotopic and transcriptomic data suggest that the fungus could transfer 15N sources to aboveground plant parts. In contrast, this mutualistic interaction was heavily disrupted under both NH4+ and NO3- conditions. Intrinsically, C. hobsonii appears to be intermediate between obligate saprotrophs and ectomycorrhizal/endophytic fungi inferred from comparative genomic analysis of total carbohydrate-active enzymes (CAZomes). Further in vitro (feeding on three nitrogen forms and sterile litters) and in planta fungal transcriptomes revealed contrasting gene expression patterns related to CAZomes, secondary metabolite biosynthetic clusters, and nitrogen uptake processes (transportation and mineralization). NH4+ rendered the fungus to be more aggressive, evidenced by upregulation of representative glucoside hydrolase families (GH) acting as virulence factors and continuously increased H2O2 production in roots, while NO3- addition probably resulted in formation of potential toxins. On the organic nitrogen side, fine-tune regulations of GH5, GH28, and AA9, which serving as key genetic determinants of mutualism, were recorded. Together, our work encourages both a more mechanistic and generalizable understanding of nutrient form-dependent mutualisms. Along with previous observations, we propose that this conditional mutualism may be more widespread than previously thought in natural forests. Such pattern of holobiont response is also likely to be ecologically important, particularly in the context of increasing global nitrogen deposition (primarily in the inorganic form).

769V *Fusarium oxysporum* induces the expression of the gene encoding the plant specific tissue protein 6 (ST6) involved in root and plant vasculature development *Virginia Casado del Castillo*[1,3], Lucía Albornos[2,3], Ignacio Martín[2,3], José María Díaz Mínguez[1,3], Berta Dopico[2,3] 1) Departamento de Microbiología y Genética, University of Salamanca; 2) Departamento de Botánica y Fisiología Vegetal, University of Salamanca; 3) Instituto de Investigación en Agrobiotecnología (CIALE).

Specific tissue (ST) proteins found in some dicotyledons plants belong to the protein family PF10950 and are encoded by multigenic families. In *Medicago truncatula*, a plant model for the economically important leguminous crops, the *ST1* and *ST6* coding genes are mainly expressed in roots where they participate in the development of primary and lateral roots and also in specific biotic interactions. *F. oxysporum* f. sp. *medicaginis* (*Fom*) is a soil inhabitant that behaves as a root colonizer and causes vascular wilt in *M. truncatula*. As other members of the *F. oxysporum* species complex, *Fom* first colonizes the epidermis and then progresses through the cortex towards the root vascular cylinder. Infected plants show reduced growth, clearly visible from 7 days post infection (dpi).
The activities of the *ST1* and *ST6* promoters are differentially activated in the root apex and during lateral root development, with only *ST6* being related to meristem activity. Also, both genes show different roles in response to *Fom* infection. *ST6* transcripts increased up to 6 times 1 dpi and continued to increase as the infection spread. On the contrary, transcript accumulation of *ST1* showed no change in response to *Fom*, except for a transient decrease at 7 and 10 dpi. These results were confirmed by analyses of *ST1* and *ST6* promoter activities in pST::GUS transgenic plants. *pST6* activity was particularly intense in the vascular cylinder of

lateral roots. Discontinuities in the basal area of emerging lateral roots are preferred sites of attachment and penetration by *F. oxysporum*. Transcript accumulation of several gene markers showed that SA is the main signalling pathway induced during *Fom* colonization, as was previously found in the *F. oxysporum* f. sp. *phaseoli*-common bean interaction.
This work was supported by grants BFU-2013-44793-P and AGL2015-66131-C2-1-R (MINECO, Spain).

770V ***Fusarium oxysporum FOSP1G_05432,*** **the orthologous of** ***B. cinerea Bcin04g03490*****, is involved in growth, sporulation and virulence** Jenny Jasbleidy Molina Imbachi[1], *Virginia Casado del Castillo*[1], Ernesto Pérez Benito[1], José María Díaz Mínguez[1] 1) Instituto de Investigación en Agrobiotecnología (CIALE), University of Salamanca.

We have identified the gene orthologous to *Bcin04g03490*, which controls development and pathogenicity in the necrotroph *B. cinerea*, in *F. oxysporum*. *FOSP1G_05432* is a single locus gene located in the chromosome 7 of the core genome of *F. oxysporum*. It encodes a 680 amino acids protein with two functional domains: a GAL4-like Zn(II)2Cys6 binuclear cluster DNA binding domain and an acetyltransferase domain.
Gene replacement mutants obtained by homologous recombination were verified by PCR and Southern hybridization. Single-conidial isolates of three selected mutants were phenotypically analyzed.
Mutants of *F. oxysporum* f.sp. *phaseoli* SP1 strain with a complete deletion of *FOSP1G_05432* show increased radial growth when cultured in continuous dark or light, or under a 16:8 photoperiod. The colony morphology is thin and flat, with a drastic reduction in aerial mycelium, leading to a significant decrease in surface hydrophobicity. This phenotype is reminiscent of that displayed by nitrate non-utilizing and velvet protein complex mutants. However, unlike the sporulation phenotype observed in velvet mutants, the production of microconidia is greatly reduced and a statistically significant increase in the production of macroconidia could be observed in continuous light cultures. Mutant mycelia grown in solid cultures for a few days showed a strong violet pigmentation, which is characteristic of aged wild type colonies.
Pathogenicity assays conducted on common bean (*Phaseolus vulgaris* L.) plants indicated that abolition of *FOSP1G_05432* gene expression resulted in a drastic reduction of virulence, as shown by the decrease in the disease scale index, disease progression rate and longer internodal lengths of infected plants.
This work was supported by grant PID2019-110605RB-100 from Ministry of Science and Innovation (Spain).

771V **Deep learning-based quantification of fungi in plant roots** *Edouard Evangelisti*[1], Carl Turner[2], Alice McDowell[1], Liron Shenhav[1], Temur Yunusov[1], Aleksandr Gavrin[1], Emily K Servante[3], Clément Quan[1], Sebastian Schornack[1] 1) Sainsbury Laboratory, University of Cambridge (Cambridge, UK); 2) Department of Applied Mathematics and Theoretical Physics, University of Cambridge (Cambridge, UK); 3) Department of Plant Sciences, University of Cambridge (Cambridge, UK).

Quantifying the extent of host colonisation by symbiotic, endophytic and pathogenic fungi is essential to research on fungal-plant associations. For instance, quantification of arbuscular mycorrhizal (AM) fungi relies on visual inspection of roots, a labour-intensive method that is prone to variations between experimentalists. We developed AMFinder, a computer-based alternative to assess AM colonisation phenotypes. AMFinder uses deep learning to automatically label colonised root sections and intraradical hyphal structures in stained root images. The software adapts to a wide array of experimental conditions and is compatible with multiple host plants and fungal endosymbionts. It enables accurate, reproducible analyses of plant root systems and supports better documentation of AM fungal colonisation analyses. AMFinder is fully trainable and can thus easily adapt to other types of interactions and hyphal structures.

772V **Genomic and associated soil microbioal community comparisons of two** ***Armillaria*** **species with different ecological behaviors** *Jane Stewart*[1], Jorge Ibarra Caballero[1], Bradley Lalande[1,2], Mee-Sook Kim[3], John Hanna[4], Ned Klopfenstein[4] 1) Agricultural Biology, Colorado State University, Fort Collins, CO; 2) USDA Forest Service, Forest Health Protection, Gunnison, CO; 3) USDA Forest Service, Pacific Northwest Research Station, Corvallis, OR; 4) USDA Forest Service, Rocky Mountain Research Station, Moscow, ID.

Armillaria species show considerable variation in ecological roles and virulence, from mycorrhizae and saprophytes to important root pathogens of trees and horticultural crops. We studied two *Armillaria* species that can be found in coniferous forests of northwestern USA and southwestern Canada. *Armillaria altimontana* is considered as a weak, opportunistic pathogen of coniferous trees, but it also appears to exhibit *in situ* biological control against *A. solidipes*, formerly North American *A. ostoyae*, which is considered a virulent pathogen of coniferous trees. We describe genome assemblies and present a functional annotation of the predicted genes and proteins for*Armillaria* altimontana and A. solidipes which exhibit contrasting ecological roles. In addition, we examined soil microbial (bacterial and fungal) communities in association with the two *Armillaria* species in a 45-year-old plantation of western white pine (*Pinus monticola*) in northern Idaho, USA, where *A. altimontana* was associated with improved tree growth and survival, while *A. solidipes* was associated with reduced growth and survival.

773V **Genetic mapping of new QTL conferring virulence in** ***Pyrenophora tritici-repentis*** *Jingwei Guo*[1], Gongjun Shi[1], Zhaohui Liu[1] 1) North Dakota State University.

The ascomycete *Pyrenophora tritici-repentis* (*Ptr*) is the causal agent of tan spot of wheat, a common and economically important disease in all wheat growing regions. Three necrotrophic effectors (NE), including Ptr ToxA, Ptr ToxB and Ptr ToxC, have been identified from the fungal pathogen as important virulence factors. However, many studies have suggested the fungal pathogen produces additional NEs. To identify new NEs from the race 2 isolate 86-124, we developed a fungal population from a cross between this isolate and the race 5 isolate DW5. The population was subjected to genotyping with SNP and SSR markers as well as *ToxA*, *ToxB*, and mating type genes. For phenotyping, each progeny was inoculated onto the Ptr ToxA-insensitive line CDC-Osler which is susceptible to 86-124, but resistant to DW5. The constructed genetic map consisted of 11 linkage groups (LG) which corresponded to the 11 *P. tritici-repentis* chromosomes (Ch.) of the optical map of the reference genome Pt-1C-BFP. Five of six *ToxB* copies are tightly linked with each other residing at the distal end of Ch.11 while the sixth copy was located to the distal end of Ch.5. Virulence of 86-124 toward CDC-Osler, designated as *VirOsler*, mapped to two genomic regions with one being a major QTL (*VirOsler1*) on Ch.2 and the other being a minor QTL (*VirOsler2*) on Ch.7. The identification of new virulence factors is a significant step to further understand fungal virulence and host pathogen interaction in wheat tan spot system.

774V ***Marchantia polymorpha*** **model reveals conserved mechanisms governing infection by the vascular wilt fungal pathogen** ***Fusarium oxysporum*** *Amey Redkar*[1], Selena Gimenez Ibanez[2], Mugdha Sabale[1], Bernd Zechmann[3], Roberto Solano[2], Antonio Di Pietro[1] 1) Departamento de Genética, Universidad de Córdoba, Córdoba, Spain; 2) Departamento de Genética Molecular de Plantas, Centro Nacional de Biotecnología CSIC, Madrid, Spain; 3) Baylor University, Center for Microscopy and Imaging, Waco,Texas, USA.

Root-infecting vascular fungi cause wilt diseases and provoke devastating losses in hundreds of crops. It is currently unknown how these pathogens evolved and whether they can also infect non-vascular plants, which diverged from vascular plants over 450 million years ago. We established a pathosystem between the non-vascular liverwort *Marchantia polymorpha* (Mp) and the root-infecting vascular wilt fungus *Fusarium oxysporum* (Fo). On angiosperms, Fo exhibits exquisite adaptation to the plant xylem niche as well as host-specific pathogenicity, both of which are conferred by effectors encoded on lineage-specific (LS) chromosomes. Fo isolates with different host specificities and lifestyles on angiosperms have the ability to infect Mp. Moreover, Fo isolates displaying contrasting lifestyles on angiosperms - pathogenic versus endophytic – are able to infect Mp and cause tissue maceration and host cell killing. Mp senses the externally applied fungal PAMP chitohexose and responds by upregulating pathogen-associated molecular patterns (PAMP) responsive genes, which are also induced in response to crude extracts from different Fo strains. Using isogenic Fo mutants we define a set of conserved fungal pathogenicity factors, including mitogen activated protein kinases, transcriptional regulators and cell wall remodeling enzymes, that are required for infection of both vascular and non-vascular plants. Remarkably, two host-specific effectors and a morphogenetic regulator, which contribute to vascular colonization and virulence on tomato plants are dispensable on Mp. Collectively, these findings suggest that vascular wilt fungi employ conserved infection strategies on non-vascular and vascular plant lineages but also have specific mechanisms to access the vascular niche of angiosperms.

775V **Screening of small secreted proteins of** ***Epichloë bromicola*** **for Hypersensitive Response–Associated effectors in** ***Nicotiana spp.*** *Pranav Chettri*[1], Benjamin Moody[1], Christine R. Voisey[1], Rosie E. Bradshaw[2], Carl H. Mesarich[3], Richard D. Johnson[1], Wayne R. Simpson [1], Linda J. Johnson[1] 1) AgResearch (Grasslands Research Centre); 2) School of Fundamental Sciences, Massey University, Palmerston North, New Zealand; 3) School of Agriculture and Environment, Massey University, Palmerston North, New Zealand.

Effectors are proteins expressed by microbes that aid infection of specific plant species. Effectors of fungal pathogens play crucial roles in actively suppressing host defence responses or preventing their recognition by corresponding plant immune receptor proteins; however, little is known about their roles in mutualistic symbioses. The fungal endophyte *Epichloë bromicola* forms mutualistic symbioses with grasses of the *Elymus* complex. Artificial inoculation of *Epichloë* endophytes into non- host plant to form novel symbioses has produced a range of infected plant phenotypes (including severe plant stunting and/or death). To understand the molecular basis of endophyte-host interactions, a transcriptome analysis was conducted with *E. bromicola* in association with its native host, and a non-host grass from the tribe Hordeae. Many *E. bromicola* small secreted protein genes (putative effectors) were differentially expressed during these contrasting interactions.
To evaluate the roles of *E. bromicola* effectors in a non-host plant, 29 candidate effector genes were identified using a bioinformatics pipeline and transiently expressed in leaves of *Nicotiana tabacum* and *Nicotiana benthamiana by* Agroinfiltration. Significantly, four of the putative effectors triggered cell death in *Nicotiana spp* when expressed in the apoplast or the cytoplasm, suggesting that they are recognised as potential invasion patterns by non-host plants. This supports the existence of an evolutionarily-conserved recognition mechanism in plants in response to effectors associated with symbiotic fungi. To our knowledge, these are the first *Epichloë bromicola* effectors identified. Future work will focus on molecular characterisation.

776V **Discovery and selection of fungal endophytes for disease resistance of barley** *Olga Lastovetsky*[1], Brian Murphy[2], Fiona Doohan[1], Trevor Hodkinson[2], Angela Feechan[1] 1) University College Dublin, Dublin, Ireland; 2) Trinity College Dublin, Dublin, Ireland.

Ramularia leaf spot (RLS) is an emerging disease of barley, caused by the Ascomycete fungus *Ramularia collo-cygni*. RLS can result in up to 70% yield losses with current control strategies reliant on intensive fungicide programs. However, due to increased incidence of fungicide resistance and recent global strategies to reduce the use of pesticides (e.g. EU “Farm to Fork” and “Biodiversity 2030”), there is an urgent need for development of alternative RLS control strategies. Wild relatives of cereals represent a rich source of beneficial microbial endophytes. These endophytes can increase disease resistance in crops and represent a previously unexplored resource for RLS disease control. Working with fungal endophytes isolated from wild barley relatives, we identified 44 isolates which inhibited the growth of *R. collo-cygni* in culture. Of these, 11 were strong inhibitors and 33 were weak inhibitors. Using a combination of genomics, transcriptomics and glass-house experiments, these 44 candidates are being explored for production of secreted compounds and as biocontrol agents for RLS disease control.

777V **Characterization of urease in** ***Aspergillus fumigatus*****: Biochemistry and implications for virulence** *Daniel Scharf*[1,2], Zhenzhen Xiong [1], Nan Zhang [1], Liru Xu[1], Zhiduo Deng [1] 1) School of Basic Medical Sciences, Zhejiang University; 2) Children›s Hospital, Hangzhou.

Urease is an enzyme that catalyzes the hydrolysis of urea into ammonia and carbamic acid. This reaction is of great importance for a wide variety of organisms from bacteria to fungi and plants. The role of urease for pathogenic bacteria like *Helicobacter pylori* or pathogenic yeasts has been reported previously. However, the potential of urease as a virulence factor in pathogenic filamentous fungi like *Aspergillus fumigatus* remains unknown. We identified the urease gene and accessory maturation genes in *A. fumigatus* based on sequence similarities. RNA-Seq shows that the genes for urease and urease maturation are up-regulated with urea as sole nitrogen source. Using different *in vivo* methods, we could also identify the interactions between the urease and the respective assembly complex subunits. We investigated the role of urease in a systemic murine infection model and could show that the urease deletion strain shows reduced virulence. Investigating the interaction of a urease deletion strain with phagocytotic cells revealed that the enzyme plays a role for the survival of *A. fumigatus* within phagocytotic cells. Screening several known urease inhibitors lead to the identification of compounds which reduce the survival of *A. fumigatus* conidia after phagocytosis. This observation might lead to an interesting new avenue for antifungal therapy.

778V The pathogen-host interaction database in 2022: Providing FAIR data to explore human, animal and plant infecting filamentous pathogens *Kim Hammond-Kosack*[1], Alayne Cuzick[1], James Seager[1], Kim Rutherford[2], Valerie Wood[2], Martin Urban[1] 1) Rothamsted Research; 2) Cambridge Systems Biology and Department of Biochemistry, University of Cambridge, UK .

PHI-base, www.phi-base.org, is a gold-standard manually curated phenotype database storing molecular information on genes implicated in virulence[1]. Our Sep 2021 release (version 4.12) of the database provides information on 278 pathogens tested on 228 hosts, covering 18,190 interactions and 8,411 genes curated from more than 4,300 peer reviewed articles. Information is also given on the target sites of commercial and experimental anti-infective chemistries and the first host targets of pathogen effector genes. PHI-base's mission is to be a primary information source for researchers studying plant, animal, and/or human pathogens. This curated information is made accessible and searchable to provide relevant molecular and biological facts on pathogenicity, wild-type/mutant genes, fungicide target sites and first host targets. Species neutral high-level phenotypes are used to describe the overall pathogen-host interaction outcomes using our newly developed PHI Phenotype Ontology (PHIPO) registered at the OBO Foundry. Together this allows comparative phenotype analysis across a wide spectrum of pathosystems. In addition, we recently developed a web-based community annotation tool, called PHI-Canto, canto.phi-base.org, to permit authors to capture wild-type and mutant phenotype data once their original research articles are published through peer review. This new curation tool will be rolled out for community use in 2022.

PHI-base phenotype data and ontology terms are disseminated to Ensembl Genomes, NCBI, UniProtKB, FungiDB and the KnetMiner knowledge graph tool[2]. This allows linking of phenotypes to genomes and enables enhanced computational analyses which include variant analysis, RNAseq results and mapping to biochemical pathways.

In this poster, the new genic centric version of PHI-base will be described as well as how the early roll out of the new author curation tool for community use will be implemented.

[1] Urban et al., (2021) Nucleic Acids Res, doi 10.1093/nar/gkab1037; [2] Hassani-Pak et al., (2021) Plant Biotech. J. doi.org/10.1111/pbi.13583

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779V The diversity of mold warfare: a taxonomically wide investigation of *Trichoderma* - *Pythium* antagonism *Siqiao Chen*[1,2], Paul Daly[2], Deyu Zhuo[2], Taiqiang Xue[2], Yifan Chen[2], Rong Wang[2], Dongmei Zhou[2], Qirong Shen[1], Lihui Wei[2], Irina Druzhinina[1] 1) Fungal Genomics Laboratory (FungiG), Nanjing Agriculture University, Nanjing, China.; 2) Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, Nanjing, China..

The next-generation bioeffectors for plant protection require understanding of the molecular mechanisms governing microbial dialogues. However, the advances of genomics reveal an outstandingly broad range of potential options that fungi and other microorganisms can harness for combative interactions with each other and for cross-talking with plants. Therefore, we studied the diversity of *Trichoderma* (Hypocreales, Ascomycota) – *Pythium* (Peronosporales, Oomycota) antagonistic interactions on a taxonomically-wide scale. For this purpose, we selected 38 *Trichoderma* species that represent the major infrageneric clades and have their whole-genomes available, and investigated their interactions with five species of *Pythium*, including the causative agents of plant diseases. The direct confrontation assays revealed that the ability to suppress *Pythium* is a generic property of *Trichoderma* as most species showed profound antagonism regardless of the partner identity. However, the soil-dwelling species, such as *T. asperellum*, *T. atroviride* and *T. virens* showed to be the most efficient. In contrast, canopy-associated and putatively strictly mycoparasitic species (*T. minutisporum, T. parepimyces,* and *T. strictipile*) were moderately antagonistic to *Pythium* spp. These results suggest that the interactions with *Pythium* spp. benefit from the shared (core-genome) genetic traits of *Trichoderma*, such as hydrolytic enzymes that can degrade cellulose, one of the main polysaccharides of the water mold cell wall. Therefore, we exploited the mutant of the highly cellulolytic species *T. reesei* lacking XYR1, which is the major transcriptional activator of cellulase gene expression to investigate the contribution of cellulases to the antagonism. The *T. reesei* Δ*xyr1* mutant showed reduced antagonism levels to all of the five *Pythium* plant pathogens species analyzed compared to the wild-type. Still, the ability of *Trichoderma* to inhibit *Pythium* spp. was not abolished, suggesting other factors contributed to the antagonism. At the next stage, we will investigate the synergistic involvement of proteolytic enzymes and secondary metabolites. In this presentation, we will discuss the multi-faceted nature of microbial warfare focusing on the evolutionary history of *Trichoderma* - water mold interactions and highlight the directions for the development of *Trichoderma*-based bioeffectors to control plant diseases caused by *Pythium*.

780V Heterologous expression of *Hanseniaspora sp.* transporters in *Saccharomyces cerevisiae* confirms their activity as pantothenate symporters, used by the yeast to obtain this vitamin from other organisms. *Maria P. Rueda-Mejia*[1,2], Laurin Müller[1], Florian Freimoser[1] 1) Agroscope, Research Division Plant Protection, Wädenswil, Switzerland; 2) ETH Zürich, Institute of Microbiology, Zürich, Switzerland.

The genus *Hanseniaspora* is formed by cosmopolitan yeasts that are found in association with plants in bark, flowers and fruits. These yeasts are also present in fermented fruit products, have importance for aroma, and in flavor development. In a screen of 40 naturally occurring yeasts, one *Hanseniaspora sp.* isolate (APC 12.1) was identified as a strong fungal antagonist against a variety of plant pathogens.

While testing the interactions between plant pathogenic fungi and our *Hanseniaspora* isolate in different minimal media, it was observed that it grew only in proximity of other fungi or plant roots. Meanwhile, the same experiment conducted in yeast nitrogen base and potato dextrose media showed normal growth. Revising and testing the components of the different media, we found that calcium panthotenate is essential for the growth of the yeast, while the absence of biotin or folic acid had negative effects on colony size.

A search in the genome of *Hanseniaspora sp.* (APC 12.1) revealed six predicted pathotenate transporters (named *PANT1-6*) and the lack of critical enzymes in the pantothenate biosynthetic pathway. In *S. cerevisiae*, the panthotenate synthase Pan6 is required to produce this vitamin, while the plasma-membrane symporter Fen2 transports it into the cell. Employing a *pan6* mutant, we constructed a strain with *FEN2* under the inducible GAL.L promoter. This strain obtains panthotenate from the medium when galactose is present, growing comparably to the wild type, but shows a strong growth defect in media with glucose as carbon source. With the goal of confirming and describing their function, we used this strain for heterologous expression of the six putative pantothenate transporters. Of the six genes, *PANT2* and *PANT4* expression rescued normal growth in media without galactose, supporting their function as pantothenate transporters. These results show that *Hanseniaspora* (APC 12.1), though metabolically limited, has mechanisms to obtain essential nutrients from neighboring organisms, which likely supports its fast growth and successful antagonism of other fungi.

781V Secreted proteins during interaction of mushroom-forming fungi against their competitors *Marieke H. van Maanen*[1], Erik P.W. Beijen[1], Robin A. Ohm[1] 1) Utrecht University, Utrecht, The Netherlands.

Mushroom-forming fungi are prone to pests and diseases from a range of fungal and bacterial pathogens, which can lead to devastating crop losses. To counteract predation, fungi have evolved several methods to defend themselves, including the secretion of effector proteins capable of suppressing the competitor's growth. However, few secreted proteins involved in defence have been identified in mushroom-forming fungi. To elucidate the secreted arsenal of mushroom-forming fungi against their competitors, we determined gene expression during interactions between vegetative mycelium of the mushroom-forming fungus *Schizophyllum commune* and four fungal or bacterial competitors. Additionally, RNA-seq was performed on the mushroom-forming fungus *Pleurotus ostreatus* against a fungal competitor to study conserved defence responses. The upregulation of 161 transcripts encoding putative secreted proteins was observed in *S. commune* during interaction with at least one of the competitors, of which 16 were upregulated during all interactions. For *P. ostreatus*, 89 genes were differentially expressed during interaction with a pathogenic fungus compared to mono-cultivation. Upregulation of transcripts encoding thaumatins and glycosyl hydrolases were observed as the main annotated groups in *S. commune*, whereas redox-related proteins were predominantly present during interaction in *P. ostreatus*. Furthermore, more drastic changes in expression were observed in unannotated genes, which lack known domains. Homology was found between 31 and 23 of the secreted proteins of *S. commune* and *P. ostreatus*, respectively, which showed similar expression regulation during interaction. These orthologues are all widely conserved among fungi. A reporter strain was designed to study the induction of secreted proteins suspected of being involved during defence. The red fluorescent dTomato gene was placed under the control of the promotor of one of the most upregulated genes of *S. commune* during interaction with all competitors. No fluorescence was observed during mono-cultivation, while strong fluorescence was found during interaction. This method can be used to quickly screen activation of putative effector proteins during interaction with a wide range of fungal and bacterial antagonists.

782V Ensembl Fungi: Melding data sets to explore species interactions *Manuel Carbajo Martinez*[1], Nishadi H. De Silva[1], Andrew D. Yates[1] 1) EMBL - EBI.

Biological communities, in both healthy and diseased states, are a myriad of complex and dynamic interactions between species. Diseases, for instance, are a consequence of interactions between pathogen virulence factors and host cell molecules. Understanding these interactions is fertile ground for uncovering crucial biological mechanisms that can lead to better management of disease and agricultural practises, and a clearer understanding of many ecosystems from soil to the human gut.
In Ensembl Fungi, we have developed a new data model to capture any pair of interacting entities (for example, a protein in a pathogen and a protein in a host) along with meta information about them using terms from controlled vocabularies such as experimental details of how the interaction was uncovered. We have integrated inter-species protein-protein interactions from PHI-base and have infrastructure in place to capture similar, manually curated data. This new data is combined with the 1500+ genomes in Ensembl Fungi, the Ensembl Variant Effect predictor, transcriptomic data in track hubs and the homologous relationships across fungi. Together, they provide a powerful toolkit to explore host-pathogen, and other, relationships between species. Here we present our data capture pipelines, underlying storage and search strategies.
Furthermore, we are formulating methods to make conservative predictions of other potential participants in these interactions from related species. These methods will use a combination of metadata about species (for instance, pathogens infecting similar hosts), orthology and sequence similarity, and will be available to view/download with clear labelling to indicate prediction methods. We believe that these will provide an exciting opportunity for plant, medical, animal and environmental researchers to explore scientific hypotheses before committing to experimentation.

783V Deciphering the mycovirome of *Botrytis cinerea* *Ana Ruiz-Padilla*[1], Julio Rodríguez-Romero[1, 2], Irene Gómez-Cid[1], Davide Pacífico [3], Maria A Ayllón[4, 2] 1) Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid/Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Pozuelo de Alarcón, Madrid, Spain.; 2) Departamento de Biotecnología-Biología Vegetal, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid, Spain.; 3) Institute of Bioscience and Bioresources, National Research Council of Italy, Palermo, Italy.; 4) Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid/Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Pozuelo de Alarcón, Madrid, Spain mariaangeles.ayllon@upm.es..

The ascomycete necrotroph *Botrytis cinerea* Pers.: Fr. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel) produces gray mold or gray rot in more than 200 crops spread throughout the world and it is considered the second most significant fungal plant pathogen. It causes economically significant losses in winter crops of tomato, cucumber, bean, pepper, etc.; in grapevine it develops mainly in mature grapes in autumn; and it significantly affects both strawberry plants in field and fruits in post-harvest.
Fungal control strategies include cultural practices, biological control agents, host resistance, and fungicides. Nevertheless, traditional chemical fungicides are not a sustainable solution as a treatment anymore, especially due to the gene plasticity of the fungal genomes, which makes them easily resistant. To date, several botrycide products, based on microorganisms as active ingredients, have been developed for *B. cinerea* biocontrol. The discovery of mycoviruses that decrease the virulence of their fungal hosts could provide another

alternative as biological control agents. Several mycoviruses have been already associated to hypovirulence in *B. cinerea* indicating that it is feasible to use mycoviruses in biocontrol strategies of this fungus.
In this line, we have explored the mycovirome of 248 *B. cinerea* field isolates from grapevine of Italy and Spain to increase the knowledge about mycoviral diversity and evolution, and to search for new widely distributed mycoviruses that could be active ingredients in biological products to control this hazardous fungus. A total of 92 viruses were identified, 62 of them constituting putative novel viral genera and families. Of these mycoviruses, 57 had a positive-sense single-stranded RNA (ssRNA) genome, 19 contained a double-stranded RNA (dsRNA) genome, 15 had a negative-sense ssRNA genome, and 1 contained a single-stranded DNA (ssDNA) genome. Some of the identified mycoviruses belong to genera that have previously been associated with hypovirulence, as for instance, *Mitovirus*, *Hypovirus*, *Partitivirus*, etc. Moreover, some of them, as the ssDNA mycovirus, has been already proved to decrease the virulence of *B. cinerea*. This study not only have expanded our knowledge of mycoviral diversity, horizontal transfers, and putative cross-kingdom events; but also, it has generated a collection of mycoviruses, some of which could be potential candidates of biological control agents of *B. cinerea*.
Ruiz-Padilla et al.. Novel Mycoviruses Discovered in the Mycovirome of a Necrotrophic Fungus. mBio. 2021 May 11;12(3):e03705-20. doi: 10.1128/mBio.03705-20
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784V Exploring the Divergence of Interactions between Fungi and Bacteria *Gayan Abeysinghe*[1], Meng Wu[1], Shunsuke Masuo[1], Naoki Takaya[1], Norio Takeshita[1] 1) Graduate School of Science and Technology, University of Tsukuba, Japan.

Fungi and bacteria comprise a large fraction of biomass in the soil and since they interact with each other, bacterial-fungal interactions are crucial for understanding the microbial ecosystem which is closely related to agriculture, medicine and the environment. It is apparent that microbial interactions promote the activation of cryptic biosynthetic pathways leading to the production of various secondary metabolites and other bioactive compounds that confer defense functions, cell to cell communication and other interactive dynamics. Now the majority of the studies based on the dynamics of microbiota employ coculturing which is proven to be an effective method to mimic the conditions existing among the microbial interactions in the natural environment, which in turn could potentially facilitate the production of novel bioactive compounds like antimicrobials.

Our recent study characterized the mutualistic relationship between the filamentous fungus *Aspergillus nidulans* and gram-positive bacterium *Bacillus subtilis* providing evidence to show their spatial and metabolic interaction that facilitates the communication in between species to explore untraveled environmental niches and obtain nutrients. Addressing this interactive nature, the current study comprised of coculturing of 35 environmental fungal species and 20 bacterial species to investigate their interaction dynamics in the cocultures. Parameters such as the effect on the fungal growth, the affinity of the bacterial cells to the fungal hyphae, bacterial cell dispersal distance and the velocity of movement of bacteria were analyzed to define the interaction specificity. Depending on the nature of interactions, the combinations were then classified as positive, negative, and neutral. Selected combinations were then subjected to LCMS analysis and subsequent transcriptomic analysis to visualize their genomic potential and expression in coexistence compared to their monoculture state.
This study impart insights to the ecological context of interactions of the environmental microbiota and utilization of the metabolic capacity of the chemically prolific microorganisms.

785V Mycovirus influences secondary metabolite production in *Aspergillus flavus* *Misa Kuroki*[1], Syun-ichi Urayama[1,2], Takashi Yaguchi[3], Daisuke Hagiwara[1,2] 1) University of Tsukuba, Ibaraki, Japan; 2) Microbiology Research Center for Sustainability, University of Tsukuba, Ibaraki, Japan; 3) Medical Mycology Research Center, Chiba University, Chiba, Japan.

In nature, almost all kind of fungi have mycovirus with some frequency. They are considered to be hypovirulence because fungus harboring mycovirus shows the same phenotype with ones not harboring mycovirus in many cases. In our previous study, however, tenuazonic acid production is drastically increased by mycovirus in *Magnaporthe oryzae* (Ninomiya *et al.*, 2020), which suggests a possibility that mycoviruses can affect host fungal physiology in a wider way.
In this study, we explored the RNA virus from environmental and clinical strains of *Aspergillus flavus*. *A. flavus* generally produce some mycotoxin. Especially, aflatoxin is serious carcinogen and result in threat of food supply. Out of 72 *A. flavus* strains, nine strains had mycovirus(es) : four had partitivirus; two had narnavirus; one had deltaflexvirus; one had polymycovirus; one had vivivirus; one had novel RNA virus. We cured the viruses to obtain virus-free strains, and compared the phenotype between a set of virus-infected and free strains. The virus effects on colony growth and the morphology on PDA plates and secondary metabolites production on YES plates were examined. In strain IFM65242, harboring partitivirus, the virus-infected strain was unable to form sclerotia and produced significantly more aflatoxin and cyclopiazonic acid compared with the virus-free strain. Production of other secondary metabolites were increased or decreased in virus-strain. On the other hand, the strain IFM63847 that also harbors partitivirus was not affected by virus infection regarding sclerotia formation and aflatoxin production. In strain IFM49866 harboring novel RNA virus, there was no difference in colony growth and morphology between virus-infected strain and virus-free strain, but some secondary metabolites were drastically decreased in virus-infected strain. In summary, we identified some kinds of virus in *A. flavus* and revealed these various effects on host fungi.
In the future study, we try to transmit the virus into other *A. flavus* strains to examine if the virus can confer the phenotypic changes in a similar manner such as aflatoxin production. In parallel, we will perform transcriptome analysis in virus-infected and virus-free strains to reveal genes whose expression is upregulated or downregulated in virus-strain. Our study will provide new insights into fungal-viral interaction and lead to improvement of fungal properties with mycovirus.

786V *Lactobacillus*-secreted Yak1 inhibitor, 1-acetyl-beta-carboline, blocks *Candida albicans* morphogenesis and biofilm formation *Jessie MacAlpine*[1], Martin Daniel-Ivad[2], Zhongle Liu[1], Junko Yano[3], Nicole Revie[1], Robert Todd[4], Peter Stogios[5], Hiram Sanchez[6], Teresa O'Meara[7], Thomas Tompkins[8], Alexei Savchenko[5], Anna Selmecki[4], Amanda Veri[1], David Andes[6], Paul Fidel Jr.[3], Ni-

cole Robbins[1], Justin Nodwell[2], Luke Whitesell[1], Leah Cowen[1] 1) Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada; 2) Department of Biochemistry, University of Toronto, Toronto, ON, Canada; 3) Center of Excellence in Oral and Craniofacial Biology, Louisiana State University Health Sciences Center School of Dentistry, New Orleans, LA, USA; 4) Department of Microbiology and Immunology, University of Minnesota Medical School, Minneapolis, MN, USA; 5) BioZone, Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, ON, Canada; 6) Department of Medical Microbiology and Immunology, University of Wisconsin, Madison, WI, USA; 7) Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI, 48109, USA; 8) Rosell Institute for Microbiome and Probiotics, 6100 Avenue Royalmount, Montreal, QC, Canada.

Interactions between bacteria and fungi are ubiquitous in nature, yet little is known about the phenotypic consequences of these interactions. In humans, the opportunistic fungal pathogen *Candida albicans* is a common member of the mucosal microbiota that can cause both superficial infections and life-threatening systemic disease. Vaginal candidiasis occurs in approximately 75% of healthy people with a vagina at least once in their lifetime, with fungal overgrowth often developing after a decline in bacterial abundance due to antibiotic use. *Lactobacillus* species are prominent constituents of the vaginal microbial community and the most common industrial probiotic. With the goal of identifying the mechanism(s) by which *Lactobacillus* affects *C. albicans* virulence, we observed that several species of *Lactobacillus* secrete a factor that can repress *C. albicans* hyphal morphogenesis, a cellular transition important for pathogenicity. Bioassay-guided fractionation linked this activity to 1-acetyl-beta-carboline (1-ABC), and genetic approaches identified the target of 1-ABC as the kinase Yak1. Additionally, we found beta-carbolines inhibited *C. albicans* biofilm formation both *in vitro* and *in vivo*. To further explore the role of Yak1 in regulating filamentation, epistatic analysis was employed to assess whether Yak1 governs this developmental transition through the Ras1/Protein Kinase A (PKA) pathway. While overexpression of *TPK2,* a catalytic subunit of PKA, resulted in filamentous growth in the absence of an inducing cue, homozygous deletion of *YAK1* in this background blocked filamentation, suggesting Yak1 signals downstream of PKA. In follow up, we also selected mutants with a restored capacity to filament in the presence of 1-ABC, identifying amino acid substitutions in the putative phosphatase Oca6 and the transcription factor, Rob1. Additional genetic analyses suggested Oca6 functions upstream of Yak1, whereas Rob1 acts downstream of Yak1 in regard to regulation of morphogenesis. Interestingly, the importance of Yak1 in mediating filamentation appeared to be environmentally contingent as the kinase was dispensable for filamentation upon exposure to physiological concentrations of CO_2. Ongoing work is continuing to probe the role of Yak1 in regulating *C. albicans* morphogenesis in response to diverse environmental cues. Overall, these insights reveal *Lactobacillus*-secreted 1-ABC as a Yak1 inhibitor capable of blocking the yeast-to-filament transition in *C. albicans* and illuminate the complex circuitry by which Yak1 regulates a key virulence trait in this major human fungal pathogen.

787V The phospholipase VIPLA$_2$ from the plant pathogen *Verticillium longisporum* is a virulence factor targeting host nuclei and suppressing PTI-related hypersensitive response Vahideh Rafiei[1], Heriberto Vélëz[1], Anna Törnkvist[2], *Georgios Tzelepis*[1] 1) Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden; 2) Department of Plant Biology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Verticillium longisporum is an amphidiploid species infecting plants mainly in Brassicaceae family. It is one of the most important pathogens in rapeseed (*Brassica napus*) cultivation, causing severe annual yield losses worldwide. In general, pathogens secrete a plethora of small proteins, termed effectors, to evade plant immunity and to establish a successful infection. Genome analysis of the *V. longisporum* stain VL1, isolated from Swedish infested soils, where rapeseed has intensively been cultivated, showed that it contains more than 80 candidate effector genes. Among them, the *VlPLA2* gene, putatively encodes an A2 phospholipase, was highly induced upon early infection of *B. napus* and previous data showed that this gene was acquired through horizontal gene transfer from a prokaryotic ancestor. Phospholipases are the enzymes responsible to hydrolyse phospholipids, which are essential structural components of plasma membranes. They produce phosphatidic acid (PA), diacylglycerol (DAG), free fatty acids (FFAs), and lysophospholipids (LPLs). These products play important roles in different aspects of cell physiology, being involved in signal transduction, cytoskeletal dynamics and protein secretion. However, their precise role in fungal virulence remains to be elucidated.
In the currect study we investigated the role of the VlPLA2 candidate effectort in *V. longisporum* infection biology. Protein expression in *E. coli* cells, showed that it is an active A2 phospholipase, while its catalytic dyad HD is crucial for its enzymatic function. Transient expression in *Nicotiana benthamiana* plants showed it to be localized to host nuclei 48hpi, while it is translocated to chloroplasts 72hpi. Except for the catalytic domain, VlPLA$_2$ contains a signal peptide at the N-terminus and two active NLS; one monopartite and one bipartite, with the latter one to be more crucial for its localization. However, the inactive version of this protein failed to enter the nucleus and localized to chloroplasts even 48hpi. Since any attempt to delete this gene in *V. longisporum* and in parent species *V. dahliae* continuously failed, overexpression strains were constructed instead. Our preliminary data from *Arabidopsis thaliana* showed that the overexpression strain is more virulent as compared to strain that the inactive version of *VlPLA$_2$* was overexpressed. Finally, this effector was able to suppress hypersensitive response (HR) triggered by the Cf4/Avr4 complex, but not by *Pseudomonas syringae* pv. *tomato* DC3000 (PsPto). It was also able to induce genes that encode pathogen related (PR) proteins. In conclusion, these data show that VlPLA$_2$ is a virulence factor targeting host nuclei and suppresses Pattern Triggered immunity (PTI)-related HR, possibly through interference in phospholipid signal transduction. More analyses are in progress to identify the precise role of this protein in manipulation of plant defense.

788V Dog9, a fungal protein involved in effectors secretion during plant infection *María Dolores Pejenaute Ochoa*[1], Ramón Ramos Barrales[1], José Ignacio Ibeas Corcelles[1] 1) Andalusian Centre for Developmental Biology, Pablo Olavide University, Seville, Spain..

Plant-fungus pathogenic interaction involves numerous elements from both organisms. The plant prevents fungal infection by inducing defence response mechanisms. Meanwhile, fungi secrete effectors which modify host cell structure and function to promote plant colonization. Although the role of these effectors for the successful plant infection has been demonstrated *(Daniel Lanver et al. 2017)*, the mechanism that underlies effectors delivery into plant cells is poorly understood. Here we identify a novel protein in the maize pathogen fungus *Ustilago maydis* which could help decipher these mechanisms.

U. maydis is a biotrophic fungus, which causes smut disease in maize and has been considered an excellent model to study

plant-pathogen interaction. To better understand these interactions, we carried out a screening to identify proteins involved in virulence *(Marin-Menguiano et al. 2019)*, highlighting the uncharacterized protein Dog9, also conserved in other phytopathogenic fungi.

Dog9 is required for full virulence in maize plant. Specifically, Δ*dog9* mutant compromises efficient appressorium progression, which allows the fungus to colonize the plant, leading to defective tissues invasion. Consistent with its potential role during the early stages of the virulence process, *dog9* has an early expression pattern during the pathogenic development and localizes in small vesicles which move towards the tip of the filament and accumulate in the appressorium. Remarkably, Dog9 is involved in effectors secretion, which in *U. maydis* facilitates plant penetration and colonization.

Furthermore, we have also found five other putative Dog9 homologs also conserved in phytopathogenic fungi. Phylogenetic analysis showed close distances between the proteins, and the single deletion of four of the five genes reduced fungal virulence. These data suggest that all members could be conforming a family of proteins involved in virulence with similar functions to Dog9.

789V Phosphoproteomic analysis of the Pmk1 MAP kinase pathway reveals novel phosphorylated virulence determinants in *Magnaporthe oryzae* *Neftaly Cruz Mireles*[1], Miriam Osés-Ruiz[1], Paul Derbyshire[1], Lauren Ryder[1], Alice Eseola[1], Xia Yan[1], Weibin Ma[1], Frank L.H. Menke[1], Nicholas J. Talbot[1] 1) The Sainsbury Laboratory, University of East Anglia, Norwich Research Park.

Rice blast is among the most devastating diseases affecting global agriculture. It is caused by the ascomycete fungus *Magnaporthe oryzae*. The blast fungus enters the plant using a specialised dome-shaped infection structure called an appressorium. The Pmk1 MAP kinase (MAPK) signalling pathway is necessary for appressorium development, plant penetration and host colonisation. However, the mechanisms by which Pmk1 regulates these complex morphogenetic changes is poorly understood. Here, we report a quantitative phosphoproteomic approach to identify direct downstream targets of the Pmk1 MAPK during plant infection. Using discovery phosphoproteomics followed by Parallel Reaction Monitoring (PRM) from a time series study of appressorium samples, we identified 30 putative direct downstream targets of Pmk1. These putative targets include proteins related to cytoskeleton remodelling, vesicle trafficking, and cell cycle control. One of the targets, named Vts1, is a SAM domain-containing protein of unknown function. Using *in vitro* and *in vivo* assays, we have demonstrated that Vts1 phosphorylation is Pmk1-dependent and occurs at two proline-directed sites. Vts1 associates with Pmk1 in both yeast-two-hybrid assays and by co-immunoprecipitation in early-stage appressoria. Targeted mutation showed that Vts1 is necessary for mycelium growth, sporulation, appressorium development and pathogenicity. Additionally, Vts1 phosphomimetic and phosphodead mutants demonstrated the importance of its phosphorylation. Taken together, our results show how quantitative phosphoproteomics can identify novel regulators, such as Vts1 which are essential for rice blast disease.

790V Interactions Between the Diet and Mycobiome of Long-tailed Macaques (*Macaca fascicularis*) Vary Across Islands with Evidence of a Role for Antifungal Plants *Benjamin Gombash*[1], Amanda Acevedo[1], Carson Smith[1], Chissa Rivaldi[1], Hope Hollocher[1] 1) University of Notre Dame, Notre Dame, IN.

The mycobiome is the group of fungal biota that are detected in samples from other organisms. Research focused on variation in the mycobiome has expanded greatly as we try to understand its role in the microbiotic community, with wildlife mycobiomes serving as natural systems to investigate. When variation is detected in the mycobiome of wildlife, it is frequently ascribed to variation in the host's diet. While the host's diet is often implicated, it is rarely assessed alongside the mycobiome. To assess the diet and mycobiome of 127 fecal samples from long-tailed macaques (*Macaca fascicularis*) on the islands of Singapore and Bali, Indonesia we amplified the V9 hypervariable region of the 18S ssu rRNA. Linear regressions and partial Mantel tests confirm that there are general associations between the diet and mycobiome occurring on both islands. These interactions appear to be stronger in Singapore than Bali. To run Multiple Factor Analyses to search for more specific interactions, we first categorized dietary and fungal taxa into several relevant groups (e.g., for diet items: crop plants and anti-fungal plants; for fungi: animal pathogens and saprotrophs). Although dietary taxa are more numerous on both islands, Multiple Factor Analyses show taxa in fungal groups are the most important for explaining variation. Despite sharing ~70% of their detected taxa, the interactions between dietary and fungal groups on the two islands are distinct. When the most important taxa were identified, only 31 to 35% of the taxa were shared between the two islands. Linear regressions comparing richness values of specific groups of taxa suggest that, in Bali, plants and plant pathogens are significantly related and that plants with anti-fungal properties are significantly related to animal pathogens. Both relationships are more significant than the relationship between diet and mycobiome richness. On the other hand, no relationship in Singapore was more significant than the relationship between diet and mycobiome richness. These results confirm that the diet and mycobiome do interact in long-tailed macaques. However, these interactions are not necessarily uniform between different populations of the same host organism, which may be related to specific fungal taxa. Our future work will incorporate other factors that are important for host organisms, such as the environment, which may interact with the diet, mycobiome, or both groups of taxa, in interesting and synergistic ways.

791V A major effect gene, *Bcin04g03490*, controls development and pathogenicity in *Botrytis cinerea* *Ernesto Pérez Benito*[1], Wilson Acosta Morel[1], Francisco Anta Fernández[1], Riccardo Baroncelli[1], Michael R. Thon[1], Jan A.L. van Kan[2], José María Díaz Mínguez[1] 1) University or Salamanca. Salamanca. Spain; 2) Wageningen University, Wageningen, The Netherlands.

A survey of *Botrytis cinerea* isolates in the vineyards of Castilla y León (Spain) allowed for the identification of several non-pathogenic isolates showing a characteristic mycelial morphotype. These isolates do not sporulate and are unable to produce sclerotia. Crosses between a representative mycelial non-pathogenic isolate and a highly aggressive field isolate able to sporulate profusely revealed that differences in pathogenicity, sporulation and production of sclerotia cosegregated in the progeny and are determined by a single genetic locus.
A bulked segregant analysis of progeny, based on the comparison of the two parental genomes, allowed to map the locus to a 110 Kb region in chromosome 4. Subcloning and transformation experiments revealed that the polymorphism explaining the phenotypic differences is a SNP affecting gene *Bcin04g03490*. Genetic complementation analysis and sequencing of the *Bcin04g03490* alleles demonstrated that the mutations harbored by the mycelial non-pathogenic isolates are allelic and responsible for the phenotypes observed. The integration of the wild type *Bcin04g03490* allele into the non-pathogenic isolate fully restored the ability to cause disease,

to sporulate and to produce sclerotia, supporting the central role of *Bcin04g03490* in development, pathogenicity and sporulation. *Bcin04g03490* encodes a protein with two functional domains: a GAL4-like Zn(II)Cys6 binuclear cluster DNA binding domain and an acetyltransferase domain. The presence of these two domains suggests that this DNA binding protein might regulate gene expression by means of modifications in the chromatin architecture. Further research is underway to validate this hypothesis.
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792V Impairment of the cellulose degradation machinery enhances *Fusarium oxysporum* virulence but limits its reproductive fitness Francisco M Gamez-Arjona[1], Stefania Vitale[2,3], Antonio Di Pietro[2], *Clara Sanchez-Rodriguez*[1] 1) Department of Biology, ETH Zurich; 2) Departamento de Genética, Universidad de Córdoba; 3) IPSP-Instituto per la Protezione Sostenibile delle Piante, CNR.

Fungal pathogens grow in the apoplastic space, in constant contact with the plant cell wall (CW) that hinders microbe progression, while representing a source of nutrients. Although numerous fungal CW modifying proteins have been identified, their role during host colonization remains underexplored. Here we show that the root-infecting plant pathogen *Fusarium oxysporum* (Fo) does not require its complete arsenal of cellulases to infect the host plant. Quite the opposite, Fo mutants impaired in cellulose degradation become hypervirulent by enhancing the secretion of virulence factors. On the other hand, the reduction on cellulase activity had a severe negative effect on saprophytic growth and microconidia production during the final stages of the Fo infection cycle. These findings enhance our understanding on the function of plant CW degradation on the outcome of host-microbe interactions and reveal an unexpected role of cellulose degradation in a pathogen›s reproductive success.

793V Effector proteins of *Pseudocercospora fijiensis* as tools in resistance breeding of banana *Maikel Steentjes*[1], Rahim Mehrabi[1], Gert Kema[1] 1) Wageningen University and Research, Laboratory of Phytopathology, Wageningen, the Netherlands.

Pseudocercospora fijiensis is the causal agent of Black Leaf Streak Disease or Sigatoka Disease, the most destructive and costliest foliar disease of banana worldwide. The disease affects the vast majority of banana varieties including the popular Cavendish banana and several types of cooking banana that serve as a staple food for hundreds of millions of people worldwide. The fungal infection process has a long biotrophic phase, similar to other Dothideomycete pathogens such as *Zymoseptoria tritici*, and after three to four weeks the fungus switches to a necrotrophic phase. In this stage of the disease characteristic necrotic streaks appear on the leaves from which airborne ascospores are released. Currently the disease is controlled by preventative fungicide treatments with up to 70 sprays a year. This is not only an environmental burden, but also an enormous selection pressure for reduced fungicide sensitivity in field populations of *P. fijiensis*. This project aims to identify and characterize effector proteins of *P. fijiensis* that can be used as a tool to screen banana germplasm for resistance. Sensitivity of banana genotypes to the effectors is hypothesized to confer resistance to the fungus that is producing the effector and therefore such effector proteins can be used as a direct tool to screen banana germplasm for resistance in breeding programmes. We will use a combination of three approaches to identify effector proteins. We start with sequencing a large global panel of *P. fijiensis* isolates that enables analyses of presence/absence polymorphisms and genetic diversity of candidate effector genes. Secondly, we will perform a transcriptome analysis during various phases of infection to determine which candidate effectors are highly expressed during colonization. For the third approach, we will produce culture filtrates and purify them based on their cell death-inducing activity in a range of banana varieties including resistant accessions. Bioactive fractions will be analysed using mass spectrometry, allowing the identification of effector proteins. Once effectors of *P. fijiensis* are identified they will be heterologously expressed enabling the screening for resistance of banana germplasm.

794V Identification of the *Avr9B* avirulence effector gene from the tomato leaf mould pathogen *Cladosporium fulvum* *Silvia de la Rosa*[1], Christiaan Schol[2], David Winter[3], Joanna Bowen[4], Rosie Bradshaw[3], Matthieu Joosten[2], Carl Mesarich[1] 1) School of Agriculture and Environment, Massey University, Palmerston North, New Zealand; 2) Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands; 3) School of Natural Sciences, Massey University, Palmerston North, New Zealand; 4) The New Zealand Institute for Plant and Food Research, Auckland, New Zealand.

Tomato leaf mould disease is caused by the biotrophic fungus, *Cladosporium fulvum*. During infection, the pathogen resides in the apoplast where it secretes an arsenal of effector proteins to promote host colonization. In disease-resistant tomato cultivars, however, one or more of these effectors is recognized by corresponding Cf immune receptor proteins, leading to a localized cell death response that renders the plant resistant. As in this case the pathogen is unable to cause disease, these recognized effectors are termed avirulence (Avr) proteins. Many of the commercial tomato cultivars deployed worldwide carry at least two *Cf* immune receptor genes, *Cf-9* and *Cf-9B*, which form part of the *Cf-9* locus. Unfortunately, due to the intensive use of these cultivars, resistance mediated by *Cf-9* has been rapidly overcome by *C. fulvum* strains through deletion of the corresponding *Avr9* gene. Furthermore, a strain of *C. fulvum* has been identified from New Zealand (NZ) that overcomes resistance provided by both *Cf-9* and *Cf-9B*. To determine the molecular mechanism responsible for the circumvention of *Cf-9B*-mediated resistance, we set out to identify the corresponding *Avr9B* gene. Genome comparisons between a reference strain (carrying a functional *Avr9B* gene) and the NZ strain (lacking a functional *Avr9B* gene) led to the identification of an *Avr9B* candidate with *in planta*-induced expression that was deleted in the NZ strain. This candidate encodes a small secreted protein with an intrinsically disordered repeat-rich N-terminus, followed by a structured C-terminal cysteine-rich region. Using *Agrobacterium*-mediated transient transformation assays in *Nicotiana tabacum*, the Avr9B candidate specifically triggered cell death, with or without its intrinsically disordered region, when co-expressed with Cf-9B, suggesting that this protein is indeed Avr9B. Structure prediction of the cysteine-rich region from Avr9B using AlphaFold2, revealed a novel fold consisting of three alpha helices and two β-strands stabilized by four disulfide bonds. Homologs of Avr9B were found only in Dothideomycete plant-pathogens, including *Pseudocercospora fuligena*, a closely related black leaf mould pathogen of tomato. Unlike Avr9B, however, the *P. fuligena* homolog does not trigger a Cf-9B-dependent cell death response. Upon identifying *Avr9B*, resistance-breaking strains can now be rapidly identified, which will in turn direct tomato cultivar selection and deployment for protection against *C. fulvum*.

795V Understanding the virulence molecular mechanisms of *Neonectria ditissima*, a necrotrophic fungal pathogen of ap-

ple. *Liz Florez*[1,2], Brogan McGreal[1,2], Saadiah Arshed[1], Reiny Scheper[3], Brent Fisher[3], Paul Sutherland[1], Matt Templeton[1], Joanna Bowen[1] 1) Bioprotection, The New Zealand Institute for Plant & Food Research Limited, Auckland, New Zealand ; 2) School of Biological Sciences, University of Auckland, Auckland, New Zealand; 3) Bioprotection, The New Zealand Institute for Plant & Food Research Limited, Havelock North, New Zealand.

European canker, caused by the necrotrophic fungal phytopathogen *Neonectria ditissima,* is one of the most damaging apple diseases in New Zealand and north-western European countries. Understanding the molecular basis of *N. ditissima* virulence may ultimately lead to the formulation of novel control strategies. Therefore, expression profiles of four candidate virulence genes (*g8150*, predicted to encode a protein kinase, and *g4542*, *g5809* and *g7123*, predicted to encode effectors [proteins that enhance virulence often by the suppression of host defence]) were measured during a comprehensive infection time course using reverse transcriptase real-time PCR. *g8150* transcription was the most highly upregulated *in planta,* however the expression of the remaining genes was also upregulated, peaking 5 to 6 wpi, suggesting a role for effectors in the *N. ditissima*-apple interaction. To gain insight into the molecular basis of *N. ditssima* during disease progression, RNA-based transcriptional profiling (RNA-seq) is currently undergoing to identify differentially expressed genes (DEGs) in *N. ditssima* during apple infection. To enable functional characterisation of candidate virulence genes in *N. ditissima*, an optimised protoplast-mediated transformation protocol was developed and used to trial gene knockout mutation in *N. ditissima* for the first time, targeting *g8150.* Use of a homologous recombination knockout vector with ~600-800bp flanks resulted in the generation of a single *g8150* knockout isolate (*Δg8150*). *Δg8150* produced smaller lesions in apple fruit and less severe symptoms on apple twigs compared to wild type (WT) and ectopic transformants (ET), whereas *in vitro* growth was unaffected, suggesting a role in virulence. Crispr-Cas9 gene editing is currently being trialled to accelerate targeted mutation of the candidate genes to facilitate functional characterisation in *N. ditissima*.

796V Functional characterisation of candidate *Fusarium graminearu*m effectors *Claire Kanja*[1, 2], Matt Dickinson[2], Kim Hammod-Kosack[1] 1) Rothamsted Research, West Common, Harpenden, AL5 2JQ; 2) Nottingham University School of Biosciences, Bonington Hall, Sutton Bonington, Loughborough LE12 5RD.

The effector repertoire of plant pathogens is a key determinant of the success of pathogen-host interactions and could mean the difference between a compromised or a successful crop harvest. One notorious pathogen, the fungus *Fusarium graminearum* is the causal agent of fusarium head blight (FHB), one of the most destructive diseases threatening wheat production worldwide.
Effectors are small secreted proteins produced by a pathogen that manipulate the host to aid colonisation. A main challenge facing *F. graminearum* effector characterisation is pinpointing high quality effector candidates from the predicted proteome. Despite the publication of the refined *F. graminearum* secretome in 2012, finding candidates suitable for functional studies from a pool of almost 300 secreted proteins with unknown functions remains problematic.
I have adopted *in silico* bioinformatic pipelines that consider a multifaceted approach to effector discovery such as transcriptional (RNA-seq and microarray), proteomic, taxonomic distribution analysis and the genome location of candidates. This has proven to be successful in finding clusters of candidate effectors in multiple filamentous phytopathogens.
By taking a two-pronged approach for the functional characterisation of candidates, I have identified a paralogous pair of effectors that are expressed during the early symptomless stage of *F. graminearum* infection. These effectors, FgSSP34 and FgSSP53 are adjacent to each other on the *F. graminearum* chromosome , divergently orientated and share 38% protein sequence identity . Both effectors, in this orientation, are highly conserved within the wider *F. graminearum* species complex (FGSC). Of the pair, FgSSP53 induces cell death in the non-host *Nicotiana benthamiana,* however, the second isoform of FgSSP53 found within FGSC does not. Interestingly, FgSSP34 does not induce cell death responses in *N. benthamiana* however a chimera formed by replacing a predicted 9 amino acid loop in FgSSP34 with the equivalent region from FgSSP53 can. Within the natural host system, viral overexpression of FgSSP53 using BSMV-VOX in wheat, reduces FHB disease severity. FgSSP53 deletion mutants have reduced pathogenicity in both wheat coleoptiles and mature wheat spikes.

797V Characterization of the role of the *Parastagonospora nodorum* effector SnTox267 in virulence on wheat using confocal microscopy *Ashley Nelson*[1], Gayan Kariyawasam[1], Pawel Borowicz[2], Justin Faris[3], Zhaohui Liu[1], Timothy Friesen[1,3] 1) Department of Plant Pathology, North Dakota State University, Fargo, North Dakota 58102, USA; 2) Department of Animal Sciences, North Dakota State University, Fargo, North Dakota 58102, USA; 3) Edward T. Schafer Agricultural Research Center, USDA-ARS, Fargo, North Dakota 58102, USA.

Parastagonospora nodorum is an economically important fungal pathogen that causes septoria nodorum blotch (SNB), a foliar disease on wheat. *P. nodorum* is known to secrete necrotrophic effectors that aid in the manipulation of the plant immune system, resulting in necrotrophic effector triggered susceptibility. Necrotrophic effectors *SnToxA*, *SnTox1, SnTox3*, *SnTox5* and *SnTox267* have been validated and functionally characterized, and have been shown to be involved in triggering the oxidative burst and programmed cell death, PR gene upregulation, chitin binding, binding to PR proteins and/or facilitating colonization of the host. SnTox267 was recently shown to target at least two independent pathways involving host genes *Snn2*, *Snn6*, and *Snn7*. However, apart from induction of PCD, the function of SnTox267 is not well characterized. In this study we used confocal microscopy to observe the role that SnTox267 plays in plant colonization. We compared Sn79-1087, an avirulent isolate of *P. nodorum,* and Sn79+*SnTox267*, an Sn79-1087 strain transformed with *SnTox267*, both tagged with red fluorescent protein (RFP) to identify differences in germination, penetration, and colonization of the leaf. Infection was evaluated at 4, 12, 24, 48, 72, 96 and 120 hours post inoculation (hpi) in three different wheat lines including BR34 (*snn2Snn6*; SnTox267-insensitive), BG223 (*Snn2Snn6*; SnTox267-sensitive), and ITMI37 (*Snn2Snn6*; SnTox267-sensitive). In the compatible interaction (Sn79+Tox267 on BG223 or ITMI37), spores germinated by 4 hpi and fungal penetration of the leaf began between 12 hpi and 24 hpi with substantial colonization of the epidermis being complete at 48 hpi. The colonization of the mesophyll began at 48 hpi and continued through 72 hpi without visible cell death. Host cellular disruption began at 96 hpi and increased in severity with complete cell death adjacent to mycelia by 120 hpi. The incompatible interaction (Sn79-1087 on BG223 or ITMI37) by comparison showed similar germination, penetration, and colonization of the epidermis by 48 hpi but did not progress beyond the epidermal layer of the leaf and never induced significant cell death. The incompatible interaction involving BR34 did not show any colonization of the mesophyll by 120 hpi in either the 79-1087 or 79+Tox267 interactions. Collectively, these preliminary experiments suggest that

SnTox267 facilitates colonization of the mesophyll by targeting the Snn2/Snn6 pathway ultimately resulting in cell death.

798V Understanding the Molecular Bases of Adaptation of the Fungal Pathogen *Zymoseptoria* to Specific Host Species Lukas Meile[1], Zoe Bernasconi[3], Jules Peter[2], Alissa Schneller[2], Bruce McDonald[2], Alessio Bernasconi[2], Julien Alassimone[2], María Garrido-Arandia[1], *Andrea Sánchez-Vallet*[1,2] 1) Centro de Biotecnología y Genómica de Plantas (CBGP-UPM/INIA). Universidad Politecnica de Madrid. Spain; 2) Department of Environmental Biology. ETH Zurich. Switzerland; 3) Department of Plant and Microbial Biology. University of Zürich. Switzerland.

Successful host colonization by plant pathogens requires the circumvention of host defence responses, typically through sequence modifications in secreted pathogen proteins known as avirulence factors (Avrs). However, the contribution of Avr sequence diversity to virulence diversity in natural pathogen populations remains largely unexplored. We determined how natural sequence polymorphisms of Avr3D1 in the wheat pathogen *Zymoseptoria tritici* contributed to adaptive changes in virulence and showed that there is a continuous distribution in the magnitude of resistance triggered by different Avr3D1 isoforms. We showed that virulent isoforms emerged independently at least three times. We also found evidence indicating that, following a gene-for-gene interaction, Avr3D1 recognition may contribute to resistance against two non-adapted *Zymoseptoria* species. Our results demonstrate that natural variation in an *Avr* gene can lead to a quantitative gene-for-gene resistance phenotype.Successful host colonization by plant pathogens requires the circumvention of host defence responses, frequently through sequence modifications in secreted pathogen proteins known as avirulence factors (Avrs). However, the contribution of Avr sequence diversity to virulence diversity in natural pathogen populations remains largely unexplored. We determined how natural sequence polymorphisms of the avirulence factor Avr3D1 in the wheat pathogen *Zymoseptoria tritici* contributed to adaptive changes in virulence and showed that there is a continuous distribution in the magnitude of resistance triggered by different Avr3D1 isoforms. We showed that virulent isoforms emerged independently at least three times. We also found evidence indicating that, following a gene-for-gene interaction, Avr3D1 recognition may contribute to resistance against two non-adapted *Zymoseptoria* species. Our results demonstrate that natural variation in an *Avr* gene can lead to a quantitative gene-for-gene resistance phenotype.

799V Disruption of a *Dothistroma septosporum* cell death elicitor through CRISPR-Cas9 *Mariana Tarallo*[1], Carl Mesarich[2], Rebecca McDougal[3], Rosie Bradshaw[1] 1) School of Fundamental Sciences, Massey University, Palmerston North, New Zealand; 2) School of Agriculture and Environment, Massey University, Palmerston North, New Zealand; 3) Scion (New Zealand Forest Research Institute Ltd.), Rotorua, New Zealand.

D. septosporum is a hemibiotrophic Dothideomycete fungus, responsible for the disease Dothistroma needle blight (DNB), one of the most important foliar diseases of pine trees. The fungus secretes multiple proteins, termed effectors, that can be recognized by plant receptors to activate the plant immune system. Amongst those, Ds74283 is a small, secreted protein from *D. septosporum* whose expression is strongly up-regulated at the transcriptional level in the Mid and Late *in planta* stages that mark the start and end of the necrotrophic stage of pine infection, when compared with the Early (biotrophic) stage and *in vitro*. Ds74283 has the ability to trigger plant cell death in the non-host species *Nicotiana benthamiana*. Exploiting host cell death for nutrients is a strategy used by necrotrophic pathogens or hemibiotrophs, during their necrotrophic stage. We investigated if this elicitor in *N. benthamiana* also promoted cell death in the host *Pinus radiata* by directly infiltrating the purified protein, heterologously produced in the yeast *Pichia pastoris*, into pine needles. As a result, the negative control (buffer) had no effect on any of the infiltrated pine shoots, but the Ds74283 protein triggered a cell death response in all pine genotypes used in the experiment. This could mean that the Ds74283 protein is being recognized by a conserved immune receptor or some other plant target in both *N. benthamiana* and *P. radiata*. To determine the biological role of the Ds74283 protein during *P. radiata* infection, *Ds74283* mutants were generated through CRISPR-Cas9-mediated genome editing via protoplast-based transformation. Co-transformation with DNA template, with a Geneticin resistance marker gene, for homologous recombination resulted in 82 transformants, of which 42 were stable and were deemed to be targeted mutants based on PCR analysis. Next, Southern blot analysis confirmed that the targeted locus had been disrupted as expected. Next steps include phenotypic characterization and pathogenicity assays of these *D. septosporum* mutants. This work will provide information that can help develop durable control strategies against DNB.

800V Functional characterization of *Fusarium graminearum* effectors inducing cell death *Martin Darino*[1], Claire Kanja[1], Kim Hammond-Kosack[1] 1) Department of Biointeractions and Crop Protection, Rothamsted Research, Harpenden, AL52JQ, UK.

Fusarium Head Blight (FHB) is a destructive fungal disease of wheat and other cereals. FHB occurs in all major cereal growing areas of the world and has the potential to devastate wheat fields. In the UK, *Fusarium graminearum* (Fg) is the predominant causal agent of FHB. Fg is a haploid organism for which a sequenced and well annotated genome exists. During the infection process, Fg secretes small secreted proteins (SSPs) or effectors that suppress the plant immune response and thus promote infections. The identification of SSPs as well as their functional characterization are essential to establish novel resistance strategies that can control FHB disease. The secretome of Fg was previously established to comprise ~870 predicted proteins. In order to identify SSP candidates, we performed a bioinformatic pipeline that combined two published sources of transcriptomics data to identify proteins highly expressed during *in planta* infection together with EffectorP1.0 for effector prediction. This resulted in the identification of 24 high quality SSP candidates. Next, we removed those SSPs that possess a predicted protein domain using InterPro and/or were already reported in the literature. This resulted in 14 candidates SSPs for functional evaluation. During FHB infection, the fungus induces cell death to obtain access to plant nutrients to sustain the colonization process. To identify potential SSPs able to induce cell death, we cloned the 14 candidates SSPs into an Agrobacterium binary vector and we tested cell death induction in the heterologous system of *Nicotiana benthamiana*. Two SSPs, FgSSP39 and FgSSP46 were able to induce cell death after 5 days post Agrobacterium infiltration compared to a control construct expressing the fluorescence protein mCherry. In addition, both SSPs still induced cell death when fused at the C-terminal with a HA tag. These recombinant proteins will now be used to identify the potential target(s) in the plant by co-immunoprecipitation followed by mass spectrometry.
In summary, the bioformatic pipeline devised resulted in a suitable strategy to identify Fg SSP candidates able to induce cell death in *N. benthamiana*. Further molecular and cell biology characterization will be performed to identify the SSPs targets as well as study their

expression and localization during wheat infection.

801V The conserved trichothecene biosynthetic cluster gene *TRI14* is required for growth of *Fusarium graminearum* in wheat but not for trichothecene production *Guixia Hao*[1], Robert Proctor[1], Susan McCormick[1], Daren Brown Brown[1], Todd Naumann[1] 1) USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Research Unit, Peoria, IL.

Trichothecenes are terpene-derived toxins produced by diverse ascomycetes, including species of *Aspergillus*, *Fusarium* and *Trichoderma*. The trichothecene deoxynivalenol (DON) produced by the Fusarium head blight (FHB) pathogen *Fusarium graminearum* (Fg) is both a virulence factor on wheat and a major food and feed safety concern. The trichothecene biosynthetic gene (*TRI*) cluster consists of 7-14 genes depending on fungal species, but only three genes are common to all cluster homologs. Two of these genes, *TRI3* and *TRI5*, encode enzymes required trichothecene biosynthesis, whereas a third gene, *TRI14*, encodes a protein of unknown function that is not required for DON production by Fg in culture. In the current study, following inoculation of single florets of wheat cultivar Norm, FHB symptoms caused by a *tri14* deletion mutant did not spread beyond the inoculated floret. Also, DON levels and Fg biomass were markedly lower in florets inoculated with the *tri14* mutant compared to wild type. When entire heads were inoculated by dipping in conidial suspensions, disease levels caused by the mutant and wild type were similar. Further, relative expression of other *TRI* genes (*TRI5*, *TRI6* and *TRI12*) did not differ in mutant and wild-type inoculated heads. However, DON levels and Fg biomass were markedly lower in mutant inoculated heads. Our results indicate that *TRI14* is required for wild-type growth in wheat tissue and that low levels of DON in mutant-inoculated heads resulted from reduced growth of Fg rather than a direct role of *TRI14* in trichothecene biosynthesis. We are currently conducting experiments to test the hypothesis that *TRI14* protects Fg from trichothecenes and/or plant metabolites.

802V The *Venturia inaequalis* effectorome is expressed in waves, and is dominated by expanded families with predicted structural similarity to avirulence effector proteins *Mercedes Rocafort*[1], Joanna K. Bowen[2], Berit Hassing[1], Murray P. Cox[3], Silvia de la Rosa[1], Brogan McGreal[2], Kim M. Plummer[4], Rosie E. Bradshaw[3], Carl H. Mesarich[1] 1) Bioprotection Aotearoa, School of Agriculture and Environment, Massey University, Palmerston North, New Zealand; 2) The New Zealand Institute for Plant and Food Research Limited, Mount Albert Research Centre, Auckland, New Zealand; 3) Bioprotection Aotearoa, School of Natural Sciences, Massey University, Palmerston North, New Zealand; 4) Department of Animal, Plant and Soil Sciences, La Trobe University, AgriBio, Centre for AgriBiosciences, La Trobe University, Bundoora, Victoria, Australia.

Venturia inaequalis is a subcuticular pathogen that causes one of the most devasting apple diseases, known as scab or black spot. The pathogen has a large repertoire of secreted proteins, with many of these anticipated to function as virulence factors (effectors) in promoting host infection or avirulence factors (Avr effectors) in triggering host resistance. Strikingly, most effector candidate (EC) proteins from *V. inaequalis* belong to expanded families ranging in size from five to 75 members. We performed the first comprehensive gene expression (RNA-seq) analysis of *V. inaequalis* during biotrophic colonization of apple leaves. Based on this analysis, we determined that genes encoding EC proteins are mostly expressed in waves corresponding to two specific infection stages: early and mid-late infection. The early expression wave is dominated by genes that encode EC families with an annotated functional domain. One such example is a family of 39 proteins with a stress up-regulated Nod19 domain. We hypothesize that this domain may be required for modulating oxidative stress during host-colonization. Contrarily, most genes expressed during the mid-late wave encode expanded EC families with no or little amino acid similarity to other proteins. Therefore, to glean more information about their functions, we used AlphaFold2 to predict the structural fold of the most highly expressed member from each EC family. Strikingly, many EC families were predicted to share structural similarity to Avr effector proteins from other plant-pathogenic fungi. For example, members of the largest expanded EC family were predicted to have a β-sandwich fold with structural similarity to MAX (*Magnaporthe* AVRs and ToxB) effectors. Three further families were predicted to have structural similarity to members of the ToxA effector superfamily, while two other families were predicted to have structural similarity to LARS (*Leptosphaeria* AviRulence-Suppressing) effectors. Additionally, many EC families were predicted to adopt a similar structure to proteins with a killer protein 6 (KP6)-like and knottin-like fold. These results further reinforce the idea that fungal effectors share a limited number of structural folds, and potentially provide an enriched list of ECs from which Avr effectors can be identified. In conclusion, we show how the use of transcriptomics together with structural biology can be a powerful tool to identify and prioritize ECs for further studies.

803V Spore-type specific chemotropic growth to maize roots determines root infection by the hemibiotrophic pathogen Colletotrichum graminicola Anina Rudolph[1], Christoph Sasse[2], Jennifer Gerke[2], Carolin Schunke[1], Gerhard Braus[2], Stefanie Pöggeler[1], *Daniela Nordzieke*[1] 1) Genetics of Eukaryotic Microorganisms, Georg-August University Göttingen, Germany; 2) Molecular Microbiology and Genetics, Georg-August University Göttingen, Germnay.

The hemibiotrophic fungus Colletotrichum graminicola is a maize pathogen infecting several plant tissues like leaves, stems, and roots. In the field, the typical anthracnose symptoms are observed late in the season: brown lesions are formed on leaves and stems which harbor the disease-spreading falcate conidia. In contrast, for young plants severe stunting is observed in the early season. In our recent project, we follow the hypothesis that oval conidia, a second asexual spore type formed by C. graminicola, are causing maize plant stunting by efficient root infection. We have recently shown that oval and falcate conidia exhibit specialized leaf infection strategies determined by spore-type specific secretion patterns. To test whether such specialization exists also for the infection of maize roots, we have analyzed symptom development of both conidia types. When we dipped maize roots in conidia suspensions, both conidia types were able to infect the maize roots and to grow inside the stem of the plant. However, when we mimicked the root infection process in the field and planted maize seeds in spore-enriched soil, only oval conidia provoked severe stunting of the young maize plants. From this, we concluded that a host recognition process has to take place before the infection process is initiated. We therefore tested the ability of maize root exudate (MRE) to provoke directed-growth of both conidia types using a recently developed 3D printed device combined with a fluorescent marker for polar growth. Intriguingly, only oval conidia showed a strong chemotropic growth to MRE after 6 h of incubation. As HPLC/MS analyses of MRE coupled with the analysis of directed growth of oval conidia demonstrates, a so far unknown secreted signaling molecule from MRE is responsible for the attraction of C. graminicola oval conidia. Together, our research reveals

for the first time that two distinct asexual conidia of C. graminicola are adapted for plant organ-specific infections. The identification of disease-relevant differences of oval and falcate conidia is part of our current investigations.

804V Cross-kingdom RNA interference in early phases of the *Botrytis cinerea* - tomato interaction *Si Qin*[1], Javier Veloso[2], Guido Puccetti[1], Tim Bosman[1], Mirna Baak[3], Britt Boogmans[1], Xiaoqian Shi-Kunne[1], Sandra Smit[3], Thomas Leisen[4], Matthias Hahn[4], Jan van Kan[1] 1) Laboratory of Phytopathology, Wageningen University, The Netherlands; 2) FISAPLANT, University of A Coruña, Spain; 3) Bioinformatics Group, Wageningen University, The Netherlands; 4) Department of Biology, University of Kaiserslautern, Germany.

Botrytis cinerea, a broad host range plant pathogen, was previously reported to deliver small RNAs (sRNAs) as effector molecules capable of interfering with the host immune response. Conversely, a host plant produces sRNAs that may interfere with the infection mechanism of the intruder. We performed high-throughput sequencing to identify the sRNAs produced by *B. cinerea* and tomato during their interaction and to examine the expression profile of their (predicted) mRNA targets in the other organism. Most of the ~28,000 sRNAs (with the length of 20-24 nt) derived from *B. cinerea* originated from ribosomal RNA (47% of the total) and transposable element regions (33% of the total). About a quarter of the sRNAs produced by *B. cinerea* were predicted to target tomato transcripts. Of the predicted tomato target genes, 56 were indeed transcriptionally down-regulated during the early phase of infection.
In order to study a causal relation between the production (by the fungus) of sRNAs and the down-regulation (in tomato) of predicted target genes, we generated *B. cinerea* mutants in which a transposon region that is the source of ~10% of the fungal sRNAs was deleted, as well as mutants in which both Dicer-like (*BcDcl1/BcDcl2*) genes were removed. Neither of these mutants was significantly reduced in virulence on tomato or other tested host plants. We also generated a fungal mutant in an effector gene (*BcSpl*1) which was predicted to be targeted for silencing by a tomato sRNA. The predicted target sequence for the sRNA was replaced by synonymous substitutions and the effect of these substitutions on the fungal transcript was analyzed. As a result, the *BcSpl1* transcript in the mutant did not show any evasion of downregulation regardless of the substitutions in the predicted target sequence.
I will present the concept and results in this study that illustrate the complexity and many possible variables that operate in the cross-kingdom RNA warfare between *B. cinerea* and its host plants.
Keywords: small RNA; *Botrytis cinerea*; tomato; high-throughput sequencing

805V A novel RNA binding protein in table beet fungal pathogen, *Cercospora beticola*, regulates growth, secondary metabolism, and virulence. *Sandeep Sharma Khatiwada*[1], Braham Dhillon[2], Sarah Pethybridge[1] 1) Cornell University; 2) University of Florida.

mRNA splicing is an important biological process that regulates post-transcriptional gene expression in eukaryotes. Various components of mRNA splicing machinery have been characterized in budding yeast, *Saccharomyces cerevisiae* and other filamentous fungi. But the role of mRNA splicing in plant pathogenic fungi is poorly understood, especially in context of host-pathogen interaction. A forward genetic screen to identify genes required for virulence conducted in *Cercospora beticola*, the causal agent of Cercospora leaf spot of table beet, identified a gene expressing an RNA binding protein CbGbp2, an ortholog of *S. cerevisiae* Gbp2p. In *S. cerevisiae*, Gbp2p along with Hrb1 prevent the export of mature mRNA to cytoplasm until splicing of introns is complete. Disruption of the gene in *C. beticola* led to a complete loss of virulence phenotype. In order to characterize this gene, deletion strains carrying targeted gene knock-out of CbGbp2 were generated using *Agrobacterium* mediated transformation. One knock-out strain ΔCbgbp2p-71-2 selected for phenotypic characterization failed to produce any lesions on the leaf with a complete loss of virulence, a phenotype demonstrated by the original insertional mutant strain. Furthermore, ΔCbgbp2p-71-2 demonstrated impaired growth and an inability to produce the red-pigmented polyketide toxin, cercosporin, on potato dextrose agar medium. However, this deletion strain was not compromised in spore germination, hyphal growth and appressorium formation on table beet cv. Ruby Queen. *CbGbp2p* represents a novel RNA binding protein which belongs to a family of proteins rich in serine and arginine residues called SR proteins and contains three RNA binding domains (RNA recognition motifs; RRMs). Previously, a link between alternate splicing and pathogenic lifestyles was established using a comparative genomics approach in several fungal species. Our work here establishes a direct genetic link between two seemingly disparate processes, i. e. regulation of mRNA splicing and virulence, in filamentous fungi. Further studies will be designed to understand *CbGbp2p*-mRNA interactions during host-pathogen interaction and utilize the information to develop effective strategies towards management of Cercospora leaf spot of table beet.

806V Understanding the Molecular Bases of Adaptation of the Fungal Pathogen Zymoseptoria to Specific Host Species *Coraline Praz*[1], Jaime Huerta-Cepas[1], Andrea Sánchez Vallet[1] 1) Centro de Biotecnologia y Genomica de Plantas, Universidad Politecnica de Madrid (UPM) – Instituto Nacional de Investigacion y Tecnologıa Agraria y Alimentaria (INIA), Campus de Montegancedo-UPM, Madrid, Spain.

Zymoseptoria tritici is a devastating pathogen causing dramatic losses on wheat yield. *Z. tritici* is highly specialized and can only infect wheat but is not pathogenic on other grasses. In contrast, species related to *Z. tritici*, such as *Z. pseudotritici*, *Z. ardabiliae*, *Z. brevis* and *Z. passerinii*, infect a variety of wild grasses species, but have been shown to be incapable of infecting wheat[1,2]. *Z. tritici* emerged from these closely related *Zymoseptoria* species around 10,500 years ago[3].
Recognition of a specific avirulence factor (Avr) of a pathogen by the corresponding resistance protein in an adapted host is the molecular base of so-called race-specific resistance. Remarkably, it has been shown that homolog genes of *Avrs* from *Z. tritici* are induced during wheat colonization in *Z. ardabiliae* and *Z. pseudotritici* and that a homolog of one of these avirulence factors in the non-adapted species induces a very strong immune response in wheat (Meile and Sánchez-Vallet, unpublished results). This suggests that avirulence factors and resistance proteins contribute to the resistance of plant species to non-adapted pathogens, so-called non-host resistance. We therefore hypothesized that an accumulation of Avrs in the closely related *Zymoseptoria* species and their recognition by specific host resistance genes in wheat are responsible for their non-ability to infect wheat.
Combining, genomics and molecular approaches, we investigated the evolutionary history of effector genes in *Zymoseptoria* species. These analyses led to the identification of candidate Avrs recognized by wheat in non-adapted *Zymoseptoria* species. Functional assays are performed to test if the candidate Avrs are recognized by wheat and to determine the contribution of accumulation of Avrs in

non-adapted species to non-host resistance.
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807V Analysis of the microbial war between the Bioeffector *Pythium oligandrum* and the Pythium Soft-Rot of Ginger Pathogen *Pythium myriotylum* *Taha Majid Mahmood Sheikh*[1], Chen Siqiao[1,2], Xue Taiqiang [1,3], Li Jingjing[3], Zhang Qimeng [1], Chen Yifan [1,3], Sheng Deng [1], Jiu Min [4], Zhou Dongmei [1], Jamie McGowan[5], David Fitzpatrick[5], Paul Daly[1], Irina Druzhinina[2], Wei Lihui [1,3] 1) Key Lab of Food Quality and Safety of Jiangsu Province – State Key Laboratory Breeding Base, Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, Nanjing, China.; 2) Fungal Genomics Laboratory (FungiG), Jiangsu Provincial Key Lab of Organic Solid Waste Utilization, Nanjing Agricultural University, Nanjing, China.; 3) School of Environment and Safety Engineering, Jiangsu University, Zhenjiang, China.; 4) College of Food and Bioengineering, Henan University of Science and Technology, Luoyang, Henan, China.; 5) Genome Evolution Laboratory, Maynooth University, Co. Kildare, Ireland..

Pythium oligandrum (Peronosporales, Oomycota) is a non-plant pathogenic soil-inhabiting water mold that can colonize the root ecosystem of many crops. Unlike most members of the *Pythium* genus, *P. oligandrum* can parasitize fungal and oomycete hosts including other *Pythium* spp. In direct interaction, *P. oligandrum* can parasitize plant-pathogenic *Pythium* spp. and thus reduce the disease load while in indirect interactions, this microorganism can induce plant resistance and promote plant growth which contributes to disease suppression. We isolated a strain of *P. oligandrum* called GAQ1 from a field site where ginger was growing and found this strain could suppress Pythium soft rot of ginger caused by *Pythium myriotylum*. Using dual transcriptomics, we investigated the changes that occurred in gene expression when *P. oligandrum* antagonized *P. myriotylum* in plate-based confrontation assays. During antagonism, the techniques of viability staining, light and scanning electron microscopy demonstrated how *P. myriotylum* became non-viable as the hyphae of *P. oligandrum* coiled around and penetrated into *P. myriotylum* hyphae. In response to the presence of *P. oligandrum*, *P. myriotylum* genes encoding putative Kazal-type protease inhibitors were strongly upregulated along with changes in expression of putative cellulases and elicitin-like proteins. In *P. oligandrum*, genes encoding putative proteases, cellulases, and peroxidases were significantly upregulated. Despite the upregulation of putative protease inhibitors from *P. myriotylum*, a strong biocontrol effect of *P. oligandrum* on soft rot disease of ginger was measured. Further work is ongoing to dissect how *P. oligandrum* suppresses Pythium soft rot disease, and whether *P. myriotylum* can counter-antagonize *P. oligandrum*.

808V Functional analysis of putative protein glycosylation related genes in the plant pathogenic fungus *Fusarium graminearum* *Heeji Moon*[1], Eun Jung Thak[3], Yejin Choi[1], Sieun Kim[1], Jiyeun Park[1], Soyoung Choi[1], Nahyun Lee[1], Jung-Eun Kim[2], Ji Young Shin[1], Hyun Ah Kang[3], Hokyoung Son[1,2] 1) Department of Agricultural Biotechnology, Seoul National University, Seoul, Republic of Korea; 2) Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea; 3) Department of Life Science, Chung-Ang University, Seoul, Republic of Korea.

Glycosylation, one of the most common types of post-translational modifications, contributes to various molecular and cellular functions by transferring different polysaccharides to proteins. During the last years, the role of protein glycosylation in plant pathogenic fungi has received significant attention, and glycoproteins have been expected to play essential roles in various biological processes including pathogenicity. However, the genes involved in the *N*- and *O*- glycosylation pathways and glycan structures of phytopathogenic fungi are still largely unknown. Fusarium head blight, caused by the fungus *Fusarium graminearum* is a devastating disease of wheat, barley, and maize worldwide. It causes severe yield losses and contaminates grains by accumulating mycotoxins which are harmful to human and animal health. To understand the complex molecular mechanisms underlying development and pathogenesis of this fungus, we attempted to generate a mutant library covering putative 78 *N*- or *O*-glycosylation-related genes (GLY). Until now, deletion mutants for 64 genes and comprehensive phenotypic database of them were successfully generated. To better understand systematic roles of protein glycosylation in *F. graminearum*, quantitative proteomics analyses of *N*- or *O*-glycosylated proteins and structural analysis of cell wall glycans are also being conducted. Further works will focus on elucidation of the potential roles of protein glycosylation and its components in diverse biological processes including virulence in *F. graminearum*. This study is the first comprehensive functional analysis of GLY genes in filamentous fungi and would be the fungal glycoproteome study initiative.

809V A spot type net blotch susceptibility locus in barley. *Mariano Jordi Muria Gonzalez*[1], Simon Ellwood[1] 1) Centre for Crop and disease Management, Curtin University, Western Australia, Australia 6102.

The major diseases of barley, net blotch, is caused by two forms of the fungal pathogen *Pyrenophora teres*: *P. teres* f. *teres*, with characteristic net-like lesions on leaves, and *P. teres* f. *maculata* (*Ptm*), responsible for the spot form net blotch (SFNB). In model pathosystems, the gene-for-gene interaction between the fungus and its host is governed by a pathogen effector molecule that interacts with a receptor in the plant promoting the onset of disease in necrotrophic interactions or preventing disease in biotrophic pathogens. Commercial cultivars are often susceptible to SFNB due to a lack of genes conferring resistance and the effectors and their receptors are yet to be characterised. Extreme qualitative virulence was found with *Ptm* isolate M3 in barley cv. Baudin. Using a doubled haploid population (Eth069 x Baudin) we mapped the susceptibility to *Ptm* M3 to a QTL in the barley chromosome 1H explaining more than 90 % of the phenotype. We presuppose this susceptibility is the result of an effector-receptor interaction and further research is required to fine map the host susceptibility gene as well as to identify the corresponding fungal gene encoding the cognate effector. This discovery represents one step in breeding-out individual susceptibilities to SFNB, although the complexity of the *P. teres*- barley pathosystem requires the identification of additional susceptibility or resistance loci for robust resistance in commercial cultivars.

810V Spray-induced gene silencing (SIGS) against fungal and oomycete diseases in forest system *Irene Teresa Bocos Asenjo*[1, 2], Jonatan Niño Sánchez[1, 2], Julio Javier Diez[1, 2] 1) Universidad de Valladolid; 2) Instituto Universitario de Investigación Gestión Forestal Sostenible (iuFOR).

The diseases affecting trees seriously compromise our life quality as well as the resources obtained from the forest. Fungi and oomycetes are causal agents of diseases in important forest species worldwide. Our works focusses in two important forest diseases

caused by the fungus *Fusarium circinatum* and the oomycete *Phytophthora cinnamomi*. These diseases have for a long time been treated with chemical products, which have a high ecological impact on nature. Moreover, these methods are not entirely effective in controlling the diseases. Thus, other sustainable alternatives are in the spotlight of forest plant pathology now. One such alternative is spray-induced gene silencing (SIGS) which implies the inhibition of plant pathogens through a direct spray of dsRNA targeting essential pathogen genes on plant tissues. This strategy is environmentally friendly and could be a sustainable alternative to chemical disease control methods. Therefore, we are studying the potential of SIGS in the treatment of *F. circinatum* and *P. cinnamomi* by the design and production of dsRNA molecules that are able to silence essential genes that have already been tested in other pathogenic fungi, as well as by seeking new targets which inhibit these forest diseases. So, here we offer a sustainable alternative for the treatment of these diseases and the results of this research will help to explore the use of this technology in forest pathogens where it is not widely developed.

811V Differential physiological prerequisites and gene expression profiles of conidial anastomosis tube fusion and germ tube formation in *Colletotrichum gloeosporioides* *Nikita Mehta*[1,2], Ravindra Patil[1,2], Abhishek Baghela[1,2] 1) Agharkar Research Institute, Pune, India; 2) Savitribai Phule Pune University, Pune, India.

The conidia of a hemibiotrophic fungus, *Colletotrichum gloeosporioides,* can conventionally form a germ tube (GT) and develop into a fungal colony. While under certain conditions, they tend to get connected through a conidial anastomosis tube (CAT) to share the nutrients. CAT fusion is believed to be responsible for the generation of genetic variations in few asexual fungi, which appears problematic for effective fungal disease management. The physiological and molecular requirements underlying the GT formation versus CAT fusion remained underexplored. In the present study, we have deciphered the physiological prerequisites for GT formation versus CAT fusion in *C. gloeosporioides*. GT formation occurred at a high frequency in the presence of nutrients, while CAT fusion was found to be higher in the absence of nutrients. Younger conidia were found to form GT efficiently, while older conidia preferentially formed CAT. Whole transcriptome analysis of GT and CAT revealed highly differential gene expression profiles, wherein 11050 and 9786 genes were differentially expressed during GT formation and CAT fusion, respectively. A total of 1567 effector candidates were identified; out of them, 102 and 100 were uniquely expressed during GT formation and CAT fusion, respectively. Genes coding for cell wall degrading enzymes, germination, hyphal growth, host-fungus interaction, and virulence were highly up-regulated during GT formation. While, genes involved in stress response, cell wall remodeling, membrane transport, cytoskeleton, cell cycle, and cell rescue were highly up-regulated during CAT fusion. To conclude, the GT formation and CAT fusion were found to be mutually exclusive processes, requiring differential physiological conditions and sets of DEGs in *C. gloeosporioides*. This study will help in understanding the basic CAT biology in emerging fungal model species of the genus *Colletotrichum*.

812V Spray-induced gene silencing (SIGS) using organic and inorganic based-nanoparticles ensures a steady RNAi effect against *Botrytis cinerea* infection on plant material *Jonatan Niño Sanchez*[1,2], Rachael Hamby[1], Hailing Jin[1] 1) University of California, Riverside; 2) iuFOR - University of Valladolid, UVa, Spain.

Fungal plant diseases cause severe crop losses worldwide and are routinely controlled with chemical fungicides. Spray-induced gene silencing (SIGS) is an alternative eco-friendly fungal disease management strategy that has emerged in recent years based on the natural phenomenon of cross-kingdom RNA interference (RNAi). This phenomenon consists of the capacity of fungal pathogens, such as *B. cinerea,* to deliver small RNAs (sRNAs) to plant hosts during the infection process to silence host defense genes, and of host plants to also send sRNAs to the fungal pathogen to inhibit virulence genes. Based on this, and the subsequent discovery by our group that several fungal pathogens can efficiently take up environmental sRNAs that silence fungal genes with complementary sequences, SIGS has proven to be effective in controlling several fungal diseases on pre- and post-harvest plant material. However, the durability of the RNAi effect is limited by the stability of double-stranded RNA (dsRNA) after the spray application and its uptake efficiency by the fungal pathogen. Here, we used organic (lipid-based) and inorganic (nanoclay-based) nanoparticles to develop coated-dsRNA sprays against *B. cinerea*, targeting key virulence-related genes (Dicer-like, *DCL1* and *DCL2*) and essential fungal growth genes (*VPS51*, *SAC1*, *DCTN1*), to control grey mold disease on a range of plant materials, including leaves, flowers and fruits. These nanoparticles ensured a steady RNAi effect, as well as higher silencing efficacy than naked dsRNA applications over time, offering higher potential and broader applications for sequence-specific SIGS technologies to control fungal plant diseases.

813V Fungal Chemical Warfare: How Secondary Metabolites Influence Relationships in Maize Associated Fungi *Tim Satterlee*[1], Trevor Mitchell[1], Jaci Hawkins[1], Anthony Glenn[1], Scott Gold[1] 1) USDA/ARS.

Abstract: Contamination of maize by mycotoxins is a global problem affecting food safety and security worldwide. Exposure to mycotoxins can lead to a variety of health problems for both humans and animals. Additionally, there is a large economic cost associated with mycotoxin contamination including reduced product market value and lower animal performance. Two mycotoxins commonly contaminating maize are aflatoxin and fumonisin that are produced by the plant pathogens *Aspergillus flavus* and *Fusarium verticillioides,* respectively. Multiple studies have found these pathogens together in field colonized maize but have not examined their direct interaction. Another maize-associated fungus that comes into contact with these mycotoxigenic fungi is the endophyte *Sarocladium zeae.* Pyrrocidine, produced by *S. zeae,* was recently shown to inhibit fumonisin production in *F. verticillioides*. In this study, we evaluated pairwise interactions between *A. flavus, F. verticillioides* and *S. zeae*. Our results indicated that when grown in proximity, *F. verticillioides* can inhibit the growth of *A. flavus,* and that fumonisin is the primary cause of this growth inhibition. While *F. verticilliodes* inhibition was not seen with aflatoxin, production of both fumonisin and aflatoxin was suppressed in the presence of the antagonist's primary mycotoxin. The responses also varied with *A. flavus* demonstrating a localized response to fumonisin where a more general response was seen with aflatoxin treated *F. verticillioides.* While pyrrocidine had no effect on aflatoxin production, *S. zeae* produced other unidentified compound(s) that inhibit the growth of both *A. flavus* and *F. verticillioides.* This compound(s), unlike pyrrocidine, inhibits aflatoxin production in *A. flavus.* This work gives insights into the ecological role of fungal secondary metabolites in the interspecies battle for resource acquisition.

814V Establishment of functional symbioses between *Epichloë* endophytes and the modern cereals rye (*Secale cereale*)

and hexaploid wheat (*Triticum aestivum*). Wayne Simpson[1], Hisashi Tsujimoto[2], S Kato[2], David Hume[1], Wade Mace[1], Joanne Drummond[3], Phil Rolston[3], *Richard Johnson*[1] 1) AgResearch Limited, Grasslands Research Centre, Palmerston North, New Zealand.; 2) Arid Land Research Center, Tottori University, Tottori, Japan.; 3) Foundation for Arable Research, Templeton, New Zealand.

Asexual *Epichloë* endophytes have been used successfully in pastoral systems to enhance the agronomic performance of important temperate grasses such as perennial ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*) via improved tolerance to biotic and abiotic stresses.

Modern cereals such as wheat (*Triticum aestivum*) and rye (*Secale cereale*) within tribe Hordeae (Triticeae) do not naturally host *Epichloë*, but some wild relatives (e.g., *Elymus* spp.) have been shown to be infected with *Epichloë bromicola.* Inoculation of *E. bromicola* into both rye and wheat has shown that successful symbiosis depends on the genetics of both the host and the endophyte strain deployed. Endophyte compatibility in rye has been improved through recurrent selection of desirable phenotypes, which is possible due to its outcrossing nature and broad population based genetic variation. However, wheat is a selfing species and populations are genetically narrow, making recurrent selection for improved phenotype impractical. Until recently the phenotypes of *Epichloë* – wheat associations have been compromised, including host death and stunting. We have overcome this barrier by utilising experimental wheat lines containing alien chromosome additions or substitutions from wild species and have identified symbioses whereby infected wheat plants are phenotypically comparable to uninfected controls. These plants completed their full lifecycle including the transmission of *Epichloë* into the next generation of grain.

Assessment of *Epichloë* infected rye has demonstrated increased resistance to both insect pests and fungal diseases with field trials yielding between 39% to 95% more grain than the endophyte-free lines. Whilst research in wheat has not yet progressed to field trials, glasshouse experiments have shown that *Epichloë* infected wheat is more resistant to a number of insect pests including aphids.

815V Going Green: Evidence for acquisition and persistence of plant and algal plastomes in diverse fungi *Julia Kelliher*[1], Aaron Robinson[1], Demosthenes Morales[1], La Verne Gallegos-Graves[1], Karen Davenport[1], Debora Rodrigues[3], Saskia Bindschedler[2], Pilar Junier[2], Patrick Chain[1] 1) Los Alamos National Laboratory, Los Alamos, NM; 2) Institute of Biology, University of Neuchatel, Neuchatel, Switzerland; 3) Civil and Environmental Engineering, University of Houston, Houston, TX.

The endo-hyphal microbiome contains many uncharacterized inhabitants and interactions. This intra-fungal microbiome, coupled with an extensive network of extracellular interactions with bacteria and plants within the soil, contribute to the complex ecosystem services facilitated by fungi. We sought to characterize the members of the intracellular fungal microbiome as a way to better understand the roles of fungi and their associated endosymbionts. Based on a 16S rRNA amplicon screen of four distinct fungal collections from different geographic locations, we identified taxonomic signatures of many bacteria not previously known to be associated with fungi. Rather unexpectedly, one of the amplicon sequence signatures that was found across all culture collections, was a recurrent signal for various plant and algal chloroplasts. Several techniques were utilized in validating the potential associations between fungi and the plastids, including FISH staining, phylogenetic analyses, qPCR and PCR amplifications, metabolomic assays, Hi-C sequencing, screens of existing SRA data, plastid probe-based sequence enrichments, and additional genomic and transcriptomic sequencing. This discovery is leading to several new avenues of research to explore the acquisition, maintenance, evolution, as well as any physiological or ecological function of chloroplasts within fungi.

816V Interactions between algal cells and the dimorphic lichenized fungus *Umbilicaria muhlenbergii* *Yuting Hu*[1,2], Yanyan Wang[1,3], Jinrong Xu[1] 1) Purdue University; 2) Sichuan Agricultural University, College of Agronomy & Key Laboratory for Major Crop Diseases, Sichuan, China; 3) Chinese Academy of Sciences, State Key Laboratory of Mycology, Institute of Microbiology, Beijing, China.

Umbilicaria muhlenbergii is a dimorphic lichenized fungus that grows in the hyphal form in lichen thalli but as yeast cells in axenic cultures. It is uniquely suited for studying symbiotic interactions between fungal-algal cells because of its relatively fast growth rate and amenability to molecular manipulations. In our previous study, we found that contact with algal cells of its photobiont *Trebouxia jamesii* induces pseudohyphal growth of *U. muhlenbergii*. Transformants expressing the dominant active alleles of *UmGPA3* stimulated the yeast-to-pseudohypha transition and often resulted in cell death in *T. jamesii* cells. To further characterize the fungal-algal interactions, we tested different co-incubation conditions for observing the attachment of yeast cells to *T. jamesii* cells and differentiation of fungal-algal cell clusters. A transformant of *U. muhlenbergii* expressing cytoplasmic GFP was generated and used to observe pseudohyphae in direct interaction with algal cells by co-focal microscopy. Possible differentiation of appressorium- or haustorium-like structures will also be examined by TEM examinations. In addition, RNA-seq data had been generated with fungal cells, algal cells, and fungal-algal cell clusters. Results from microscopical examinations and RNA-seq analysis will be presented to show symbiotic interaction-specific gene expression and morphogenesis in *U. muhlenbergii*.

817V Genetic determinants of endophytism in the *Arabidopsis* root mycobiome *Fantin Mesny*[1], Shingo Miyauchi[1,2], Thorsten Thiergart[1], Brigitte Pickel[1], Lea Atanasova[3,4], Magnus Karlsson[5], Bruno Hüttel[6], Kerrie Barry[7], Sajeet Haridas[7], Elodie Drula[8,9], Bernard Henrissat[10], Annegret Kohler[2], Igor Grigoriev[7,11], Francis Martin[2,12], Stéphane Hacquard[1,13] 1) Max Planck Institute for Plant Breeding Research - Cologne, Germany; 2) UMR Interactions Arbres/Microorganismes, Centre INRAE Grand Est-Nancy - Champenoux, France; 3) Institute of Chemical, Environmental and Biological Engineering, Vienna University of Technology - Vienna, Austria; 4) Institute of Food Technology, University of Natural Resources and Life Sciences - Vienna, Austria; 5) Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences - Uppsala, Sweden; 6) Max Planck Genome Centre - Cologne, Germany; 7) U.S. Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory - Berkeley, CA, USA; 8) INRAE, USC1408 Architecture et Fonction des Macromolécules Biologiques - Marseille, France; 9) Architecture et Fonction des Macromolécules Biologiques (CNRS, Aix-Marseille Univ.) - Marseille, France; 10) Department of Biological Sciences, King Abdulaziz University - Jeddah, Saudi Arabia; 11) Department of Plant and Microbial Biology, University of California Berkeley - Berkeley, CA, USA; 12) Beijing Advanced Innovation Centre for Tree Breeding by Molecular Design (BAIC-TBMD), Institute of Microbiology, Beijing Forestry University

- Beijing, China; 13) Cluster of Excellence on Plant Sciences (CEPLAS), Max Planck Institute for Plant Breeding Research - Cologne, Germany.

The roots of *Arabidopsis thaliana* host diverse fungal communities that affect plant health and disease states. We sequenced the genomes of 41 fungal isolates representative of the *A. thaliana* root mycobiota for comparative analysis with 79 other plant-associated fungi. Our analyses indicated that root mycobiota members evolved from ancestors with diverse lifestyles and retained large repertoires of plant cell wall-degrading enzymes (PCWDEs) and effector-like small secreted proteins. We identified a set of 84 gene families associated with endophytism, including genes encoding PCWDEs acting on xylan (family GH10) and cellulose (family AA9). Transcripts encoding these enzymes were also part of a conserved transcriptional program activated by phylogenetically-distant mycobiota members upon host contact. Recolonization experiments with individual fungi indicated that strains with detrimental effects in mono-association with the host colonized roots more aggressively than those with beneficial activities, and dominated in natural root samples. Furthermore, we showed that the pectin-degrading enzyme family PL1_7 linked aggressiveness of endophytic colonization to plant health.

818W Long reads and Hi-C sequencing illuminate the two-compartment genome of the model arbuscular mycorrhizal symbiont Rhizophagus irregularis *Gokalp Yildirir*[1], Jana Sperschneider[2], Mathu Malar C[1], Eric C H Chen[3], Wataru Iwasaki[3], Calvin Cornell[1], Nicolas Corradi[1] 1) Department of Biology, University of Ottawa; 2) Biological Data Science Institute, The Australian National University; 3) Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo.

Chromosome folding links genome structure with gene function by generating distinct nuclear compartments and topologically associating domains. In mammals, these undergo preferential interactions and regulate gene expression. However, their role in fungal genome biology is unclear. Here, we combine Nanopore (ONT) sequencing with chromatin conformation capture sequencing (Hi-C) to reveal chromosome and epigenetic diversity in a group of obligate plant symbionts: the arbuscular mycorrhizal fungi (AMF). We find that five phylogenetically distinct strains of the model AMF Rhizophagus irregularis carry 33 chromosomes with substantial within-species variability in size, as well as in gene and repeat content. Strain-specific Hi-C contact maps reveal a 'checkerboard' pattern that underline two dominant euchromatin (A) and heterochromatin (B) compartments. Each compartment differs in the level of gene transcription, regulation of candidate effectors and methylation frequencies. The A-compartment is more gene-dense and contains most core genes, while the B-compartment is more repeat-rich and has higher rates of chromosomal rearrangement. While the B-compartment is transcriptionally repressed, it has significantly more secreted proteins and in planta upregulated candidate effectors, suggesting a possible host-induced change in chromosome conformation. Overall, this study provides a fine-scale view into the genome biology and evolution of model plant symbionts, and opens avenues to study the epigenetic mechanisms that modify chromosome folding during host-microbe interactions.

819T Emerging tree pathogen *Phellinus noxius* has a long evolutionary history in eastern Asia, Australia, and the Pacific Islands *Olga Kozhar*[1], Mee-Sook Kim[2], Jorge Ibarra Caballero[1], Ned B. Klopfenstein[3], Phil G. Cannon[4], Jane E. Stewart[1] 1) Colorado State University, Fort Collins, CO; 2) USDA Forest Service, Pacific Northwest Research Station, Corvallis, OR; 3) USDA Forest Service, Rocky Mountain Research Station Moscow, ID; 4) USDA Forest Service, Forest Health Protection, Vallejo, CA.

Emerging pathogens and diseases they cause have increased exponentially over the last century, and it is critical to determine if these pathogens are native that were present in areas of emergence for a long time, or invasive that were recently introduced to those areas. Understanding the ecological and evolutionary processes promoting pathogen emergence can help to control pathogen and disease spread. Over the last few decades, brown root rot disease, caused by the root- and wood-rotting fungus *Phellinus noxius* of unknown origin, has been causing extensive damage to diverse trees in tropical/subtropical regions. Understanding the population structure, demographic history, and potential pathways of spread for *P. noxius* is an initial step to determine if *P. noxius* is an invasive pathogen that is causing the emergence of brown root rot disease. Little is known about the pathogen's population structure, diversity, and invasion routes. Using restriction site-associated DNA sequencing data, we characterized genetic relationships, pathways of spread, and evolutionary histories of *P. noxius* collected from 15 locations in eastern Asia, Australia, and the Pacific Islands. We analyzed patterns of genetic variation using Bayesian inference, maximum likelihood phylogeny, principal component analysis, and populations splits and mixtures measuring correlations in allele frequencies and genetic drift. In addition, we applied coalescent based theory using approximate Bayesian computation (ABC) with supervised machine learning. Population structure analyses revealed five distinct genetic groups of *P. noxius* with little admixture and with signatures of a complex recent and ancient migration history among study locations. ABC analyses indicated most likely pathogen spread from ghost population to Malaysia and the Pacific Islands (Guam and American Samoa), and with subsequent spread to Taiwan and Australia. Furthermore, ABC analyses indicate that major spread by *P. noxius* occurred 1,000s of generations ago, contradicting previous assumptions that it was recently introduced in many areas. Our results suggest that *P. noxius* has a long evolutionary history in eastern Asia, Australia, and the Pacific Islands, and recent pathogen and disease emergence is likely driven by anthropogenic and natural disturbances, including deforestation, land-use change, severe weather events, and introduction of exotic plants.

820F Repeat-driven genome expansion and two-speed genome architecture of amphibian-infecting chytrids *Theresa Wacker*[1], Helmstetter Nicolas[1], Wilson Duncan[1], Fisher Matthew C.[2], Studholme David J.[3], Farrer Rhys A.[1] 1) Medical Research Council Centre for Medical Mycology at the University of Exeter, Exeter, United Kingdom; 2) MRC Centre for Global Infectious Disease Analysis, Imperial College London, London, United Kingdom; 3) Biosciences, University of Exeter, Exeter, United Kingdom.

Over the past half century, the chytridiomycosis panzootic has led to the decline of over 500 amphibian species with 90 attributed extinctions. Chytridiomycosis of amphibians is caused by two fungal species *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*). The genetic mechanisms underlying host-specificity and pathology in the *Batrachochytrium* genus remain elusive and their evolution and origins of virulence are largely unknown. Using deep nanopore sequencing, we found that *Bsal* is extremely repeat-rich with high numbers of long terminal repeats, long interspersed nuclear elements and transposable elements. This repeat-driven genome expansion in *Bsal* has resulted in a tripling of its length compared with *Bd.* Key pathogenicity genes including M36 metalloproteases have expanded compared with *Bd*, and are enriched for flanking transposable elements, suggesting its genome

expansion is connected to selective evolutionary processes. Both batrachochytrids have evidence of a two speed genome architecture, including an enrichment of functional categories in compartments of repeat richness or sparsity. Furthermore, among *Bd* lineages, M36 metalloproteases with signatures of positive selection and, both in *Bsal* and *Bd*, genes upregulated during infection *in vivo* are enriched in repeat-rich and gene-sparse compartment of the genome. This is the first evidence for a two-speed genome in an animal pathogen, shedding new light on the role of repetitive sequences on the evolution of fungal pathogens driving global declines and extinctions of amphibians.

821W Pangenomics of the 'death cap' mushroom, *Amanita phalloides*, and of *Agaricales* reveal dynamic evolution of toxin-related gene family in an invasive range. *Milton Drott*[1], Lynn Harrow[2], Yen Wen Wang[2], Nancy Keller[1], Anne Pringle[2] 1) Department of Molecular and Environmental Toxicology, University of Wisconsin, Madison, WI. ; 2) Department of Botany, University of Wisconsin, Madison, WI..

The most notorious poisonous mushroom in the phylum *Basidiomycota* is the 'death cap,' *Amanita phalloides* which produces non-canonical toxic cyclic-peptide secondary metabolites (SMs) encoded by 'MSDIN' genes. The structure and population dynamics of MSDIN genes are poorly understood, a contrast to the many well described SM biosynthetic gene clusters of phylum *Ascomycota*. We developed a bioinformatic pipeline to describe MSDIN genes in 20 *A. phalloides* genomes from native populations (Europe) and 68 genomes from an invasive population (California). We identify 44 unique MSDIN sequences in the species, including 14 previously-undescribed sequences, organized into 28 distinct loci. Pangenomic analysis of MSDINs reveals 13 MSDIN sequences across 13 loci as part of an *A. phalloides* core genome (present in all individuals) while 31 MSDIN sequences from 16 loci are part of an *A. phalloides* accessory genome. Our resolution of MSDINs to the locus-level allows us to reconstruct the evolutionary histories of toxins and to identify patterns of positive and negative selection acting on these genes. We contextualize the MSDIN content of the *A. phalloides* pangenome using an analysis of 250 *Agaricales* genomes. We find most MSDIN sequences are not conserved across species. We demonstrate that many MSDIN genes in the genus *Amanita* show a consistent and significant physical clustering. These results clarify the diversification of MSDIN genes within and between species and genera of poisonous mushrooms while also uncovering a trove of novel chemical diversity. We speculate that MSDIN genes present in the core genome are important for fundamental ecological processes in these species, but that accessory-genome MSDINs may reflect species or population-specific processes, potentially contributing to the spread of invasive *A. phalloides*populations.

822T Births, deaths and survival of a retrotransposon family in the face of repeat induced point mutations (RIP) *Ivar Westerberg*[1], Aaron Vogan[1], Lorena Ament-Velásquez[2], Hanna Johannesson[1] 1) Uppsala University; 2) Stockholm University.

The filamentous fungus *Podospora anserina* is a part of a species-complex with six other species. *P. anserina* is known to harbor a relatively small abundance of repeats (seven percent of the genome), and also possess the host defence mechanism known as repeat induced point-mutations (RIP). RIP induces C-to-T mutations in repetitive regions of the genome, and hence can introduce nonsense mutations into TEs. Typical classification relies on a number of assumptions, such as constant rates of evolution, which are violated by RIP due to its stochastic nature. Thus, classifying TEs in many fungal species is a complex and imprecise process. In this study, we utilized a combination of a sequence similarity network (SSN) approach together with the more commonly used alignment-based classification to explore the evolution of an LTR retrotransposon family, Crapaud, across the Podospora species-complex. LTR-retrotransposons rely on an RNA-mediated transposition mechanism and contain protein domains for reverse transcription and integration as well as structurally important long terminal repeat sequences that provide sequence promotors and transcription termination sites. Our initial results revealed multiple variants of the terminal repeats, where half of the terminal repeat was conserved and the other diverged, throughout the Podospora genomes. Additionally, we identify several full length variants of Crapaud that are present in multiple copies throughout the species-complex. This result is indicative of the variants either actively transposing or having done so at some point in the evolution of the species-complex. The identification of these variants reveals a recent radiation of Crapaud within the species-complex, with some variants specific to one or a few species. The utilization of SSNs to classify these repeats showcase the utility of the addition of a sequence similarity clustering method to the typical methods of studying TE evolution in genomes with RIP. These results also provide key insights into the evolution of LTR retrotransposons and how they manage to evade the host defence RIP. Crapaud is abundant in the species-complex and present in structurally important regions such as the centromeres. The evolution and radiation of this LTR retrotransposon thus give further insights into the overall genome evolution of the species-complex.

823F Evolution of a Large Effector Family in *Pyricularia* *Daniel Ebbole*[1], Meilian Chen[2], Nicholas Farmer[1], Wenhui Zheng[3], Yijuan Han[3], Guodong Lu[3], Zhenhui Zhong[4], Zonghua Wang[2, 3] 1) Texas A&M University; 2) Minjiang University; 3) Fujian Agriculture and Forestry University; 4) University of California, Los Angeles.

Investigations of the origins, evolution, and functional divergence of fungal effectors across host-adapted populations and species have been limited. We previously identified a gene encoding a suppressor of plant cell death in *Pyricularia oryzae* (syn. *Magnaporthe oryzae*). This gene, *HAG1*, is one of a 21-member gene family and we characterized sequence diversity in different *P. oryzae* populations and *Pyricularia* species. Within the rice pathogen population, nucleotide diversity is low, however; the majority of gene family members display presence-absence polymorphism or other null alleles. Gene family allelic diversity is greater between host-adapted populations and, thus, we named them host-adapted genes (HAGs). Multiple copies of *HAGs* were found in some genome assemblies and sequence divergence of alleles in two cases suggested they resulted from repeat-induced point (RIP) mutagenesis. Transfer of family members between populations and novel *HAG* haplotypes resulting from apparent recombination were observed. The apparent transfer of a RIP allele from one host-adapted population to another indicates RIP is one mechanism for generation of novel alleles conferring a selective advantage to recipient lineages. Transfer of virulence genes between Pyricularia species has previously been shown and *HAG4* was transferred from *P. grisea* to *P. oryzae*, becoming fixed in the *Seteria* and *Oryza* pathogen populations. The *HAG* family presents a unique resource for examination of gene family evolution and functional evolution of plant pathogen effectors.

824W Molecular evolution of virulence effectors of the rice blast fungus *Magnaporthe oryzae* *Pierre Gladieux*[1], Florian Charriat[1], Marie Le Naour-Vernet[1], Jérôme Gracy[2], Charis Ramsing[1], Sandrine Cros-Arteil[1], Céline Thivolle[1], Isabelle Meusnier[1], Florian Veil-

let[1], Sébastien Ravel[1], Didier Tharreau[1], Elisabeth Fournier[1], André Padilla[2], Thomas Kroj[1], Stella Cesari[1] 1) PHIM Plant Health Institute, Univ Montpellier, INRAE, CIRAD, Institut Agro, IRD, Montpellier, France; 2) Centre de Biochimie Structrurale (CBS), INSERM, CNRS, Université de Montpellier, 29 rue de Navacelles, 34090 Montpellier, France..

During infection, plant pathogens secrete proteins called effectors that target cellular processes of the host plant to promote disease. Some of these effectors are specifically recognized by plant immune receptors, triggering defense responses that abort the infection process.
Phytopathogenic fungi possess extended and highly diverse effector repertoires comprising several hundreds of secreted proteins. Recently, we have identified a large fungal effector family in the rice blast fungus *Magnaporthe oryzae*, called MAX effectors (*Magnaporthe* AVRs and ToxB-like [1]). *MAX* effectors are highly enriched among the effectors recognized by immune receptors. While MAX effectors share a conserved three-dimensional structure, their amino-acid sequences and surface properties are highly diversified, suggesting that they target a broad and diverse range of host proteins.
This talk will provide an update on our work on the molecular biology and evolution of effectors in *M. oryzae*. Using a structure-informed gene annotation pipeline, we identified 46 to 69 MAX effectors per genome (average: 61 MAX effectors) in a set of 121 isolates, representing six host-associated lineages. The expression of MAX effector genes was largely restricted to the early biotrophic phase of infection and strongly influenced by the host plant. MAX effectors harbored more standing genetic variation than non-effector genes, pointing to adaptive forces that maintain genetic variation in populations - or balancing selection - as an important factor shaping the diversity of MAX effectors. Pangenome analyses of MAX effectors demonstrated extensive presence/absence polymorphism and identified several candidate gene loss events possibly involved in host range alterations, although gene knock-in experiments did not reveal a strong effect on virulence phenotypes. Our work demonstrate that MAX effectors represent a highly dynamic compartment of the genome of *M. oryzae*, potentially reflecting intense coevolutionary interactions with host molecules.

[1] Guillen K De, Ortiz-vallejo D, Gracy J, Fournier E (2015). Structure Analysis Uncovers a Highly Diverse but Structurally Conserved Effector Family in Phytopathogenic Fungi. 1–27

825T An NLR-like system delimits individuals in the basidiomycete *Coprinopsis cinerea* *Ben Auxier*[1], Julia Marschall[1], Alfons Debets[1], Duur Aanen[1] 1) Wageningen University.

Fusion between hyphae has potential benefits, but to limit risks should be restricted to be within an individual. To acheive this, sustained fusion is restricted based on the identity of polymorphic allorecognition genes. The genes responsible for this non-self recognition have been unknown in Basidiomycetes. Since Basidiomycetes experience an extended dikaryotic phase, non-self recognition likely functions differently from known mechanisms of ascomycetes. We present results of genetically mapping the first known basidiomycete non-self recognition locus in the model mushroom *Coprinopsis cinerea*. Using set of ~600 F1 offspring, combined with independant backcross lines, we identify a region on chromsome 5 whose alleles are strongly associated with nonself recognition. Fine-mapping of this region combined with genomic comparisons of additional *C. cinerea* isolates provide evidence that nonself recognition is driven by ancient polymorphic alleles of an NLR-like system. The polyallelic locus we identify appears to involve a Leucine Rich Repeat, a novel finding for fungal non-self recognition. We speculate this locus may form part of a reader-writer system, allowing the mating and cohabitation of two genomes, yet retaining the identity in all parts of the life cycle. These results provide a first understanding of how Basidiomycetes regulate individuality.

826F Pararesistance: a non-genetic mechanism of antifungal drug resistance *Jinglin Lucy Xie*[1], Kiran Chandrasekher[2], Judith Berman[3], Daniel Jarosz[1] 1) Stanford University School of Medicine, Stanford, CA; 2) Cornell University, Ithaca, NY; 3) Tel Aviv University, Tel Aviv, Israel.

Drug resistance is a major cause of treatment failure in infectious diseases and an emerging public health crisis. Although research has largely focused on identifying mutation-based mechanisms of drug resistance, cellular variation driven by epigenetic heterogeneity may be a hidden force promoting rapid adaptation to drug-induced stress. Recent studies have demonstrated that multiple chromatin-based and protein-based epigenetic states can be induced in response to stress. These heritable 'molecular memories' often confer a fitness advantage during re-exposure to stress. However, the mechanisms that promote the establishment and maintenance of such non-genetic states remain poorly characterized, especially in pathogens. Here, we describe a non-genetic mechanism of stress adaptation that accelerates the acquisition of drug resistance in a leading human fungal pathogen, *Candida albicans*. We discovered that a transient exposure to fluconazole, the most widely prescribed antifungal, elicits a sustained protective response in a subpopulation of cells. This mode of drug adaptation, which we term 'pararesistance', is induced by low doses of fluconazole, and facilitates the rapid emergence of resistance to high doses of fluconazole. Exposure to low levels of the protein denaturant guanidine hydrochloride or a suppressor of liquid-liquid phase separation 1,6-hexanediol blocks the induction, suggesting that pararesistance may be established via a prion-like mechanism. RNA-sequencing analysis showed pararesistant isolates share similar transcriptional profiles that are distinct from those of susceptible isolates. Importantly, a number of multidrug transporters such as *CDR1* are constitutively upregulated in pararesistant cells. Consistent with this finding, pararesistant isolates exhibited increased efflux, resulting in decreased accumulation of Cdr1 substrate rhodamine 6G. Additionally, phenotypic characterization of 62 pararesistant isolates across 20 different growth conditions revealed that pararesistance confers resistance to a number of Cdr1 substrates in addition to fluconazole, including brefeldin A and terbinafine. These results suggest that the adaptive value of pararesistance is at least in part mediated by the upregulation of drug efflux. Together, this work presents a new paradigm for understanding non-genetic mechanisms that drive the rapid evolution of drug resistance, establishing a conceptual framework for developing novel therapeutic strategies that target evolutionary processes.

827W The molecular resistance mechanisms and population structure of azole-resistant *Aspergillus fumigatus* present on commercial agricultural products in the United States *Caroline Burks*[1], Natalie Miller[1], Douglas Vines[1], Michelle Momany[1], Paul Severns[1], Marin Brewer[1] 1) University of Georgia.

Aspergillus fumigatus is a common environmental saprophyte as well as a human-pathogenic fungus that can infect patients with a variety of immune function; however, the disease aspergillosis can be deadly in immunocompromised individuals. Since the late 1990's

resistance to azole antifungals - the first line of defense against aspergillosis - has been documented in *A. fumigatus*. This resistance was found in patients without prior exposure to azoles, leading investigators to believe that the azole resistance had developed in agricultural environments where azoles are frequently used against plant-pathogenic fungi. Previous studies in the United States have documented azole-resistant *A. fumigatus* across different agricultural environments but none have looked at commercial plant products. Five hundred twenty-five isolates were collected from produce and garden products and screened for tebuconazole and itraconazole resistance; and 130 isolates were chosen for further azole-resistance phenotyping. Twenty-four isolates from compost, soil, flower bulbs, and peanuts were pan-azole resistant. The primary genetic mechanisms underlying the azole resistance in the pan-azole-resistant isolates were *cyp51A*-based (TR_{34}/L98H, TR_{46}/Y121F/T289A, and H147Y), although some isolates had alternative mechanisms. To determine the genetic relatedness of azole-resistant and sensitive commercial isolates with isolates from agriculture and clinical samples, 95 isolates from this study and 80 clinical and environmental isolates from a previous study were selected for population genetics analysis. Minimum spanning networks and discriminate analysis of principal components (DAPC) were used to identify if populations were structured. The minimum spanning networks both showed a large amount of diversity in the population and had 2 primary branches distinguished from the other isolates that contained isolates with a tandem repeat in the promoter of the *cyp51A* allele and isolates collected from commercial products with a T248N/E255D allele. Three primary clusters were identified with DAPC, which appeared to separate out based on *cyp51A* genotype. This is consistent with previous studies that have found that pan-azole-resistant isolates are found in a unique clade. Overall, pan-azole resistance can be found in lawn and garden products in the United States. Further surveying is necessary to determine the extent to which azole-resistant *A. fumigatus* is present in these products and others.

828T Identifying genes involved in the temperature-dependent morphological transition in *Coccidioides posadasii* *Keith Walcott*[1], Bastian Joehnk[1], Mark Voorhies[1], Anita Sil[1], Claire Dubin[2], Rachel Brem[2] 1) University of California, San Francisco, San Francisco, CA; 2) University of California, Berkeley, Berkeley, CA.

Coccidioides posadasii is a thermally dimorphic fungal pathogen and the causative agent of Valley Fever. Coccidioides is endemic to Southern California, Arizona, Central and South America. Valley Fever poses a serious health and financial burden to those afflicted with the illness; Californians alone incur costs of close to $200M/year for health-related expenses. In the soil, Coccidioides grows as mycelia, or vegetative filaments, that develop into arthroconidia which can be easily aerosolized. Upon inhalation, arthroconidia swell and undergo nuclear division and segmentation to form spherules filled with endospores. During infection, the spherules rupture, and endospores spread throughout the body and develop into more spherules. To identify genes involved in the morphological transition between the environmental and host phase, we are profiling the population genomics and phenotypic diversity of a collection of Coccidioides posadasii clinical isolates. Sequence analysis of these isolates revealed that a subset of them form a diverse but closely related population that is suitable for a type of forward genetic screen called a Genome Wide Association Study (GWAS). Arthroconidia from 54 clinical isolates were pooled and grown in competition under conditions replicating the host or the environment, after which genomic DNA was extracted and sequenced. GWAS analysis was used to identify alleles that are differentially abundant under each condition. 9 genes were identified with statistically significant differences in allele abundance under each condition. Using CRISPR/Cas9, we are disrupting each gene in the reference Silveira strain. The growth phenotype of each knockout mutant is then determined under the environmental and host conditions. Our preliminary studies have identified one mutant strain with a growth defect under environmental conditions. The corresponding gene encodes a protein with homology to an MRC1 checkpoint mediator. We will expand this GWAS pipeline to interrogate other conditions related to virulence so that we can better understand Coccidioides and how it causes disease in the host.

829F Population genetics and microevolution of clinical *Candida glabrata* reveals recombinant sequence types and hypervariation within mitochondrial genomes, virulence genes and drug-targets *Nicolas Helmstetter*[1], Aleksandra Chybowska[2], Christopher Delaney[3], Alessandra Da Silva Dantas[1], Hugh Gifford[1], Theresa Wacker[1], Carol Munro[2], Adilia Warris[1], Brian Jones[3], Christina Cuomo[4], Duncan Wilson[1], Gordon Ramage[3], Rhys Farrer[1] 1) University of Exeter; 2) University of Aberdeen; 3) University of Glasgow; 4) Broad Institute of MIT and Harvard.

Candida glabrata is the second most common etiological cause of worldwide systemic candidiasis in adult patients. Genome analysis of 68 isolates from 8 hospitals across Scotland, together with 83 global isolates, revealed insights into the population genetics and evolution of *C. glabrata*. Clinical isolates of *C. glabrata* from across Scotland are highly-genetically diverse, including at least 19 separate sequence types (STs) that have been recovered previously in globally diverse locations, and one newly discovered ST. Several STs had evidence for ancestral recombination, suggesting transmission between distinct geographical regions has coincided with genetic exchange arising in new clades. Three isolates were missing MATa1, potentially representing one of the mating types. Signatures of positive selection were identified in several genes and gene families between every ST and reference ST15 including statistical enrichment for Epithelial Adhesins (EPA) thought to facilitate fungal adhesion to human epithelial cells. Microevolution was identified between seven sets of between 2 and 9 isolates from recurrent cases of candidiasis, revealing an enrichment for non-synonymous and frameshift indels in cell surface proteins. Microevolution within patients also affected EPA genes, and several genes involved in drug resistance including the ergosterol synthesis gene *ERG4* and the echinocandin target *FKS1*/2. In addition to nuclear genome diversity, the *C. glabrata* mitochondrial genome was particularly diverse, appearing reduced in size and with fewer conserved protein encoding genes in all non-reference ST15 isolates. Together, our work highlights the genetic diversity present within the *C. glabrata* population that may impact virulence and drug resistance, and two major mechanisms generating this diversity: microevolution and genetic exchange/recombination.

830W A cystic fibrosis patient lung environment allowed for coexistence of multiple *Exophiala dermatitidis* clades over time *Tania Kurbessoian*[1], Daniel Murante[2], Alex Crocker[2], Jason Stajich[1], Deborah Hogan[2] 1) University of California, Riverside, Riverside, CA; 2) Geisel School of Medicine, Dartmouth, Hanover, NH.

It is vital to understand how microbes persist in the lungs of individuals with cystic fibrosis (CF) and cause inflammatory responses and irreversible lung damage. While most respiratory infections that occur in CF are dominated by bacteria, some are dominated by fungi such as the slow-growing black yeast *Exophiala dermatitidis*. Here, we report that isolates of *E. dermatitidis* cultured from two samples,

collected two years apart, from a single subject. One isolate was sequenced using long-read Nanopore technology to assemble an in-population reference for comparative single nucleotide polymorphism (SNP) and insertion-deletion (INDEL) variant analysis. We then used population genomics and phylogenomics to compare 23 strains to the in-population reference and type strain *E. dermatitidis NIH/UT8656.* Three *E. dermatitidis* clades were detected, each with varying mutation rates. Additionally, all strains are MAT 1-1, which was consistent with the absence of evidence for mating or recombination between isolates. Phylogenetic analysis grouped sets of strains into clades that contained isolates from both early and late timepoints indicating there are multiple persistent lineages had adapted to the host lung environment. Functional assessment of variants unique to each clade identified alleles in genes that encode transporters, cytochrome P450 oxidoreductases, iron acquisition and DNA repair processes. The persistent population heterogeneity identified in lung-derived strains is an important factor to consider in treatment and indicates for further investigation, and the analysis of changes in fungal pathogens over time in chronic infections may provide important insights into the physiology of black yeasts and other slow-growing fungi in vivo.

831T Global evolutionary patterns and drug resistance acquisition in the human pathogen *Aspergillus fumigatus* *Johanna Rhodes*[1], Alireza Abdolrasouli[2,3], Katie Dunne[4], Thomas Sewell[1], Yuyi Shang[1], Eloise Ballard[5], Amelie Brackin[1], Norman van Rhijn[6], Harry Chown[6], Paul Dyer[7], Paul Bowyer[6], Michael Bromley[6], Elizabeth Johnson[8,9], P. Lewis White[10], Adilia Warris[5,9], Richard Barton[11], Silke Schelenz[12], Thomas Rogers[4], Darius Armstrong-James[2], Matthew Fisher[1] 1) MRC Centre for Global Disease Analysis, Imperial College London, London; 2) Department of Infectious Diseases, Imperial College London, London ; 3) Department of Medical Microbiology, King's College University Hospital, London; 4) Department of Clinical Microbiology, Trinity College Dublin, Dublin; 5) Aberdeen Fungal Group, Institute of Medical Sciences, University of Aberdeen; 6) Manchester Fungal Infection Group, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester; 7) School of Life Sciences, University of Nottingham, Nottingham; 8) National Mycology Reference Laboratory, Public Health England, Bristol; 9) MRC Centre for Medical Mycology, University of Exeter, Exeter; 10) Public Health Wales Microbiology, Cardiff; 11) Mycology Reference Centre, Leeds Teaching Hospitals National Health Service Trust, Leeds; 12) Infection Sciences, Kings College University Hospital, London.

Aspergillus fumigatus is a globally ubiquitous environmental mould capable of causing opportunistic lung disease. Invasive aspergillosis (IA) occurs in at-risk populations, such as neutropenic patients, those receiving immunosuppressive therapy or stem cell and solid organs transplants, and CF patients. It is also emerging as an important pathogen as an influenza and COVID-19 associated infection. Here, we use whole-genome sequencing of over one thousand globally sourced isolates sampled over 102 years to solve the spatio-temporal origins of *A. fumigatus*. We describe the polymorphisms associated with drug resistance, including novel drug resistance polymorphisms, and the spatiotemporal origins of the most prominent polymorphism TR_{34}/L98H. Our findings also indicate a two clade structure, with the majority of drug resistance polymorphisms assigned to Clade A; we will present data on crosses aimed at exploring whether there is a reproductive barrier between Clades A and B, and whether these polymorphisms are capable of being transferred onto new genetic backgrounds *via* recombination.

832F Parasexual recombination enables *Aspergillus fumigatus* to persist in cystic fibrosis Tobias Engel[1,2], Paul Verweij[1,2], Joost van den Heuvel[3], Dechen Wangmo[3], Jianhua Zhang[3], Ben Auxier[3], Alfons Debets[3], *Eveline Snelders*[3] 1) Radboud University Medical Center, Nijmegen, the Netherland; 2) Center of Expertise in Mycology Radboudumc/CWZ, Nijmegen, the Netherlands; 3) Laboratory of Genetics, Wageningen University and Research, Wageningen, the Netherlands.

Aspergillus fumigatus is a saprobic fungus that causes a range of pulmonary diseases, some of which are characterised by fungal persistence such as is observed in cystic fibrosis (CF) patients. Creation of genetic variation is critical for *A. fumigatus* to adapt to the lung environment, but biofilm formation, especially in CF patients, may preclude mutational supply in *A. fumigatus* due to its confinement to the hyphal morphotype. We tested our hypothesis that genetic variation is created through parasexual recombination in chronic biofilms by phenotypic and genetic analysis of *A. fumigatus* isolates cultured from different origins. As diploids are the hallmark of parasex, we screened 799 A. fumigatus isolates obtained from patients with CF, chronic pulmonary lung disease and acute invasive aspergillosis, and from the environment for spore size. Benomyl sensitivity, nuclear content measurements through fluorescence-activated cell sorting and scanning electron microscopy were used to confirm the diploid state of large size spores. Whole genome sequencing was used to characterise diploid-associated genetic variation. We identified 11 diploids in isolates recovered from six of 11 (55%) CF patients and from one of 24 (4%) chronic aspergillosis patients, but not in 368 isolates from patients with acute *Aspergillus* infection and the environment. Diploid formation was associated with accumulation of mutations and variable haploid offspring including a voriconazole-resistant isolate. Parasexual recombination allows A. fumigatus to adapt and persist in CF patients, and plays a role in azole resistance development. Our findings are highly significant for understanding the genetics and biology of A. fumigatus in the human lung.

833W Transposon mobilization elevated by heat stress causes genome-wide mutations in the human fungal pathogen *Cryptococcus deneoformans* *Asiya Gusa*[1], Vikas Yadav[1], Cullen Roth[1], Jonathan Williams[1], Eva Mei Shouse[1], Paul Magwene[1], Joseph Heitman[1], Sue Jinks-Robertson[1] 1) Duke University School of Medicine, Durham, NC.

Cryptococcus species cause disease primarily in immunocompromised populations, including a deadly cryptococcal meningitis that contributes to 15% of AIDS-related deaths. Compared to other model yeast species, *Cryptococcus* has the highest concentration of transposable elements (TEs) in the genome (roughly 5-6%), and yet little is known about transposon mobility during ambient growth or during infection. These TEs include both DNA transposons and retrotransposons, and most are concentrated in the centromeres of each chromosome. We recently reported transposon mutagenesis as a significant driver of spontaneous mutations in *Cryptococcus deneoformans* in a murine model of infection. Mutations by TEs in reporter genes for drug resistance were dramatically elevated at high temperature (37° versus 30°) *in vitro*, suggesting that heat stress stimulates TE mobility in the cryptococcal genome. To further explore temperature-dependent TE mutagenesis in the genome and its impact on gene expression, we generated transposon accumulation lines by *in vitro* passage of *C. deneoformans* at 30° and 37°. Through whole genome analysis, utilizing both long-read and short-read sequencing methods, we identified movements of the T1 DNA transposon, the Tcn12 retrotransposon and the Cnl1 non-LTR retrotransposon. Each has a unique mechanism of mobility and apparent preferences for insertion sites in the genome.

T1 insertions occurred at both 30° and 37° and were distributed throughout the genome with a bias for insertion between genes. Tcn12 insertions occurred primarily within gene-coding regions and were found only during growth at the host-relevant temperature of 37°. Cnl1 movements, evident at both temperatures, were localized to the subtelomeric regions of chromosomes, with dramatic amplifications in copy number at elevated temperature. Importantly, movements of all three TEs were detected in the genomes of *C. deneoformans* recovered from infected mice, providing further evidence of a role for transposons in adaptive evolution and enhanced pathogenesis during infection.

834T Plants vs Botrytris: a model for quantitative interactions? *Celine Caseys*[1], Gongjun Shi[1,2], Nicole Soltis[1], Daniel Kliebenstein[1] 1) Department of Plant Sciences, University of California, Davis, Davis, CA 95616, USA; 2) Department of Plant Pathology, North Dakota State University, Fargo, ND 58102, USA.

Botrytis cinerea can cause necrosis and decay on more than a thousand known hosts widely spread across the plant kingdom. How B. cinerea can affect so many plants remains largely unknown. To address this question, we generated a large infectivity matrix by the inoculation of leaves of eight host plants (3 Rosids, 5 Asterids) with 98 isolates. This experiment revealed that the lesion area is quantitative and dependent on the plant resistance, strain virulence and their interactions (by decreasing effect). Furthermore, the disease outcomes cluster among genotypes of a host species but are independent of the relatedness between hosts. To uncover the genetic architecture of Botrytis virulence on various hosts, genome-wide association studies (GWAS) were performed. It revealed that, in this generalist pathogen, hundreds of functionally diverse genes might be involved in adjusting the infection to hosts that evolved various defenses.

835F Human mediated contact between amphibian-killing chytrid variants results in repeated recombination *Thomas Jenkinson*[1], Timothy James[2], Erica Rosenblum[3] 1) California State University, East Bay; 2) University of Michigan; 3) University of California, Berkeley.

Global biodiversity is under threat from introductions of non-native fungal disease. The pathogenic chytrid Batrachochytrium dendrobatidis (Bd) causes chytridiomycosis – the infectious disease implicated in frog, toad, and salamander population declines and extinctions worldwide. Where this fungus has been introduced, a single hypervirulent strain (Bd-GPL) proliferates through host populations. In the southern Atlantic Forest of Brazil, recent human introduction brought the globally invasive Bd-GPL strain into secondary contact with a distantly related, endemic strain, Bd-Brazil. In most anthropogenically mediated secondary contact scenarios such as this one, the epidemiological and evolutionary consequences of strain interaction remain unknown. We show that Bd, long considered obligately asexual, is capable of second-generation introgression following the human induced contact of divergent lineages. Using whole-genome sequencing of fungal isolates cultured from wild-infected Brazilian frogs, we characterize the hereditary relationships among disease populations in this strain invasion zone. Our analyses reveal regions of the Bd genome that are potentially driving adaptive variation among invasive and endemic strains. The patterns of hybrid inheritance we observe offer new insights into the genetic underpinnings of fungal reproductive isolation, the process which ultimately results in speciation of emerging fungal diseases. These new southern Brazil hybrid strains we describe are of particular ecological and evolutionary concern because they demonstrate the ability of anthropogenic change to drive novel recombinant genetic variation in a deadly pathogen. These findings show how humans are actively creating new evolutionary trajectories for emerging diseases, such as chytridiomycosis, by creating novel mating opportunities between previously allopatric strains.

836W Mushrooms without mating: the discovery haploid sporocarps in the invasive habitat of the heterothallic death cap, *Amanita phalloides* *Yen-Wen Wang*[1], Holly Elmore[1], Jacob Golan[1], Anne Pringle[1] 1) University of Wisconsin-Madison, Madison, WI.

Successfully invading plants often reproduce both asexually and sexually, or generate seed via selfing and outcrossing. By analogy, the ability to develop sporocarps without mating (haploid fruiting) may facilitate the colonization of new habitats by invasive fungi. Haploid fruiting in otherwise heterothallic fungi is reported for multiple species in laboratory settings, but haploid mushrooms have not been observed in nature. We sequenced 86 *Amanita phalloides* mushrooms collected from an invasive range (California) and from native ranges (Europe). Unexpectedly, we identified two individuals as genetically haploid. These individuals are homozygous with patterns of k-mer and allele depth frequencies typical of haploids. The mycelia of haploid sporocarps are either binucleate or uninucleate but diploid sporocarps are exclusively binucleate. Surprisingly, kinship analyses suggest these two haploid individuals are regularly mating with other haploid mycelia. The genotype of one of the individuals was collected in both 1993 and 2015: it has persisted in different fungal bodies at Point Reyes National Seashore, CA for at least 20 years. To understand the molecular machinery underpinning mating in these fungi, we next investigated the mating type loci–both the homeodomain (HD) and pheromone receptor (PR) loci–within *A. phalloides* and across the genus *Amanita*. The genetic diversity, and structural and phylogenetic analyses, of these two loci suggest *A. phalloides* is a bipolar heterothallic species. However, the haploid individuals have zero heterozygosity at the HD locus and no additional copies of HD genes are present in their genome. We tentatively hypothesize the haploid fruiting bodies are the results of either self-compatible HD genes or a mechanism bypassing HD control, and we speculate the death cap's reproductive plasticity facilitated its invasion of California.

837T Analysis of mitochondrial diversity in the smut fungus *Sporisorium reilianum f. sp. zeae* reveals potential recombination events that alter predicted patterns of uniparental inheritance. *Hector Mendoza*[1], Emma Lamb[1], Luke Schroeder[1], Sunita Khanal[1], Michael Perlin[1] 1) University of Louisville.

Modern understanding of the concept of genetic diversity must include the study of both nuclear and organellar DNA, which differ greatly in terms of their structure, organization, gene content and distribution. The present study comprises an analysis of genetic diversity for the smut fungus, *Sporisorium reilianum f. sp. zeae* (SRZ), from a mitochondrial perspective. Bioinformatic analysis of whole-genome sequencing data revealed a wide array of single-nucleotide polymorphisms among the mitogenomes for strains of different geographical origins. One of these, present in all strains examined, is predicted to result in truncated *nad6* alleles. Notably, for *cox1*, a mitochondrial gene encoding one of the subunits that make up complex IV of the mitochondrial respirasome, a unique apparent insertion was

detected in the Chinese strains. This unique sequence had high percent identity to the mitogenome of the related species, *S. scitamineum* and *Ustilago bromivora*, hinting at potential recombination events during the evolutionary history of these species. A PCR-based methodology was developed around the *cox1* polymorphism to follow mitochondrial inheritance patterns of SRZ crosses between German and Chinese isolates. These studies were done in the context of examining the molecular function of the SRZ orthologues of the *Ustilago maydis* Lga2/Rga2 system, shown in that organism to favor a2 mating partner mitotype inheritance. Teliospores were collected from test crosses used in pathogenicity assays and subjected to mitotype screening. Surprisingly, the Chinese mitotype was predominant in the offspring populations generated, regardless of the origin of the a2 mitotype partner involved in a mating event. These results suggest potential recombination events between mtDNA molecules, with outcomes that deviate from predicted patterns of uniparental inheritance of mitochondria in the context of the Lga2/Rga2 molecular mechanism.

838F Extensive parasexual recombination promotes genetic diversity in *Candida albicans* progeny Robert Fillinger[1], Abhishek Mishra[1], Anna Mackey[1], Scott Filler[2,3], Richard Bennett[4], *Matthew Anderson*[1] 1) The Ohio State University; 2) Lindquist Institute for Biomedical Innovation at Harbor-UCLA-Medical Center; 3) David Geffen School of Medicine at UCLA; 4) Brown University.

Meiosis is a hallmark of eukaryotes that promotes the generation of genetically diverse offspring through processes of segregation and independent assortment. However, many fungal species lack evidence of meiosis, including those among the medically relevant CUG paraphyletic group. The most clinically relevant of these, *Candida albicans*, is able to undergo an alternative mating program termed parasex that includes fusion of cells of opposing mating types and an uncoordinated process of ploidy reduction. Use of genetically identical backgrounds has hampered previous attempts to quantify the degree of genetic diversity produced through this alternate mating program. Here, we induced mating between two pairs of diploid *C. albicans* lineages that were distinct from each other and analyzed the outcomes of parasex. Ploidy reduction in the tetraploid mating product through concerted chromosome loss overwhelmingly produced highly aneuploid progeny, ranging from near tetraploid to less than diploid. Genotyping of parasexual progeny revealed distinct biases in each mating, one cross produced primarily heterozygous offspring whereas the other cross resulted in progeny dominated by alleles from a single parent. Surprisingly, recombination was more frequent in parasexual progeny than is commonly observed in meiosis of other ascomycetes. Whole genome sequencing of a subset of parasexual progeny identified progeny genomes that contained ~500 recombination events on average. Sites of recombination were typically depleted of repetitive sequences and suggests that independent methods of recombination are acting in parasex and mitosis. Progeny diversity was linked to genotypic diversity among parasexual progeny and provides a roadmap for applying quantitative genetics to this and other parasexual species.

839W *In-silico* cross-contamination affects inference of genetic relationships in *Saccharomyces cerevisiae* *Audrey Ward*[1], Eduardo Scopel[2], Brent Shuman[3], Michelle Momany[3], Douda Bensasson[1,2,3] 1) Department of Genetics, University of Georgia, Athens, GA; 2) Institute of Bioinformatics, University of Georgia, Athens, GA; 3) Department of Plant Biology, University of Georgia, Athens, GA.

Population genetic analysis depends on the quality of whole genome sequences. Contamination of sequence data may occur *in vitro* prior to sequencing or *in silico* during multiplex sequencing as a result of cross-contamination or barcoding issues. Testing for interspecies contamination is common practice. In contrast, identification and prevention of within-species contamination is more difficult. To test the effects of contamination on genome analyses, we contaminated short read genome data of *Saccharomyces cerevisiae in silico* with genome data from another *S. cerevisiae* strain. We repeated the contamination experiment using strains with varying degrees of relatedness and ploidies, in addition to varying level of cross-contamination along a range from 0% to 50%. Using a standard base calling pipeline, we found that cross-contaminated genomes appeared to produce good quality genome-wide data. Past studies estimated relationships among *S. cerevisiae* lineages using maximum likelihood trees inferred from whole-genome data after excluding strains showing recent admixture. We similarly estimated trees with that include single simulated cross-contaminated genomes to assess if within-species contamination affects the inference of their genetic relationships. We found that between 5% and 10% contamination is enough to significantly change tree topologies, making contaminated strains look like hybrids in maximum likelihood trees. These results suggest that even low levels of contamination significantly change trees and may lead to misunderstanding of evolutionary relationships within species.

840T Genomic and genetic analyses of antifungal drug resistance in *Aspergillus fumigatus* Yuying Fan[1], Yue Wang[1], Greg Korfanty[1], Meagan Archer[1], *Jianping Xu*[1] 1) McMaster University.

Aspergillus fumigatus is an airborne opportunistic human fungal pathogen and a major cause of aspergillosis. Aspergillosis is commonly treated using triazole antifungals and amphotericin B (AMB). However, there is increasing prevalence of drug-resistant strains and associated with it, an increasing number of studies reporting specific mutations in these strains. Here, based on the genome and antifungal drug susceptibility information of over 200 strains, we used multiple stepwise genome-wide association analyses to investigate the influences of these known mutations on antifungal resistance as well as to identify novel mutations that impact drug susceptibility. Our analyses confirmed several known mutations, rejected others, and revealed many novel mutations related to triazole and AMB resistance. A subset of the mutations showing putative associations with AMB susceptibilities was further investigated using a laboratory genetic cross, which allowed us to reveal that SNP-SNP interactions contributed to quantitative growth differences among progeny at different AMB concentrations. Together, our results demonstrated the power of combining population genomics and laboratory crosses for providing novel quantitative insights into drug resistance in *A. fumigatus*.

841F Lineage structure of the *Fusarium oxysporum* Species Complex (FOSC) based on a dataset of 41 full-length genes from the core genomes of 545 isolates: its implications in taxonomy and diagnostics *David Geiser*[1], Seogchan Kang[1], María del Mar Jiménez-Gasco[1], Frank Martin[2], Ningxiao Li[2] 1) Penn State University; 2) USDA-ARS, Salinas, CA.

The *F. oxysporum* Species Complex (FOSC), encompassing the taxa *F. oxysporum* and *F. foetens*, represents one of the most important groups of fungal plant pathogens, causing economically devastating vascular wilt and other diseases on over 100 hosts. In addition, some isolates can be endophytic, exist as saprobes, and/or cause serious infections in humans and animals. Despite its importance, *F.*

oxysporum has remained taxonomically unsettled, and the non-monophyletic nature of most of its host associations and lack of appropriate genomic markers have confounded diagnostics. We identified 41 protein-coding genes that are presumably orthologous and map to core genome regions and extracted their full-length DNA sequences from genome assemblies of 535 diverse *F. oxysporum* isolates generated in this and previously published studies. Sequences were aligned with *F. commune, F. foetens* and *F. hostae* as outgroup taxa, producing an alignment totaling 69,434 sites, clone-corrected to 302 unique ingroup taxa, and subjected to partitioned maximum likelihood phylogenetic analysis using the IQ-TREE software package. The resulting phylogenetic structure resolved nodes with strong (>95%) bootstrap support that correspond to lineages identified in previously published work, including both phylogenomic studies and analyses utilizing 3-4 portions of protein-coding genes. However, most of these lineages were resolved in only a minority of individual gene trees, consistent with high levels of incomplete lineage sorting and recent common ancestry. Some taxa resolved phylogenetically in previous studies based on a small number of markers were unresolved within broader lineages in this larger dataset, while others corresponded well. Important patterns of diversity within host-specific FOSC pathogens (*formae speciales*) and endophytic isolates from the same host are informative, indicating the utility of a phylogenomic perspective in studying the evolution of these pathogens and developing novel diagnostic tools. The dataset presented in this study provides a framework for genome-based identification of FOSC isolates and is adaptable to an amplicon-based approach to study populations. The implications of these findings in comparison to other studies is discussed, in particular whether and to what extent lineages within FOSC are reasonably delimited as species in an evolutionary context.

842W Genetic diversity of the pea root pathogen *Aphanomyces euteiches* in Europe *Carol Kälin*[1], Anna Berlin[1], Agnese Brantestam[2], Mukesh Dubey[1], Anna-Kerstin Arvidsson[2], Paul Riesinger[3], Malin Elfstrand[1], Magnus Karlsson[1] 1) Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences SLU, Uppsala, Sweden; 2) Nomad Foods, Findus Sverige AB, Bjuv, Sweden; 3) Novia University of Applied Sciences, Ekenäs, Finland .

The oomycete *Aphanomyces euteiches* is a plant pathogen causing root rot in its broad host range of various legume species. In this study we are focusing on the green pea-infecting *A. euteiches* which is responsible for severe yield losses in areas of green pea (*Pisum sativum* L.) production. *A. euteiches* is homothallic and can reproduce both sexually and asexually. It forms oospores that persist in the soil for long periods of time. This further complicates conventional measures against the disease, leaving long periods of crop rotation and avoidance of highly infested fields as the most successful measures to reduce yield losses. This study aims at understanding the genetic diversity of *A. euteiches* in Europe, covering a north-to-south gradient spanning from Sweden, Norway and Finland to the United Kingdom, France, and Italy. We obtained a collection of 85 European *A. euteiches* strains, isolated from infected pea roots from green pea production sites. The isolates were genotyped using microsatellite markers and analysed based on their genetic diversity. We used 22 codominant markers and found a total of 67 multilocus genotypes (MLGs). Remarkably, strains from Italy have shown to cluster in a genetically distant group with no shared ancestry with other populations, while the rest of the isolates seem to be more closely related. A subset of ten *A. euteiches* strains from four countries (Finland, Italy, Sweden and France) was further phenotyped on the two susceptible pea lines Lumina and Linnea, as well as on line MN313 with partial resistance towards *A. euteiches*. All strains have proven to be pathogenic on all pea lines but with varying levels of disease severity although with low correlation with genetic diversity. Our results contribute to a better understanding of the genetic diversity of Europen *A. euteiches* populations and serve as a base for future studies of whole-genome sequence analyses investigating the genetic basis for virulence.

843T The *Parastagonospora nodorum* necrotrophic effector SnTox3 likely evolved from a duplication event of *SnTox5* *Gayan Kariyawasam*[1], Nathan Wyatt[2], Zhaohui Liu[1], Eva Stukenbrock[3], Justin Faris[2], Timothy Friesen[2] 1) North Dakota State University, Fargo, ND, USA; 2) Edward T. Schafer Agricultural Research Center, USDA-ARS, Fargo, ND, USA; 3) Max Planck Institute for Evolutionary Biology, August-Thienemann, Plön, Germany.

Parastagonospora nodorum is a destructive foliar pathogen of wheat that causes septoria nodorum blotch. The fungus follows an inverse gene-for-gene model and releases multiple necrotrophic effectors that elicit programmed cell death (PCD) in the presence of the corresponding host susceptibility genes. Five genes, *SnToxA*, *SnTox1*, *SnTox267*, *SnTox3*, and *SnTox5* that encode five distinct necrotrophic effectors have been cloned and functionally characterized. *SnTox5* is the latest gene to be cloned and it encoded a protein with a signal peptide and a pro-sequence. The mature SnTox5 was 16.26 kDa in size and showed 45.13% sequence homology and 98% structural similarity to SnTox3. Functional characterization using confocal microscopy showed that SnTox5 facilitated the colonization of leaf mesophyll tissue and may suppress the host defense response prior to the induction of PCD, suggesting functional similarity to SnTox3. Therefore, we hypothesize that *SnTox3* was originated by a duplication of *SnTox5*. To test this, we evaluated the genomic regions surrounding *SnTox3* and *SnTox5*. *SnTox5* showed 63.13% homology to *SnTox3* at the nucleotide level. Furthermore, repeat annotation showed a TcMar-Fot1, a DNA transposable element 748 bp upstream of *SnTox5*. BlastN analysis of the TcMar-Fot1 element showed 44.25 % homology to a region 532 bp upstream of *SnTox3*. Furthermore, eight repeat induced polymorphisms (RIP) were identified in the TcMar-Fot1 homolog upstream of *SnTox3*. The unexpected similarity of the TE indicates a possible mechanism of gene duplication. To further investigate the direction of the duplication event we evaluated whole genome sequences of 387 *P. nodorum* isolates acquired from different wheat growing regions of the world. The presence/absence of *SnTox5* and *SnTox3* was initially evaluated, and it was shown that 48 isolates lacked both genes, 179 isolates harbored both genes, and 133 isolates had only *SnTox5*, whereas only 27 isolates had only *SnTox3*. Haplotype analysis showed that *SnTox5* had 27 haplotypes that encoded for 21 protein isoforms, whereas *SnTox3* had only 12 haplotypes that encoded for only four protein isoforms. Therefore, high prevalence and haplotype diversity of *SnTox5* relative to *SnTox3* indicate that *SnTox3* likely evolved through a duplication of *SnTox5*. Therefore, it is possible that both SnTox3 and SnTox5 target similar vulnerabilities of the host to induce PCD in the presence of *Snn3* and *Snn5*, respectively.

844F Genome-wide association studies for the genetic basis of saprophytic fitness traits in samples of isolates of *Fusarium graminearum* from the Americas *Christopher Toomajian*[1], Upasana Dhakal[1], John Leslie[1] 1) Department of Plant Pathology, Kansas State University, Manhattan, KS.

In the US, *Fusarium graminearum* (*Fg*) is the major causal agent of Fusarium head blight on wheat. The direct yield loss caused by this pathogen is compounded by its ability to contaminate the infected grains with mycotoxins. Our broader aims include identifying genes

harboring functional variation that contributes to variation in critical saprophytic and pathogenic fitness traits within *Fg* populations to provide targets for pathogen management and control, and toxin reduction. Specific goals include performing genome-wide association studies (GWAS) to find the genetic basis of these pathogen traits by scanning tag single nucleotide polymorphisms (SNPs) throughout the *Fg* genome generated by genotyping-by-sequencing (GBS). We have genotyped nearly 600 *Fg* isolates from several geographical regions in the Americas, including the Upper Midwest, New York, and Louisiana in the US as well as Uruguay. The sample includes isolates from the 3-ADON, 15-ADON, NX-2, and NIV trichothecene chemotypes, and is highly structured into four populations. To make collecting trait data feasible in lab experiments as well as more laborious greenhouse head-inoculation experiments, we have focused on subsets of ~150 of our isolates. In the subsets, we have attempted to preserve the diversity of the larger sample and avoid choosing genetically similar isolates. So far, isolates have been phenotyped for the saprophytic traits of ascospore discharge rate and mycelial growth rate at three temperatures. We screened for statistical associations between the traits and SNPs, implementing a mixed linear model that accounts for the strong population structure of *Fg* populations as well as cryptic relationships between isolates from the same populations. We performed imputation to infer the allele state where SNPs were missing genotype data, then filtered the SNPs to exclude low frequency variants for which power to detect a robust association is low. This left us with ~6,000 SNPs for the GWAS scans. Due to the correction for strong population structure in our diverse sample, the significance of our top associations remains moderate. For ascospore discharge, we identify a handful of strong candidate loci where the model predicts about half will be true positives. Subsequent attempts at mapping via GWAS will involve collecting trait data from primarily a single genetic population to avoid the reduction in power we have seen that results from correction for strong population structure.

845W Using machine learning to gain insight on how environment and diet influence the evolution of galactose metabolism across the budding yeast subphylum *Marie-Claire Harrison*[1], Abigail LaBella[1], Dana Opulente[2], Chris Hittinger[3], Antonis Rokas[1] 1) Vanderbilt University; 2) Villanova University; 3) University of Wisconsin-Madison.

The metabolisms, isolation environments, and genomes of all ~1,200 known species of budding yeasts are now characterized by the Y1000+ project, led by the Hittinger & Rokas labs. The dataset's broad evolutionary scope, coupled with its inclusion of ecological data and of quantitative and qualitative growth rate data on dozens of substrates for nearly all ~1,200 species, makes it uniquely powerful for understanding the evolution of metabolic pathways. Galactose is a monosaccharide that is abundant in nature and is found in many forms: for example, in lactose, in glycoproteins and glycolipids, or in raffinose and melibiose, common polysaccharides in grains and other plants. Even though genes from the *GAL* pathway are present in most species, there is substantial variation in the strength of growth on galactose across budding yeasts, suggesting that the *GAL* pathway varies in its genomic structure, function, and regulation across the subphylum. In my presentation, I will show that growth on galactose can be predicted with a high degree of accuracy from either qualitative and quantitative growth data on diverse substrates or from data on the environments where species are found as input, using a supervised machine learning approach and data from nearly all known budding yeast species. These findings suggest that both isolation environment and diet influence the evolution of galactose metabolism in budding yeasts, and that there is substantial variation that is yet to be studied in the *GAL* pathway. More broadly, my results raise the hypothesis that ecology is a reliable predictor of metabolic specialization in microbial eukaryotes.

846T The role of DNA methylation in the evolution of two truffle-forming ectomycorrhizal sister species *Emeline E. Pano*[1], Ana E. Chavez[1], Alija B. Mujic[1] 1) California State University Fresno.

DNA methylation plays an important role in regulating plant and mammalian development. In Fungi, epigenetic modification of DNA and histones remains under-investigated and its contribution to gene regulation and development across fungal phyla and ecological life histories is not well understood. A study conducted in the model plant-pathogenic fungus, *Magnaporthe oryzae*, demonstrated modifications in DNA methylation during vegetative, reproductive, and asexual states. This work suggested that accumulation of epigenetic modifications in and around genes regulates fungal development and maintains genomes structure by serving as a genomic defense mechanism against transposons. Prior studies of whole-genome DNA methylation profiles in Fungi mostly have focused on single species of Ascomycota and Basidiomycota, rather than between species comparisons . Here, we compare the methylomes of the ectomycorrhizal (ECM) sister species, *Rhizopogon vinicolor* and *Rhizopogon vesiculosus* (Basidiomycota:Boletales), as well as between population methylome differentiation in *R. vinicolor* across its range in the Western US. *Rhizopogon vinicolor* and *Rhizopogon vesiculosus* are truffle-forming Fungi that diverged approximately 23 MYA based on phylogenetic studies and share a high degree of genome synteny at the scale of 0.5 megabases. These species possess largely sympatric distributions and maintain an obligate ECM relationship with a single tree host, *Pseudotsuga menziesii* (Douglas fir). Interestingly, these taxa can coexist temporally on the same host tree due to vertical niche partitioning and differing host-root colonization strategies. In studying these two species, we seek a broader understanding of how epigenetic modification affects fungal macro- and micro-evolutionary processes. Analysis of the genome and methylome variation in these species allows for the identification of regions that have accumulated epigenetic modification to suggest gene regions of stasis among these sister species. The genome, methylome, and transcriptome data of these two *Rhizopogon* species provides valuable information for advancing our understanding of the evolution of genus *Rhizopogon* and Basidiomycota fungi in general.

847F Signaling Pathway Loss-of-Function Alleles and Evolutionary Hotspots in the Fungi *Paul Magwene*[1], Katherine Dura[1] 1) Duke University, Durham, NC.

A modest number of evolutionarily conserved signal transduction pathways are primary regulators of stress responses and morphogenetic processes across the fungal tree of life. Examples of these pathways include Ras-cAMP-PKA signaling, calcineurin signaling, TOR signaling, and a variety of MAPK signaling pathways. Because of the central role that these signaling networks play in regulating cellular physiology and differentiation, they have been intensively studied, both as tractable models for understanding the principles of signaling and as potential targets for strain improvement or antifungal drug design. From an evolutionary perspective, these pathways are expected to be under relatively strong stabilizing selection, as the phenotypic consequences of mutations in these pathways are pleiotropic and loss-of-function mutations (LoF) in these pathways typically lead to reduced growth rates and increased sensitivity to environmental stresses. We describe comparative population genomic analyses of signaling pathway LoF alleles for three model yeast

genera, *Saccharomyces*, *Candida*, and *Cryptococcus.* We show that several pathways exhibit unusually high frequencies of naturally occurring putative LoF alleles and that specific genes in these pathways seem to be particularly tolerant to such mutations. We discuss the implications of this finding for the evolutionary lability of signaling pathways in the fungi, and combine information on loss-of-function alleles with related evidence from QTL mapping and experimental evolution studies to identify pathways that may act as «evolutionary hotspots» for adaptation to novel environments.

848W Characterisation of the mating type loci in *Elsinoe* including the *Eucalyptus* pathogen *Elsinoe necatrix* Brenda Wingfield[1], *Mike Wingfield*[1], Nam Pham[2], Irene Barnes[1], Alvero Duran[3] 1) Forestry & Agricultural Biotechnology Institute, Department of Biochemistry, Genetics & Microbiology, University of Pretoria; 2) Department of Plant and Soil Sciences, Forestry and Agricultural Biotechnology Institute, University of Pretoria; 3) Research and Development, Asia Pacific Resources International Holdings Ltd. (APRIL), Pangkalan Kerinci 28300, Riau, Indonesia.

The genus*Elsinoe* includesnumerous aggressive plant pathogens that infect various economically important agricultural, horticultural and forestry plants. Important diseases include citrus scab (*Elsinoe fawcettii*and *Elsinoe australis*), grapevine spot anthracnose (*Elsinoe ampelina*) and the recently described *Elsinoe necatrix*that causes a devastating Eucalyptus scab and shoot malformation disease in North Sumatra, Indonesia. Given their importance as plant pathogens, it is surprising that relatively little is known regarding the biology of these fungi. To better understand the reproductive biology of *Elsinoe*spp., we characterized the structure of the mating type loci and flanking genes using whole genome sequences for six species. Results showed that the organization of the MAT locus and flanking genes is relatively conserved in most cases. Each *Elsinoe* MAT idiomorph was defined by either the *MAT1-1-1*or the *MAT1-2-1*gene. Interestingly, a unique MAT1-1idiomorph containing a truncated *MAT1-2-1*gene, and a *MAT1-1-1*gene, was identified in the genomes of *E. necatrix*and *E. fawcettii*. In addition, two idiomorph-specific hypothetical proteins were found only in the MAT1-1 and MAT1-2 idiomorphs of *E. australis*. All seven species were shown to have a typical heterothallic mating system characterized by the presence of a single idiomorph in each isolate, and this is likely true for the genus. Universal mating type markers were developed and tested on a collection of 20 different *Elsinoe*spp., which will facilitate future studies regarding the biology of these fungi.

849T Population genomics analysis of *Fusarium graminearum* isolates from the Americas *Upasana Dhakal*[1], John Leslie[1], Chris Toomajian[1] 1) Kansas State University.

Fusarium head blight (FHB) is one of the major diseases of wheat and is caused by different species in the genus *Fusarium*. In the wheat-producing areas of the United States, *Fusarium graminearum* (*Fg*) is the major causal agent. The direct yield loss caused by this pathogen is compounded by its ability to contaminate the infected grains with mycotoxins. We conducted population genomics analyses using SNPs obtained through genotyping by sequencing of 454 isolates of *Fg* from the upper Midwest, New York, Louisiana, and Uruguay. PCA and structure analyses group these isolates into four previously described populations. More than 40 isolates were assigned to each of the NA1, NA2, and Southern Louisiana (SLA) populations, while only 19 isolates were assigned to the Gulf Coast population, which was excluded from subsequent analyses. Population structure is imperfectly correlated with chemotype. Differentiation between the three populations is fairly high (F_{ST} 0.385 - 0.551), yet several admixed isolates indicate the populations are not reproductively isolated. Patterns of linkage disequilibrium (LD) decay suggest frequent recombination within populations. LD decays to half of the highest value within 7 kb and reaches background levels within 300 kb for all three populations. Demographic modeling suggests simultaneous splitting of the NA1, NA2, and Southern Louisiana (SLA) populations from the ancestral population and moderate levels of subsequent gene flow between them. Genome-wide selection scans in all three populations revealed signatures of positive selection, often more pronounced towards the chromosomal ends. Only a very small proportion of regions were shared among the three populations. Distinct patterns of selection in each population suggest unique host-pathogen interactions and environmental adaption. *Fg* populations from the US have great evolutionary potential given the high recombination rate and a large proportion of admixed isolates. Knowledge of population structure and diversity is critical to making management decisions. Genomic regions providing a selective advantage to the fungus offer new targets for disease management.

850F Computational advances in the discovery of a new class of fungal natural products *Grant Nickles*[1], Milton Drott[1], Nancy Keller[1] 1) Medical Microbiology and Immunology, University of Wisconsin-Madison; Madison, WI, USA.

Fungal secondary metabolites (SMs) are major sources of antimicrobial (e.g. penicillin, griseofulvin) and therapeutic (e.g. cyclosporine, mycophenolate) compounds. Ecologically, they provide important fitness adaptations that are finely tailored to the niche of an organism. The fungal biosynthetic genes responsible for SM synthesis and transportation are uniquely arranged in contiguous clusters within the genome, termed biosynthetic gene clusters (BGCs). Current genome mining algorithms capable of identifying putative BGCs are limited to what is considered 'canonical' (BGCs defined by biochemically characterized synthetases and synthases, i.e. nonribosomal peptide synthetases or polyketide synthases). SMs synthesized by BGCs lacking canonical structure are difficult to be incorporated into current predictive software (i.e. AntiSmash) and thus preclude informative analysis such as extensive phylogenetic studies. One such example of a noncanonical BGC class blind to existing genome mining software is the isocyanide (N⁻□C⁺) metabolite producing BGCs. Isocyanides have been a major interest of organic and synthetic chemists since the 1920s due to their unique divalent carbon, and high reactivity. While numerous bioactive isocyanide metabolites have been extracted from bacteria and fungi, the genes responsible for their synthesis were largely unknown prior to our laboratory publishing the first examples of isocyanide synthase (ICS) containing BGCs in the fungus *A. fumigatus*. We have developed paradigm-shifting software and a computational approach that allows detection of diverse ICS BGCs across the fungal kingdom, and offer initial predictions of functionality in specific ecological settings.

851W Genome-scale phylogeny of the fungal order Sordariales *Noah Lisa Hensen*[1], Lucas Bonometti[2], Markus Hiltunen[1], Anna Lipzen[5], Chris Daum[5], Fabien Burki[1], Francis Martin[3], Igor Grigoriev[5], Jasmyn Pangilinan[5], Philippe Silar[4], Robert Riley[5], Vivian Ng[5], Pierre Gladieux[2], Hanna Johannesson[1] 1) Uppsala University, UU, Uppsala, Sweden ; 2) PHIM Plant Health Institute, Univ Montpellier, INRAE, CIRAD, Institut Agro, IRD, Montpellier, France; 3) Université de Lorraine, INRAE, UMR Interactions Arbres/Microorganismes, Centre INRAE Grand Est-Nancy, Champenoux, 54 280, France; 4) UMR8236 Laboratoire Interdisciplinaire des Energies de Demain, Université de Paris, France; 5) Joint Genome Institute, Lawrence Berkeley National Laboratory, UC Berkeley,

USA.

Mutations are the main source of genetic variation, both within and between organisms in a population. As a result, knowledge about the rate of mutations and about the factors determining mutation rate in nature are central to our understanding of numerous processes, including evolution and ecological niche divergence. Mutation rate is expected to vary with organismal and ecological traits, but as of yet, the mutational processes and factors driving mutation rate variation are poorly understood.
The order Sordariales (Ascomycotina) is taxonomically diverse, with closely related species inhabiting a wide variety of natural habitats. The order therefore provides a unique opportunity to study the connection between mutation rates and life history traits (such as longevity), as well as between mutation rates and ecological traits (such as thermophili). In order to study these connections, a robust framework of phylogenetic relationships within the order is essential.
Previous molecular phylogenetic analysis of Sordariales has relied on a few genes-many taxa approach. In our project, we use whole genome data from 106 genomes publicly available and/or sequenced as part of JGI Community science Programs (proposals 504394 and 662/300789). This dataset includes representatives from different families within the order, creating a more representative source of genomic data across the order than previously available.
We compiled a genome-wide phylogenomic data matrix using the BUSCO genes present in the majority of the 106 species. Analysis of the data matrix using concatenation- and coalescence-based approaches is used to infer a robust genome-wide phylogenetic framework of the order. This phylogeny is used as a basis for the comparisons of genomic properties and inference of the direction of evolutionary change amongst Sordariales fungi. The created phylogeny will furthermore be used as a basis for ongoing studies on the influence of species-specific traits on the rate of molecular evolution. Additionally, the phylogeny will be helpful for researchers studying other biochemical, ecological, genetic and evolutionary questions in this group.

852T Analysis of 439 Cyp51 protein sequences shows 5 major Cyp51 gene family groups across Fungi *Brandi Celia*[1], Michelle Momany[1], Marin Brewer[1] 1) University of Georgia, Athens, GA USA.

Azole drugs target fungal sterol biosynthesis and are used to treat millions of human fungal infections each year. Resistance to azole drugs has emerged in multiple fungal pathogens including *Candida albicans*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Aspergillus fumigatus*. The most well-studied resistance in *A. fumigatus* arises from missense mutations in the coding sequence combined with a tandem repeat in the promoter of Cyp51A, a cytochrome P450 enzyme in the fungal sterol biosynthesis pathway. Filamentous Ascomycetes such as *A. fumigatus* have three paralogs of Cyp51 (Cyp51A, Cyp51B, and Cyp51C) with most previous research focused on Cyp51A due to its role in pan-azole resistance. We used the *A. fumigatus* Cyp51A protein sequence as the query in database searches to identify Cyp51 proteins across Fungi. We found 439 Cyp51 proteins in 297 species spanning from early-diverging fungi (Zygomycetes) to late-diverging fungi (filamentous Ascomycetes). We found these sequences formed 5 major Cyp51 groups: Cyp51, Cyp51 Erg11, Cyp51A, Cyp51B, and Cyp51C. Surprisingly, we found all filamentous Ascomycetes had a Cyp51B paralog, while only 50% of species had a Cyp51A paralog.

853F Comparative genomics and population structure of South African *Histoplasma* isolates *Rutendo Eugenia Mapengo*[1,2], Tsidiso G Maphanga[1], Nelesh P Govender[1,2,3,4] 1) Centre for Healthcare-Associated infections. Antimicrobial Resistance and Mycoses, National Institute for Communicable Diseases, a Division of the National Health Laboratory Service, Johannesburg, South Africa; 2) Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; 3) Division of Medical Microbiology, Faculty of Health Sciences, Cape Town, University of Cape Town, ; 4) Medical Research Council Centre for Medical Mycology, College of Medicine and Health, University of Exeter, Exeter, United Kingdom.

Background: Histoplasmosis is a mycosis caused by pathogenic fungi in the genus *Histoplasma*. While *Histoplasma capsulatum* has been proposed to be sub-divided into cryptic species based on analyses of whole genome sequencing (WGS) data, these designations are not currently considered taxonomically valid and no clinical differences have been observed among cases. We performed a comparative genome-wide phylogenetic analysis of South African *Histoplasma* isolates.
Materials and methods: We included six *Histoplasma capsulatum* isolates from patients collected through passive laboratory-based surveillance from 2011 to 2021 and one veterinary *Histoplasma farciminosum* isolate. Isolates were retrieved from -70^{0}C storage and species identity was confirmed by phenotypic methods followed by Sanger sequencing. WGS was then performed on all confirmed *Histoplasma* isolates using the Illumina NextSeq platform. We included publicly-available *H. capsulatum* and *Blastomyces spp* sequence reads for comparison. Quality checks were performed and raw reads were assembled using the *de novo* method following the Unmanned Genome Assembly Pipeline. For gene annotation, we used the *H. capsulatum* WU24 strain as a reference. To study the genealogical relationships among *H. capsulatum* isolates, we built a maximum likelihood (ML) phylogenetic tree.
Results: Of the 45 sequences (30 *Histoplasma* publicly available, 9 *Blastomyces,* six SA clinical *H. capsulatum*, one veterinary *H. farciminosum*), four *Histoplasma* clusters were observed corresponding to the Panama, North American 1 (Nam 1), Nam 2 and Latin American A (Lam A) groups. The four clinical *H. capsulatum* strains were most closely related to the Panama group, while one clinical isolate did not cluster with any of the groups. The *H. farciminosum* isolates closely related to the Lam A strains. Four clinical *Histoplasma* isolates clustered in a single group despite the decade-long period over which they were collected and varying provincial origins of patients. The genome sizes of the isolates ranged from 32Mbp to 35Mbp. Within group SNPs were between 17000 and 45 000 for South African strains.
Conclusions: *Histoplasma capsulatum* (Panama group) was the main cause of histoplasmosis among patients from different geographic areas of South Africa. More isolates from across Africa (including *Histoplasma duboisii*) should be included in an analysis for a better understanding of the evolution and relatedness of *Histoplasma* species.

854W Understanding the nature of the reproductive barriers within the wood decay species *Trichaptum abietinum* *Dabao Sun Lu*[1], David Peris[1], Jørn Henrik Sønstebø[2], Sundy Maurice[1], Håvard Kauserud[1], Mark Ravinet[3], Inger Skrede[1] 1) Department of biosciences, University of Oslo, Norway, Oslo Norway; 2) Department of Natural Sciences and Environmental Health, University of South-Eastern Norway, Bø Norway; 3) School of Life Sciences, University of Nottingham, Nottingham UK.

In basidiomycetes, an extreme diversity of alleles at the mating loci promote outcrossing within a species, but the genetic mechanisms

maintaining reproductive barriers between species and how these barriers arise are largely unknown. Reproductive barriers within a single morphospecies have been reported for numerous fungi, and in several wood decay fungi these have been shown to be stronger between sympatric lineages. In the wood decay fungus *Trichaptum abietinum,* crossing experiments from the 1960´s revealed two sympatric intersterility groups in North America. The monokaryons from these two groups are unable to mate with each other and form dikaryons, but both groups are in part able to mate with European isolates. In this study, we use population genomic analyses together with *in vitro* crosses to investigate the genetic basis and infer the evolutionary origin of the pre-mating barriers in *T. abietinum*. To this end, we have whole genome sequenced 350 *T. abietinum* samples from Asia, Europe and North America. Our phylogeographic analyses show four major lineages, one in Asia and one in Europe, and two in North America corresponding to the known intersterility groups. Coalescence analyses identify one of the North American lineages as early diverging, whereas the other lineages coalesce more recently, but the splits among the lineages are hard to date and link to biogeographic events due to unknown generation time of wood decay fungi. Our *in vitro* crosses reveal additional partial intersterility groups between sub-lineages within Asia which also differ in their ability to mate with the other major lineages in Europe and North America. Since reproductive isolation in *T. abietinum* is not correlated with overall genomic divergence and appears in sympatry and parapatry, we propose that reinforcement could have been involved in the development of these barriers in North America and Asia. We use demographic modelling to test if secondary contact is more likely to have occurred between lineages where reproductive barriers exist. The complex and nested pattern of incompatibility between lineages indicate that it may be governed by several loci, and we use genome scans and association studies to identify regions correlated with the ability to mate across lineages.

855T Synonymous codon usage as a lens into the metabolic ecology of budding yeasts *Abigail LaBella*[1], Dana Opulente[2], Marie-Claire Harrison[1], Chris T. Hittinger[3], Antonis Rokas[1] 1) Vanderbilt University, Nashville, TN; 2) Villanova University, Villanova, PA; 3) University of Wisconsin-Madison, Madison, WI.

Despite their purportedly “silent” role in evolution, synonymous codon changes are frequently subject to selection due to their role in transcriptional and translational regulation. Translational selection on codon usage occurs when highly expressed genes undergo selection for codon usage that matches the tRNA pool, known as optimal codon usage, resulting in fast and accurate translation. Our recent work in 327 species of budding yeasts detected translationally associated selection on codon usage in 81% of species and subsequently found that optimal codon usage in the *GAL* pathway was positively correlated with quantitative growth on galactose. Moreover, *GAL* optimal codon usage was positively correlated with human-associated ecological niches in yeasts of the CUG-Ser1 clade and with dairy-associated ecological niches in the family Saccharomycetaceae. In my presentation, I will describe the results of my analysis of the genomes of all ~1,200 known budding yeast species. This work further explores how codon metrics can reveal associations between multiple metabolic pathways, associations between metabolism and ecology, and be used to predict growth phenotypes or ecology. To date, our results highlight codon optimization as a tool for gaining insights into the metabolic ecology of microbial eukaryotes. This may be illuminating for studying fungal dark matter—species that have yet to be cultured in the lab or have only been identified by genomic material.

856F The story behind the strains: using genomes to define wild yeast lineages from woodlands *Jacqueline Peña*[1], Eduardo Scopel[1], Douda Bensasson[1] 1) University of Gerogia.

Recent work shows that human pathogenic yeast species can live on trees in woodlands. This raises questions about the ecology and evolution of yeast in this habitat. We used Saccharomyces cerevisiae as a model to understand the phylogeography of a human-associated yeast species when it lives wild in natural woodlands. There is enough information (2,000+ whole-genomes publicly available) to determine how S. cerevisiae strains from woodlands are distinct from human associated strains. By specifically focusing on S. cerevisiae strains from woodlands, we can understand past transitions between natural and human-associated habitats. The objectives are to (1) define wild S. cerevisiae lineages isolated from trees in woodlands and (2) estimate the timing of S. cerevisiae migration events. We focused on S. cerevisiae strains isolated from trees in woodlands because woodlands tend to be undisturbed and remote from human activity. We found that S. cerevisiae strains isolated from woodlands have several diverse lineages that are structured by geography and that these lineages are phylogenetically distinct from human-associated lineages. For instance, S. cerevisiae strains isolated from oak trees in Japan and North America form several distinct wild lineages and there is gene flow between these lineages. Japanese and North American lineages are additionally similar suggesting that S. cerevisiae may have been transported into the Americas by humans thousands of years ago. This suggests that wild S. cerevisiae usually live independently of humans, but occasionally humans have affected their distribution even in natural habitats.

857W Contrasting continental patterns of adaptive population divergence in a holarctic ectomycorrhizal fungus *Keaton Tremble*[1,2], Joe Hoffman[3], Bryn Dentinger[1,2] 1) University of Utah, Salt Lake City, Utah; 2) Natural History Museum of Utah, Salt Lake City, Utah; 3) Bielefeld University, Bielefeld, Germany.

Understanding the population processes and genetic mechanisms that give rise to new species remains one of the most elusive goals of modern evolutionary biology. In the hyperdiverse and ecologically important Fungi, the process of speciation is virtually unknown, including for the more than 20,000 species of obligate ectomycorrhizal mutualists that play essential roles in ecosystem function. We investigated patterns of genome-wide differentiation in the ectomycorrhizal porcini mushroom *Boletus edulis,* a globally distributed species complex with broad ecological amplitude. By whole genome sequencing 160 individuals from across the Northern Hemisphere, we identified 792,923 SNPs and used these to elucidate the demographic and adaptive processes shaping global population differentiation. We show that *B. edulis* exhibits deeply contrasting patterns of genomic divergence between continents, with multiple lineages being present across North America, while a single lineage dominates Europe over a vast geographic scale. These geographical lineages are inferred to have diverged between 2.66 and 1.62 million years ago, corresponding to a period of climatic upheaval and the onset of glaciation during the Pliocene-Pleistocene boundary. High levels of genomic differentiation were observed among lineages despite evidence of substantial and ongoing introgression. Furthermore, genome scans, demographic inference and ecological niche models all suggest that genomic differentiation is maintained by environmental adaptation and not physical isolation. Our study uncovers striking differences on a truly global scale and emphasizes the importance of local adaptation and ecologically

mediated divergence, rather than prezygotic barriers such as allopatry or genomic incompatibility in population differentiation in Fungi.

858T Molecular Characterisation of *Candida auris* Clinical Isolates at a Large Tertiary Academic Hospital in South Africa, 2016-2020 *Dikeledi Kekana*[1,2], Serisha Naicker[1,2], Liliwe Shuping[1], Sithembiso Velaphi[3], Firdose Nakwa[3], Jeannette Wadula[4,5], Nelesh Govender[1,2,6,7] 1) National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa; 2) School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; 3) Department of Paediatrics and Child Health, Chris Hani Baragwanath Academic hospital, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; 4) Department of Clinical Microbiology and Infectious Diseases, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; 5) National Health Laboratory Service, Microbiology Laboratory, Chris Hani Baragwanath Academic Hospital, Johannesburg, South Africa; 6) Division of Medical Microbiology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa; 7) Medical Research Council Centre for Medical Mycology, College of Medicine and Health, University of Exeter, Exeter, United Kingdom.

Background: Candida auris is a multidrug-resistant healthcare-associated fungal pathogen comprising of five geographical clades and was first detected in South Africa in 2009. We describe the molecular epidemiology of C. auris, using whole-genome sequencing (WGS), over 4 years at a large tertiary academic hospital in South Africa with a focus on the hospital's neonatal unit which had a large outbreak during this period.
Methods: Cases of culture-confirmed C. auris infection or colonisation were identified through laboratory surveillance across the entire hospital from March 2016 through July 2020 and viable isolates submitted to a reference laboratory. Antifungal susceptibility testing was performed using commercial broth microdilution and gradient diffusion methods. Molecular analysis was based on the WGS data of isolates, the quality of read data was assessed and bioinformatics analysis was performed.
Results: Of 287 cases, 207 (72%) had viable isolates and 188 non-contaminated isolates were available for testing. Cases across the hospital had a median age of 1.4 years (interquartile range: 22 days – 21 years), with a majority from the neonatal unit (74/207, 36%). Most isolates belonged to clade III (63%, 118/188) or clade IV (37%, 70/188). All 181 fluconazole-resistant isolates (minimum inhibitory concentration ≥32 μg/mL) had a clade-specific mutation on the ERG11 gene, a target gene for Fluconazole. Additionally, 5 fluconazole-susceptible isolates also carried a mutation on the ERG11 gene. All clade III isolates had the VF125AL ERG11 mutation while isolates in clade IV (68/70, 97%) had K177R/N335S/E343D mutations. We used a Bayesian molecular clock phylogeny and dated the emergence time for the most recent common ancestor (TMRCA) for clade III in this hospital to early 2014 and in the neonatal unit to 2018. Phylodynamic analysis showed multiple introductions of C. auris into the neonatal unit.
Conclusion: Clades III and IV co-circulated in the hospital, with clade III causing all but one case in the neonatal unit. Most isolates contained previously-described clade-specific mutations related to azole resistance. The estimated emergence of the TMRCA for the hospital and neonatal unit clade III isolates was roughly consistent with the first cases reported. The large neonatal unit outbreak may have originated from cross-unit transmission by infected/colonised patients, colonised healthcare workers or contaminated equipment.

859V Southwestern US-associated population of *Cryptococcus* isolates fills gaps in the family tree of molecular type VG-VI *Juan Monroy-Nieto*[1], Jolene Bowers[1], Parker Mortfort[1], Gillermo Adame[2], Brian Mochon[3], Shane Brady[2], Wieland Meyer[4], Ken Komatsu[2], David M. Engelthaler[1] 1) Translational Genomics Research Institute, Flagstaff, AZ.; 2) Arizona Department of Health Services, Phoenix, Arizona; 3) University of Sydney, Sydney, Australia; 4) Banner Health, Phoenix, Arizona.

Our understanding of the population structure of the pathogenic fungus *Cryptococcus gattii* and its sibling species *C. neoformans* has advanced rapidly with the advent of whole-genome sequencing and increased sampling. Genomic evidence has enabled the discrimination of subtypes of previously described major molecular type groupings and novel *C. gattii* clades and lineages. Recently a genome assembly from a single isolate, collected in Mexico in 1962, was determined to be genetically distant from any known population was published and dubbed "VG-VI".
We recently sequenced four clinical isolates and one veterinary isolate collected during 2019-2021 in the Southwest US and Argentina. Phylogenetic analysis groups the genomes closely with, but distal to, the original VG-VI isolate genome. Mean intraclade distances are ~11.5 times shorter than distances to the most closely related major molecular type (VG-III), establishing this clade as reciprocally monophyletic and distinct to other major molecular types. These recent VG-VI genomes have approximately one hundred thousand high-confidence SNPs among them, displaying a deep population structure indicative of rich natural history.
The initial epidemiological information associated with the southwestern U.S. isolates suggests the VG-VI cases were locally acquired infections, which poses several questions about adaptations of VG-VI to a warm, arid environment. Additional public VG-VI genomes WM.1802 and WM.1804, which had been misclassified initially as VG-III, were collected in Mexico possibly more than 30 years ago, reinforcing the association of this clade with the geographical region. More research is required to shed light on the origin, distribution, micro-habitat, and clinical phenotype of this novel branch of the *Cryptococcus* species tree. These results are a step forward in recognizing this pathogen›s genetic diversity and ecological range and provide evidence of a previously unrecognized endemic geographic area that will be important to public health and clinical medicine.

860V Global introduction patterns of the pine ectomycorrhizal fungus *Suillus luteus* *Yi-Hong Ke*[1], Rytas Vilgalys[1] 1) Duke University, Durham, NC.

Global afforestation efforts have been accompanied by the widespread co-introduction of many ectomycorrhizal fungi (EMF) well beyond their original native ranges. One of the most successful EMF colonizing exotic pine forests is the European slippery jack *Suillus luteus*. As a model for studying fungal natural history and mycorrhizal symbiosis, the widespread, highly replicated, and recent (<150 yrs) global introduction of *S. luteus* with pines to novel and isolated geographic regions provide an excellent opportunity to test basic hypotheses about fungal population genomics as well as identifying its parental population and sources of introduction. We investigated the *S. luteus* global introduction patterns by analyzing whole genomes from 187 *S. luteus* fruiting bodies and cultures obtained from Asia, North America, South America, New Zealand, Australia and Europe. Phylogenomic analysis and admixture analysis suggest that the introduced populations including one in North America are derived from Central Europe. Northern Europe, Asia and native Northern America populations belong to separate lineages. The introduced population have smaller effective population sizes, but the magnitude

of order is the same. To solve the detailed divergent history of Australia-New Zealand clade, demographic models with independent and sequential introduction were compared. The sequential introduction model from New Zealand to Australia was better supported than independent introduction or the opposite order of introduction. Demographic modeling also revealed all introduced populations have ongoing migration or multiple waves of introduction. In total 102 to 157 selective sweeps were detected in each introduced population through composite likelihood ratio of site frequency spectra. Among the detected selective sweeps, 18 of them are consistently present in all three introduced populations, suggesting convergent local adaptations in the introduced population.

861V Population Structure and Genomic Analysis of *Aspergillus sojae* and *Aspergillus parasiticus* *Kimberly Acevedo*[1], John Gibbons[1] 1) University of Massachusetts at Amherst.

Domestication is the genetic modification of a species by breeding it in isolation from its ancestral population in an effort to enhance its utility to humans, involving different evolutionary mechanisms such as selections, bottlenecks, and gene flow. Most domestication research focuses on the plant and animal kingdoms, leaving microorganisms understudied. Here, we phylogenetically distinguish *Aspergillus sojae* and *Aspergillus parasiticus* as two distinct populations, based on their genomic differences. Among isolates collected by the USDA, a single domestication event of *A. sojae*, followed by a clonal expansion, was observed. *A. sojae*, a putative domestic filamentous fungus which combines flavor-enhancing and enzymatic proteins to ferment several Asian foods, such as: soy sauce, miso, and mirin. *A. parasiticus,* a close relative, is a wild fungus that produces aflatoxins, making it pathogenic to plants and animals. Using whole-genome assemblies of 12 *A. sojae*, and nine *A. parasiticus* isolates, 658,329 SNPs were identified using *Freebayes1.3.5.* Using principal component analysis, and linkage disequilibrium less nucleotide diversity and recombination was observed in the *A. sojae* isolates as compared to the *A. parasiticus* population. Structural variation using copy number variation (CNV) analysis was done to assess presence or absence of secondary metabolite genes amongst the *A. sojae* and *A. parasiticus* isolates. CNV profiles were generated for each sample to investigate the quantity and function of these genes in *A. parasiticus.* A total of 12,370 genes were annotated, of these, 8% represented the coding sequence for secondary metabolites, as predicted by BLASTp. Further analysis of these genes with differing copy numbers showed variance stabilizing transformation measurements greater than 0.5. Of these, 20.6% were for secondary metabolites. High-impact mutations, defined as having significant protein structural changes, were identified using SnpEff. In summary, we predict that the genetic basis for domestication lies in the presence or absence of secondary metabolite genes, differing across populations.

862V Early divergent lineages of Ascomycota: A new hope *David Diaz Escandon*[1], Phillip Resl[2], Toby Spribille[1] 1) University of Alberta, Alberta, Canada; 2) University of Graz, Austria.

Whole-genome sequencing has proven to be reliable and stable, to resolve large scale evolutionary patterns. However, several early divergent lineages in Ascomycota have never been included in genomic studies, due to their rarity or unculturabilty. Here, we explore the relationships of several orphans and early diverging lineages in Ascomycota, using over two dozen new metagenome-derived fungal genomes, together with hundreds genomes covering all available genera in Ascomycota. We stress-tested topologies by using multiple approaches to assess internode certainties, and we used genome annotations to prove the reliability of the recovered relationships based on shared genomic profiles. The resulting topology provides new insights into the evolution of major classes in Ascomycota, injecting new data on the lifestyle and genome repertoire transitions of over half of all named ascomycete fungi.

863V Comparative genomics of *Aspergillus oryzae* genomes from different clades reveals signatures of artificial selection in primary and secondary metabolism in domesticate environments *Katherine Chacon-Vargas*[1], John Gibbons[1] 1) University of Massachusetts Amherst.

Humans domesticated different species by selecting for desired traits to enhance their benefits. Domestication is not limited to plants and animals. In parallel, microbes (bacteria, yeasts, and molds) were also domesticated for their roles in food preservation, nutrition and flavors. *Aspergillus oryzae* is a domesticated filamentous fungal species used during the fermentation of traditional Asian foods and beverages such as sake, soy sauce, and miso. The artisanal practice of continuous passage of *A. oryzae* on food substrates over thousands of years resulted in adaptation to the food environment along with genetic differentiation from its wild relative *A. flavus*, a toxin producing agricultural pest. Here, we analyzed 300 isolates of *A. oryzae* and *A. flavus* to understand how the history of domestication and how this process shaped patterns of genomic variation. Using population structure and phylogenetic analysis we identified 2 major population of A. oryzae and two major lineages of *A. flavus*. Next, we used two population genomic metrics to identify regions of the *A. oryzae* genome possessing signatures of artificial selection. We identified 30 candidate genes possessing strong signatures of artificial selection, several of which have functional annotations directly related to fermentation. Specifically, alcohol dehydrogenase, fructose transmembrane transporters and glutathione metabolism genes. Additionally, we examined differences in gene copy number variation between *A. oryzae* and *A. flavus*. Most strikingly, we found significantly more copies of the α-amylase encoding genes in *A. oryzae* compared to *A. flavus*, suggesting selection for increased carbohydrate metabolism during fermentation. Further, gene absences in *A. oryzae* compared to *A. flavus* were enriched for secondary metabolism function, suggesting selection for loss of toxicity in *A. oryzae*. Taken together, our results show the *A. oryzae* genome was significantly reshaped as a result of domestication

864V A global pangenome analysis of tan spot (*Pyrenophora tritici-repentis*) reveals an open genome and virulence factors nested in mobile elements Ryan Gourlie[1], Megan McDonald[2], Mohamed Hafez[1], Rodrigo Ortega-Polo[1], Kristin Low[1], Wade Abbott[1], Stephen Strelkov[3], *Reem Aboukhaddour*[1] 1) Agriculture and Agri-food Canada, Cereal Pathology, Lethbridge, Alberta, Canada; 2) University of Birmingham, School of Biosciences, Edgbaston, Birmingham, United Kingdom; 3) University of Alberta, Faculty of Agricultural, Life, and Environmental Sciences, Edmonton, Alberta, Canada.

Pyrenophora tritici-repentis (Ptr) is one of the most destructive wheat pathogens in the world; its genome is a mosaic of the presence and absence of effectors, and hence Ptr can serve as a model for examining the evolutionary process behind acquisition of virulence in necrotrophs to explain new disease emergence. In this work, we took advantage of a diverse collection of 41 Ptr pathogenic isolates from different global origins and applied the short and long read sequencing technologies to dissect the Ptr genome. Ptr exhibited an open-pangenome with 43% of genes in the core set, 57% of its predicted genes defined as accessory. A clear distinction between

pathogenic and non-pathogenic genomes was observed in size, gene content, and phylogenetic relationships. Ptr genome exhibited major chromosomal rearrangements, including chromosomal fusion, translocation, and segment duplications. An intraspecies translocation of ToxA, the necrosis-inducing effector coding gene, was confirmed, as ToxA and a 143 Kb crypton, was localized on two different chromosomes in the Ptr species. Additionally, ToxB, the gene encoding the chlorosis inducing effector, was clustered as three copies on a 294 Kb transposable element in the coding isolate. ToxB and its carrying transposon were missing from the ToxB non-coding reference isolate, but the homolog toxb and the transposon were present in another non-coding isolate. The Ptr genome also exhibited a 'one-compartment' organization and but may still possess a 'two-speed genome' facilitated by copy-number variation as reported in other fungal pathosystems. This study provides the most comprehensive insights into Ptr genome and highlights the structural organization of Ptr genome as an open pangenome with 'one-compartment'.

865V Analysis of pathogenicity genes in Batrachochytrium dendrobatidis Pangenome *Mark Yacoub*[1], Jason Stajich[1] 1) UC Riverside.

Batrachochytrium dendrobatidis (*Bd*) is a fungal pathogen that causes chytridiomycosis disease in amphibians. The pathogen is globally distributed and comprises distinct evolutionary and geographic clades; GPL, BRAZIL, CAPE, and ASIA. The GPL clade is panzootic while the others are considered enzootic. Efforts to understand *Bd*'s pathogenicity have focused on presence absence variation (PAV) of putative pathogenicity genes between *Bd* and its saprophytic relatives, yet less is known about the variation of these genes within the *Bd* pangenome. In this study, we assembled and annotated 343 *Bd* genomes from public Illumina sequencing projects to study PAV of pathogenicity genes among *Bd* strains. We used profile HMM searches to evaluate copy number of pathogenicity genes across the pangenome and constructed phylogenetic trees for each gene family in the *Bd* pangenome. Additionally, we used synteny analyses to confirm orthology of PAV for each pathogenicity gene. During our analyses, five putative pathogenicity gene families; S41 Protease, M36 Metalloprotease, Aspartic Protease (ASP), Crinkler Necrosis Genes (CRN), and proteins containing one or more Chitin Binding Module-18 (CBM18) domains came to our attention, as we found these genes to be expanded in copy number in *Bd* compared to saprophytic relatives. Of the five pathogenicity genes S41, M36, and CBM18 were hyper-diverse with regards to copy number, phylogenetic diversity, and conservation of loci. Our results suggest there are both conserved and variable copies for each pathogenicity gene; however, we did not observe the variation to correlate to *Bd* strain phylogeny. This analysis is the first to employ a holistic approach towards studying presence/absence of pathogenicity genes in the *Bd* pangenome and can provide more insight into the functional diversity of strains that builds on previous studies which focused on SNP and short INDEL variation.

866V The evolutionary analysis of *hac* BGC suggests the composite regulatory complex and the broad distribution of harzianic acid among metabolites of plant-associated ascomycetes *Guan Pang*[1], Feng Cai[1,2], Irina Druzhinina[1] 1) Fungal Genomics Laboratory (FungiG), Nanjing Agricultural University, Nanjing, China; 2) School of Ecology, Sun Yat-sen University, Shenzhen, China.

Fungi produce a great variety of bioactive compounds, many of which play essential roles in their biotic interactions. The biosynthetic pathway of the harzianic acid (HA), an agrochemically-relevant antifungal, plant growth promoting, and iron-binding secondary metabolite of *Trichoderma* spp. (Hypocreales, Ascomycota) belonging to the *Harzianum* clade, was recently reconstituted in the prominent biocontrol strain of *T. afroharzianum*. HA is proposed as a specific inhibitor of acetohydroxyacid synthase (AHAS), which is the first enzyme in the branched-chain amino acid biosynthetic pathway. This function further supports HA and the *Harzianum* clade of *Trichoderma* as promising targets for biopesticide and bioeffector development. Here, we studied the distribution and evolution of *hac* - the biosynthetic gene cluster (BGC) required for the HA production. We revealed that *hac* BGC was present in *T. asperellum*, a few plant-associated hypocrealean fungi, and in a number of unrelated fungi such as members of Eurotyomycetes (*Aspergillus ibericus, Penicillium digitatum*) and Leotiomycetes classes. Surprisingly, we identified that in most fungi *hac* BGC was putatively regulated by the two none-paralogous pathway-specific Zn_2Cys_6 transcription factors, HACF and HACI, respectively. The functional genetic investigation revealed that in *T. guizhouense* (the *Harzianum* clade), the overexpression of HACF was not sufficient for the trigger of the *hac* BGC, while the activation of HACI resulted in the production of HA. Furthermore, the mutant constitutively overproducing HA due to the yet unidentified genomic alteration(s) showed a superior antifungal activity by means of water-soluble metabolites secreted to the medium, including the ability to inhibit other *Trichoderma* spp. that have no *hac* BGC in their genomes. In this presentation, we will report the detailed results of the composite evolutionary history and regulation of *hac* BGC. Together our results expand the understanding of the role of HA in combative interactions between fungi and contribute to the explanations of the superior environmental opportunistic nature of *Trichoderma* spp. from the *Harzianum* clade and their high applied potential.

867V Independent Expansion of the Hyr/Iff-like (Hil) Adhesin Family in C. auris and other Candida Yeast Pathogens *Bin He*[1], Rachel Smoak[1], Lindsey Snyder[1], Jan Fassler[1] 1) University of Iowa.

Candida spp. are a paraphyletic group of yeasts, many of which are opportunistic human pathogens. Emergence of novel Candida pathogens, such as the multidrug resistant *C. auris*, poses a threat to health and raises an evolutionary question about the genomic changes that allowed for certain species to be successful in the host. Fungal adhesins are established virulence factors in Candida spp., contributing critically to biofilm formation and cell adherence. Here we characterize the sequence, structure and evolution of a putative adhesin family - the Hyr/Iff-like (Hil) family - in *C. auris*, whose members share the N-terminal Hyphal_reg_CWP (PF11765) domain. Structural prediction shows the PF11765 domain adopts a β-solenoid fold similar to a group of bacterial adhesins known as Serine-Rich-Repeat Proteins. The central domains of the Hil family proteins are Ser/Thr-rich and contain tandem repeats as well as β-aggregation prone sequences, all characteristic of fungal adhesins. Phylogenetically, the Hil family expanded independently in two pathogenic clades that include *C. auris* and *C. albicans*. Even though the N-terminal domain and the overall architecture are conserved among the Hil family proteins, the central domain of these proteins diverged extremely rapidly, driven mostly by type and copy number variation in their tandem repeats, resulting in differences in protein length and β-aggregation potential. Our results suggest that the same genetic material, i.e. the PF11765 domain, may have been used repeatedly in the evolution of distinct pathogenic Candida spp. The ensuing rapid diversification of their central domains may have then allowed for phenotypic diversification. Lastly, we identified a list of putative adhesin genes in *C. auris* that share the sequence properties of the Hil family but don't contain the PF11765 domain. This

provides a valuable resource for future experimental investigation of adhesion in *C. auris*.

868V A Spot of Bother, transcontinental genetic diversity of *Pyrenophora teres f. maculata*. *Kealan Hassett*[1], Simon Ellwood[1], Jordi Muria Gonzalez[1] 1) Curtin University.

Pyrenophora teres f. *maculata* (*Ptm*), the causal agent of spot form net blotch of barley, is an economically important disease worldwide. Current knowledge of the pathogen's genetic diversity and population structure is critical for better understanding the evolutionary capacity of the disease and the development of sustainable management strategies. We compiled a collection of 360 *Ptm* isolates from diverse geographic origins, with a particular focus in Australia. Using DArTseq SNP markers we analysed the population structure and genetic diversity of isolates internationally and within Australia. Analysis revealed *Ptm* is genotypically diverse and displays little population structure between Australian states, or between Western Australian agroecological zones, fields or host varieties. However, globally, population structure is evident between different countries. Interestingly, cryptic populations were found in Western Australia, unrelated to the general population in the broader Australia, potentially indicating the introduction of genotypes from overseas due to their strong genetic alignment to another transcontinental population.

869V Ancient introgression between highly divergent fungal sister species *Vilde Bruhn Kinneberg*[1], Dabao Sun Lü[1], David Peris Navarro[1], Mark Ravinet[2], Inger Skrede[1] 1) University of Oslo, Oslo, Norway; 2) University of Nottingham, Nottingham, UK.

The evolutionary past of lineages can be intricate despite well-resolved phylogenies. When speciation occurs on a continuous scale, reproductive barriers can remain incomplete and give way to introgression. If species have diverged over large time spans, signs of introgression can get blurred by recombination and genetic drift, leaving only small traces of admixture in the genomes. The kingdom of Fungi originated over a billion years ago, and it might contain many species exhibiting signs of ancient introgression. In this study, we investigated introgression between two fungal sister species, *Trichaptum abietinum* and *T. fuscoviolaceum.* The species constitute monophyletic taxa but are morphologically and ecologically similar, with overlapping habitats. We investigated the possibility of introgression between these species by conducting whole genome sequencing of individuals from populations in North America and Europe. We applied divergence analyses (F_{ST} and d_{XY}) to assess the genome wide nucleotide differences between the species, and ABBA-BABA analyses (*D* and *f* statistics) to investigate introgression. This study is one of few conducting such analyses to examine introgression among mushroom-forming fungi. Crossing experiments were also performed to assess reproductive barriers between the species. The results reveal *T. abietinum* and *T. fuscoviolaceum* to be highly divergent sister species with genome wide high F_{ST} and d_{XY} values. The crossing experiments further show the species incompatible in vitro. Despite the large genetic differences and incompatibility, small regions of introgression are scattered throughout the genomes. Ghost populations (both unsampled extant and extinct populations) may be involved in the introgression. Moreover, the introgression is most likely ancient. This study demonstrates that ancient introgression can be found among mushroom-forming fungi, but the implications of gene transfer across species and possible retention of introgressed genes from extinct lineages remain unknown.

870V Dynamics of *Verticillium dahliae* race 1 population under managed agricultural ecosystems Jie-Yin Chen[1], Dan-Dan Zhang[1], Jin-Qun Huang[1], Ran Li[1], Dan Wang[1], Jian Song[1], Krishna Puri[2], Lin Yang[1], Zhi-Qiang Kong[1], Bang-Zhuo Tong[1], Jun-Jiao Li[1], Yu-Shan Huang[1], Ivan Simko[3], *Steven Klosterman*[3], Xiao-Feng Dai[1], Krishna Subbarao[2] 1) State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China; 2) University of California, Davis, CA; 3) United States Department of Agriculture, Agricultural Research Service, Crop Improvement and Protection Research Unit, Salinas, CA.

Plant pathogens and their hosts undergo adaptive changes in managed agricultural ecosystems by overcoming host resistance, but the underlying genetic adaptations are difficult to determine in natural settings. *Verticillium dahliae* is a fungal pathogen that causes Verticillium wilt on many economically important crops including lettuce. We assessed the dynamics of changes in the *V. dahliae* genome under selection in a long-term field experiment. A field was fumigated before *Verticillium dahliae* race 1 strain (VdLs.16) was introduced. A derivative 145-strain population was collected over a 6-year period from this field in which a segregating population of lettuce derived from *Vr1/vr1* parents were evaluated. We de novo sequenced the parental genome of VdLs.16 strain and resequenced the derivative strains to analyze the genetic variations that accumulate over time in the field cropped with lettuce. Population genomics analyses identified 2769 single-nucleotide polymorphisms (SNPs) and 750 insertion/deletions (In-Dels) in the 145 isolates compared with the parental genome. Sequence divergence was identified in the coding sequence regions of 378 genes and in the putative promoter regions of 604 genes. Five-hundred and nine SNPs/In-Dels were identified as fixed. The SNPs and In-Dels were significantly enriched in the transposon-rich, gene-sparse regions, and in those genes with functional roles in signaling and transcriptional regulation. Under the managed ecosystem continuously cropped to lettuce, the local adaptation of *V. dahliae* evolves at a whole genome scale to accumulate SNPs/In-Dels nonrandomly in hypervariable regions.

871V Molecular determinants of host adaptation in a recombining population of *Pyricularia oryzae* infecting rice *Marie Leys*[1], Florian Charriat[1], Sonia Guillou[1], Joëlle Milazzo[2], Henri Adreit[2], Sandrine Cros-Arteil[1], Didier Tharreau[2], Thomas Kroj[1], Pierre Gladieux[1] 1) PHIM Plant Health Institute, INRAE, CIRAD, Institut Agro, IRD, Université de Montpellier, Montpellier, France; 2) PHIM Plant Health Institute, CIRAD, INRAE, Institut Agro, IRD, Université de Montpellier, Montpellier, France.

Pyricularia oryzae is a multihost fungal pathogen causing blast disease in rice and other grasses [1]. This fungus is a textbook example of a rapidly evolving pathogen, globally threatening food security through changes in host range and adaptation to new resistant cultivars. Understanding the molecular evolution of virulence genes is therefore critical to design efficient and sustainable disease management strategies. To identify virulence genes involved in adaptation of *P. oryzae* to new rice varieties, we performed genome-wide association studies (GWAS) of a highly polymorphic population inoculated on 16 isogenic rice varieties. We focused on a rice-infecting population of *P. oryzae* from Yunnan (China), where the pathogen has been characterized to reproduce sexually[2]. Illumina reads from 78 isolates from Yule (Yunnan) aligned to the Guy11 reference genome resulted in 4.9 Million SNPs for a genome size of 42.8 Mb. Population genomic analyses of the Yule population indicated a swift linkage disequilibrium decay reaching values below 0.2 within 15 kb, and no population subdivision. These results confirmed the extent of genetic recombination in the Yule population of *P. oryzae*, allow-

ing us to investigate genotype-phenotype associations by GWAS. The quantitative pathogenicity of 64 isolates was measured in the greenhouse on 16 isogenic varieties of rice, which differed from each other by introgression of different major resistance genes. Image analysis of infected leaves revealed an average Diseased Leaf Area of 18.6% and an average number of 20.3 lesions per cm². Genome-wide association mapping will allow us to identify new candidate genes involved in the adaptation of *P. oryzae* to major resistance traits introgressed in rice varieties.
[1] Gladieux *et al.* (2018). *mBio* 9: e01219-17
[2] Saleh *et al.* (2012). *Molecular Ecology* 21: 1330-1344.

872V An exploration into the differences in noncoding regions between *Aspergillus fumigatus* and close relatives and how these differences may influence virulence. *Alec Brown*[1], Matthew Mead[1], Antonis Rokas[1], Gustavo Goldman[2] 1) Vanderbilt University; 2) Universidade de São Paulo.

Several species in the genus *Aspergillus* can cause invasive aspergillosis (IA) in humans, an infectious disease that is often fatal in immunocompromised individuals. With a morbidity rate of >200,000 cases per year, the mortality rate of those inflicted with IA is 50-95%. Mortality rates are even higher for patients that have also undergone an organ transplant or cancer treatment. Of the >300 species in the genus *Aspergillus*, *Aspergillus fumigatus* is responsible for > 90% of all reported IA cases. Comparative genomic analysis between *A. fumigatus* and closely related species revealed that most of the genes in *A. fumigatus* are conserved amongst nonpathogenic close relatives. For example, nearly all genes known to be genetic determinants of virulence in *A. fumigatus* are also found in closely related nonpathogenic species. These results suggest that **the pathogenicity of *A. fumigatus* may not be solely due to differences in the composition of genetic determinants of virulence relative to its nonpathogenic relatives; rather, *A. fumigatus* pathogenicity may be better explained as the result of differences in the regulation of genetic determinants of virulence**. To begin addressing this, we performed the first (genome-wide) study on noncoding regions differences between 2 *A. fumigatus* strains (the lesser virulent Af932 strain and the more virulent A1163 strain) and 8 close relatives. We found abundant genetic variation in noncoding regions *between A. fumigatus* strains and closely related *Aspergillus* species. We identified a set of conserved noncoding regions associated with single copy orthologous genes across the phylogeny. We also identified 418 noncoding regions which exhibited a different evolution rate between *A. fumigatus* and closely related species, including noncoding regions associated with genetic determinants of virulence. Moreover, differences in the sequences of known transcription factor binding locations between *A. fumigatus* and close relatives were also determined. One of these transcription factors, CrzA, is a well-studied genetic determinant of virulence, involved in the hypoxia and drug response; and required for *A. fumigatus* virulence while being present in close relatives. These results indicate potential differences in gene regulation/expression in *A. fumigatus* compared to close relatives due to differences in CrzA binding sites in noncoding regions of known CrzA binding targets. Additionally, we found that strains of *A. fumigatus* (Af293 and A1163) differ in their transcriptomic profiles in respective CrzA knockout strains and that there exists variation in noncoding regions between these *A. fumigatus* strains. Taken together, differences in noncoding regions provides a largely unexplored framework which can help us better understand *A. fumigatus* virulence.

873T The formation of a fuzzy negative arm protein complex is important for clock robustness in *Neurospora crassa* *Meaghan Jankowski*[1], Daniel Griffith[2], Divya Shastry[1], Jaqueline Pelham[1], Garrett Ginell[2], Joshua Thomas[1], Pankaj Karande[1], Alex Holehouse[2], Jennifer Hurley[1] 1) Rensselaer Polytechnic Institute, Troy, NY; 2) Washington University School of Medicine, St. Louis, MO.

Many important biological processes are influenced by the molecular clock, the components of which form a negative feedback loop comprised of proteins that are inherently flexible or disordered. The lack of a fixed three-dimensional structure means that identifying interaction regions on core clock proteins is difficult using typical biophysical methods. Given that disordered proteins often interact through short linear binding motifs (SLiMs), it is feasible to use a divide-and-conquer approach. We co-opted a synthetic biology method to take the primary sequence of a disordered clock protein in *Neurospora crassa* called FREQUENCY (FRQ, NCU02265) and divided it into a set of short overlapping peptides, synthesized, then printed them in a microarray format. After challenging the FRQ-microarray with an overexpressed interactor, FRQ-interacting RNA Helicase (FRH, NCU03363), we were able to investigate FRH binding behavior to different regions of FRQ and identify SLiMs within FRQ's sequence. Rationally designed peptides were then used to investigate the specificity of candidate SLiMs through scrambles, point mutations, and truncations. All together we have termed this novel application of peptide microarray technology to a highly disordered protein as **l**inear m**o**tif dis**c**overy using r**at**ional d**e**sign (LOCATE). We gained insight into the importance of electrostatics for this dynamic and "fuzzy" complex, as conserved positive electrostatic "islands" within FRQ's sequence were consistently bound by the mainly negatively surface-charged FRH. We were also able to replicate FRH binding to FRQ-based peptides containing the genetically identified FRH-binding domain as validation of our method. However, we demonstrated the true FRH/FRQ SLiM depended on "hotspot" residues outside the genetically-identified region, establishing the importance of biochemical analysis of clock protein interactions. Targeted *in vivo* mutations of this SLiM hotspot led to the unexpected discovery that, though it was thought that FRH was essential for feedback, FRQ can close the negative feedback loop without FRH, while FRH is necessary for clock robustness. Overall, we found that LOCATE is a new high-resolution yet high-throughput method for identifying SLiMs within difficult-to-study clock proteins that will allow researchers to dissect clock protein interactions and learn more about the molecular basis of circadian timekeeping and output regulation.

874F Program the Future with Ginkgo's Cell Development Kit Sneha Srikrishnan[1], Swami Srinivas[1], Istvan Weyda[1], Earl Kang[1], Ming-Yueh Wu[1], Sam Jactel[1], Kathryn Loving[1], Deeya Burman[1], Sushmita Venkatraman[1], Yifei Li[1], Peter Punt[1], Joia Ramchandani[1], Patrick Boyle[1], *Jesse Dill*[1] 1) Ginkgo Bioworks, Boston, Massachusetts, United States.

The most important thing to program this century isn't computers. It's DNA.

Ginkgo Bioworks is building a cell programming platform to make biology easier to engineer with *Pichia* and *Aspergillus* driven protein production platforms. The **Cell Development Kit (CDK)** provides modular access to our extensive cell programming capabilities, expertise, and scale. With target protein or enzyme sequence as primary input, Ginkgo›s CDK provides data on validated key metrics including: process, rate, titer and proteomics using the Ginkgo Design-Build-Test-Ferment and Learn cycle.

Design: *We generate multiple unique designs with high potential for expression.*
Build: *We synthesize and assemble designs in a validated host*
Test: *We use high-throughput screening to evaluate strain performance at lab scale*
Ferment: *We validate the highest-performing strains in a scalable fermentation mode*

The Cell Development Kit is a first step to de-risking future investments in protein production. Ginkgo's CDK unlocks the potential of biology with a unique modular combination of our codebase, automation, software, computational capabilities, and experts in the field.

875W CRISPR/Cas9-based engineering of *Aspergillus oryzae* mycelium for meat-like flavor and appearance *Vayu Maini Rekdal*[1,2], Jay Keasling[1,2,3,4,5] 1) Department of Bioengineering, University of California, Berkeley, CA; 2) Joint BioEnergy Institute, Emeryville, CA; 3) Biological Systems and Engineering, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, United States; 4) Department of Chemical & Biomolecular Engineering, University of California, Berkeley, CA, 94720, United States; 5) Novo Nordisk Foundation Center for Biosustainability, Technical University Denmark, DK 2970 Horsholm, Denmark.

Filamentous fungi are predicted to play key roles in a more sustainable food system, including the transition from resource-intensive animal agriculture towards sustainable meat alternatives. Although their nutritional value and filamentous textures make fungi promising meat replacements, most products based on fungal biomass require processing and additives to mimic meat in flavor, texture, and appearance. Genetic engineering could remove the need for processing fungal biomass and has the potential to both decrease cost and increase the meat-like properties of fungal foods. Here we develop a CRISPR/Cas9-based method to genetically engineer *Aspergillus oryzae*, an edible fungus that is involved in traditional fermentations and is currently used in alternative meat products available on the market. The CRISPR/Cas9 method involves protoplast transformation with Cas9-sgRNA Ribonucleotide (RNP) complexes and linear fixing templates, followed by selection and locus-specific loop-out of a *pyrG* marker. The method is precise and efficient, does not necessitate the use of a *ku70* mutant, and allows for unlimited repeatable engineering. In addition to validating the method for both targeted mutagenesis and protein overexpression, we identify 8 neutral chromosomal integration loci that enable high expression of proteins of interest. Finally, we apply this method to the production of heme, a key flavor compound in red meat. By overexpressing both heme biosynthetic enzymes and heme-binding hemoglobins, we engineered a strain that has deeply red mycelia and produces heme at more than 10-fold higher levels than the wild-type. Moving forward, the method will be used to overexpress additional flavor pathways and to alter fungal morphology to change texture, laying the foundation for a new generation of fungal meat substitutes.

876T Use of CRISPR/Cas9 editing to generate mutations in *erg27* gene of *Botrytis cinerea* associated with resistance to hydroxyanilides *Anastasios Samaras*[1,2], Janina Werner[3], Ioannis Rodovitis[2], Matthias Hahn[3], George Karaoglanidis[2] 1) University of California, Davis,CA; 2) Aristotle University of Thessaloniki, Greece; 3) University of Kaiserslautern, Germany.

Botrytis cinerea is a necrotrophic plant pathogen causing gray mold. The wide range of hosts makes *B. cinerea* counts as one of the major agents of causing losses in several important agricultural crops. The control of the disease is mainly achieved by repetitive fungicide applications. However, the intensive use of fungicides has led to the development of resistances against most of the botryticides, related in different mechanisms such as point mutations in target protein, target site overexpression, decreased uptake and fungicide detoxification. Fenhexamid is a fungicide from SBIs class and therefore acts as a ergosterol biosynthesis inhibitor. Resistance in fenhexamid is connected with point mutations in *erg27* gene, a large number of point mutations has reported until today giving different resistance factors. Recently, several genome editing technologies using bacterial endonucleases have been established. Especially CRISPR/Cas9 genome-editing method has sparked a revolution in genetic research due to its high efficiency, versatility and easy operation. The CRISPR/Cas9 editing for *Botrytis cinerea* is based on sgRNA-Cas9 ribonucleoprotein (RNP) complex – transfection. A delivery of RNP together with a telomere vector revealed a high editing frequency without selection markers. The goal of this study is to edit previously found point mutations in *erg27* with CRIPR/Cas9 editing system and the investigation of resistance profiles from strains with multiple combinations of these mutations.The most well-known point mutations in *erg27* gene are replacements in 412 position of phenylalanine to serine, valine, cysteine and isoleucine. The editing was performed using a sgRNA in close proximity to the mutation site, a telomere plasmid with hygomycin resistance as well as a 160 bp repair template with the desired editing. These components were added to protoplasts in a PEG-mediated transformation approach. Transformants were isolated ans selected for several rounds in hygromycin petri dishes and later checked for the wanted mutation by digestion and sequencing. Lab strains were examined in fitness parameters and compared with field strains. Results highlight the accuracy and high efficiency of the CRISPR/Cas9 technology in *B. cinerea.* Using teleomere vectors enables unlimited rounds of editing without the need for selection markers and generation of multiple editings in one gene. With this tool fungicide research can massively be improved.

877F Detection and engineering of rapidly evolving genomic regions in anaerobic microbes *Amy Eisenberg*[1], Kerri Luttrell[1], Hugo Doré[1], Lizzy Wilbanks[1], Dave Valentine[1], Michelle O'Malley[1] 1) UC Santa Barbara, Santa Barbara, CA.

Rapidly evolving genomic regions can be used to track cell changes over short time scales and indicate where and how beneficial diversification is occurring. Several distinct mechanisms for generating mutations at specific loci have been characterized including immunoglobulin recombination in mammals and diversity generating retroelements (DGRs) in bacteria. Sequencing technologies allow us to quickly and deeply sequence genomes and metagenomes at low cost, tracking mutations as they emerge and change, even when they are only present in a small fraction of the cellular population. We implemented a method to sequence and identify variable genomic regions in microbes and calculate the sequence diversity index and compare variants to a reference sequence. This is being used to understand DGRs that we have identified in bacterial strains from complex communities of rumen-associated gut microbes, where bacteria and fungi work together to digest complex lignocellulosic substrates. Fungal LTRs can also be targets of rapid diversification and are being sequenced and compared from time points over years of lab culturing lab to understand how these strains have evolved since their isolation. Determining how these variant regions evolve over time can provide insight into the mutations that are most useful to the organism, as well as what environmental factors lead to their active diversification. The systems that generate fast-evolving variants are being developed as synthetic biology tools to create optimized variants in the genomes with great precision, which could be applied to

engineer strains with improved bioconversion and biodegradation properties.

878W Heterologous production of the fungal quinone polyketide bostrycoidin in *Yarrowia lipolytica* *Mihaela Bejenari*[1], Eva Mie Lang Spedtsberg[1], Tobias Bruun Pedersen[1], Mikkel Rank Nielsen[1], Jens Laurids Sørensen[1] 1) Aalborg University.

Fungal polyketides are a large group of secondary metabolites, valuable due to their diverse spectrum of pharmacological activities. Polyketide production in fungi is associated with several challenges: small yield and low-purity titers. To tackle these aspects, we switched from fungi to the yeast *Yarrowia lipolytica*, an easily cultivable heterologous host.
As an oleaginous yeast, *Yarrowia lipolytica* displays a high flux of acetyl- and malonyl-CoA precursors used in the fatty acid biosynthesis. Likewise, acetyl- and malonyl-CoA are the building blocks of fungal polyketides, and we explored the possibility of redirecting this flux towards polyketide production. Despite its promising prospect, *Y. lipolytica* has so far only been used for heterologous expression of simple type III polyketide synthases (PKSs) from plants. We therefore decided to examine the potential for more complex polyketide production in *Y. lipolytica* by targeting fungal polyketide derived from type I PKSs. We employed a CRISPR-Cas9-mediated genome editing method to achieve markerless gene integration in *Yarrowia lipolytica* of the *fsr1*, *fsr2*, and *fsr3* genes, responsible for bostrycoidin biosynthesis in *Fusarium solani*.
Additionally, we integrated a fourth gene encoding the activating co-enzyme phosphopantetheinyl transferase, *FsPPT1*, found in the same native host. This resulted in production of bostrycoidin with a yield of 6 mg/L in the initial trial. Extraction of bostrycoidin was however problematic due to a high lipid production, which is being addressed in our ongoing research. However, this work demonstrates the potential of *Yarrowia lipolytica* as a platform for heterologous production of complex fungal polyketides.

879T Nature's Silent Pharmacy: Mining fungal genomes in the discovery of novel antibiotics. *Sarah Dodd*[1], Andy Bailey[1], Gary Foster[1] 1) Univerity of bristol.

As humanity faces the possible return to an antibiotic "dark age", the need for new therapeutics has never been more urgent. Through the revolutionary discovery of penicillin, fungi continue to be a useful yet underutilised source of antimicrobials. With advances in genome analysis, fungi have been found to encode genes for more secondary metabolites than previously imagined which leaves a hidden and untapped silent pharmacy waiting to be explored.
We analysed the genomes of various fungi looking for clusters involved in the biosynthesis of polyketide and non-ribosomal peptides as well as genes for secreted antimicrobial peptides. To further validate our bioinformatics-based theories, selected gene clusters were expressed in *A. oryzae* and their resulting compounds were analysed using a myriad of cloning and chemical analytical techniques. The analysis pipeline was validated through the successful heterologous expression of a naphthapyrone cluster from *Byssochlamys. fulva* which resulted in the discovery of two novel compounds, as well as the characterisation of a siderophore biosynthetic pathway also in *B. fulva*.
Where our theories were vaguer and a product could not fully be predicted, we report progress on the expression of the RAL-like double PKS cluster in *Cladobotryum sp.* with a unique halogenase/O-methyltransferase tailoring gene which gives the possibility of finding novel RALs. In the same fungus, a cluster with peculiar PKS without a termination domain was also studied. Additionally, we explore yeast-based expression, extraction and purification of recombinant copsin-like peptides from *Coprinopsis. strossmayerii*.
With such a variety of possible compound scaffolds and biosynthetic pathways, the number of molecules created by fungi cannot be underestimated and within this library lies the next important antibiotic.

880F Structural characterization of secondary metabolites from filamentous fungi. Shu Yi Lin[1], C Elizabeth Oakley[2], Cory Benjamin Jenkinson[2], Berl Oakley[2], *Clay C. C. Wang*[1] 1) Univ Southern California; 2) Univ Kansas.

Genome sequencing has revealed that filamentous fungi contain many secondary metabolite gene clusters. Our labs have been actively engaged in chemically characterizing the metabolites produced by Aspergillus species. A time-consuming part of our research has been on the scale-up and purification of metabolites to isolate sufficient products for NMR characterization, typically several milligrams of purified compounds. In this presentation, I would like to present our efforts to structurally determine secondary metabolites using a sub-milligram of material.

881W CRISPR-based transcriptional activation tool for silent genes in filamentous fungi *Laszlo Mozsik*[1,2], Mirthe Hoekzema[2], Niels A W de Kok[2], Roel A L Bovenberg[3,4], Yvonne Nygård[5], Arthur F J Ram[1], Arnold J M Driessen[2] 1) Leiden University, Dept. of Molecular Microbiology and Biotechnology, Groningen, the Netherlands; 2) University of Groningen, Dept. of Molecular Microbiology, Groningen, the Netherlands; 3) University of Groningen, Dept. of Synthetic Biology and Cell Engineering, Groningen, the Netherlands; 4) DSM Biotechnology Center, Delft, the Netherlands; 5) Chalmers University of Technology, Department of Biology and Biological Engineering, Gothenburg, Sweden.

Filamentous fungi are historically known to be a rich reservoir of bioactive compounds that are applied in a myriad of fields ranging from crop protection to medicine. The surge of genomic data available shows that fungi remain an excellent source for new pharmaceuticals. However, most of the responsible biosynthetic gene clusters are transcriptionally silent under laboratory growth conditions. Therefore, generic strategies for the activation of these clusters are required. We constructed a genome-editing-free, transcriptional regulation tool for filamentous fungi, based on the CRISPR activation (CRISPRa) methodology. Herein, a nuclease-defective mutant of Cas9 (dCas9) was fused to a highly active tripartite activator VP64-p65-Rta (VPR) to allow for sgRNA directed targeted gene regulation. dCas9-VPR was introduced, together with an easy-to-use sgRNA "plug-and-play" module, into a non-integrative autonomously replicating AMA1-vector, which is compatible with several filamentous fungal species. The AMA1 sequence is known to self-replicate within a number of fungal species within the genera of *Aspergillus*, *Penicillium*, *Giberella*, and *Trichoderma*. To demonstrate its potential, this vector was used to transcriptionally activate a fluorescent reporter gene under the control of the transcriptionally silent synthetic *penDE* core promoter in *Penicillium rubens* (previously identified as *P. chrysogenum*). Subsequently, we activated the transcriptionally silent, native *P. rubens* macrophorin biosynthetic gene cluster by targeting dCas9-VPR to the promoter region of the transcriptionally silent, *macR* positive transcriptional regulator. This resulted in the transcriptional activation of the complete biosynthetic gene cluster of the antimicrobial macrophorins. This CRISPRa technology can be used for the rapid and convenient activation of silent fungal

biosynthetic gene clusters, and thereby aid in the identification of novel compounds such as antimicrobials.

882V Modular Synthetic Biology Toolkit for Filamentous Fungi *Carsten Pohl*[1], László Mózsik[2,5], Vera Meyer[1], Roel A.L. Bovenberg[3,5], Yvonne Nygård[4], Arnold J.M. Driessen[5] 1) Technische Universität Berlin, Berlin, Germany; 2) Leiden University, Leiden, The Netherlands; 3) DSM Biotechnology Center, Delft, The Netherlands; 4) Chalmers University of Technology, Gothenburg, Sweden; 5) University of Groningen, Groningen, The Netherlands.

Filamentous fungi are highly productive cell factories, often used in industry for the production of enzymes and small bioactive compounds. Recent years have seen an increasing number of synthetic biology-based applications in fungi, emphasizing the need for a synthetic biology toolkit for these organisms. Here, we present a collection of 96 genetic parts, characterized in Penicillium or Aspergillus species, that are compatible and interchangeable with the Modular Cloning system. The toolkit contains natural and synthetic promoters (constitutive and inducible), terminators, fluorescent reporters, and selection markers. Furthermore, there are regulatory and DNA-binding-domains of transcriptional regulators, and components for implementing different CRISPR-based technologies. Genetic parts can be assembled into complex multipartite assemblies and delivered through genomic integration or expressed from an AMA1-sequence-based, fungal-replicating shuttle vector. With this toolkit, synthetic transcription units with established promoters, fusion proteins or synthetic transcriptional regulation devices can be more rapidly assembled in a standardized and modular manner for novel fungal cell factories. Here, we present application examples of utilizing this toolbox for engineering of *Penicillium rubens* and *Aspergillus niger*.

883V Development of CRISPR-Cas editing tools in *Sphaerulina musiva* towards controlling its establishment and pathogenicity in the model ecosystem, *Populus* *Joanna Tannous*[1], Cole Sawyer[1,2], David Kainer[1], Alyssa Carrel[1], Mindy Clark[1], Jesse Labbe[1,3], Wellington Muchero[1], Melissa Cregger[1], Carrie Eckert[1], Paul Abraham[1] 1) Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA; 2) Graduate School of Genome Science and Technology, University of Tennessee, Knoxville, TN, USA; 3) Invaio Sciences, Cambridge, MA, USA.

The genus *Populus* are economically important biofuel crops cultivated worldwide, but mainly in the Northern hemisphere to fulfill the demands for bioenergy and fiber production. Poplars and their hybrids› widespread distribution and usage are limited by their instability to various diseases, of which the leaf spot and canker disease caused by the fungal pathogen *Sphaerulina musiva* is the most detrimental one. Breeding and cultivation of resistant plant species have been the primary approaches adopted to control the damage caused by this pathogen.
In our effort to mitigate Poplar disease caused by *S. musiva* we focused on advancing both the fundamental knowledge on the *S. musiva -Populus* pathosystem and the applied research by developing genetic engineering tools on the pathogen as a strategy to control the fungal abundance and disease severity. The above-ground part of the *S. musiva*'s lifecycle is relatively well studied; however, the underground establishment of *S. musiva*'s spores, from fallen infected leaves, in the presence of *Populus* soil microbiota had remained obscure. Therefore, using RNA-sequencing, we first aimed to identify genetic markers implicated in the establishment of *S. musiva* within native *Populus* soil microbial communities. Later we developed and validated the first CRISPR-Cas9 gene-editing tool to successfully transform *S. musiva*. The development of this genetic tool, along with the molecular markers identified from this study would allow for specific gene targeting to disrupt *S. musiva*'s establishment in soil. Lastly, we are also in the process of leveraging from this established tool to develop a self-propagating synthetic gene drive designed to suppress leaf spot disease caused by *S. musiva* in *Populus*.

884V Elucidation of pyranone pigment biosynthesis in fungi *Yanfang Guo*[1,3], Caroline Rodenbach[1], Olga Mosunova[1], Jorge Navarro-Muñoz[1], Bert Gerrits van den Ende[1], Ferry Hagen[1,2], Arnold J.M. Driessen[3], Jérôme Collemare[1] 1) Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; 2) University Medical Center Utrecht, Utrecht, The Netherlands; 3) University of Groningen, Groningen, The Netherlands.

Industrial production of dyes relies on petrol-based methods with corrosive chemicals that are harmful to both environment and humans. While the dyestuff industry and its derivatives (textile, paint, etc) greatly contributes to the economic growth on the short term, they cause serious pollution issues that are detrimental over the long term. Hence, we urgently need new sustainable production routes. Dyes of natural origin represent an attractive alternative. Especially, pigments produced by fungi are promising because these microbes can be used as cell factories to produce natural dyes in fermentation-based processes. The chemical family of pyranone compounds produced by fungi exhibit diverse colours and biological activities. Xylindein is a natural cyan pigment produced by the fungus *Chlorociboria aeruginascens*, and laccaridione A is a related purple pigment produced by *Laccaria amethystina*. In this study, we re-sequenced *C. aeruginascens* and mined the genomes to identify candidate gene clusters for the production of xylindein and laccaridione A. We assessed expression of the candidate genes and have embarked on the functional characterization using heterologous expression in the industrial host *Aspergillus oryzae*. Elucidation of both pathways will open the possibility to engineer novel pyranone pigments.

885V Secondary Metabolite Production in *Aspergillus niger*: methyltransferase specificity *Susannah Selber-Hnatiw*[1], Marie Beigas[1], Marcos Di Falco[1], Adrian Tsang[1], Isabelle Benoit-Gelber[1] 1) Concordia University.

Secondary metabolites are compounds not directly involved in the growth or viability of an organism, but represent a source of pharmacologically or industrially relevant compounds that provide a selective advantage[1]. Biosynthetic gene clusters (BGCs) encode the production of secondary metabolites and generally include genes responsible for the synthesis, chemical modification, and transport of products outside the cell. In addition, the presence of a transcriptional regulator may activate the production of secondary metabolites[2]. The methylation of secondary metabolites is a chemical modification resulting from methyltransferase activity. For instance, the neurokinin receptor antagonist BMS-192548 is tautomerized from the neuropeptide Y antagonist TAN-1612 through an *O*-methyltransferase tailoring enzyme that regulates the selective addition of C9-methyl groups[3]. In general, methyltransferase specificity is important to our understanding of redesigning molecules, in which the product of altered methylation may lead to pharmacologically or industrially relevant secondary metabolites[4]. As such, to examine late-stage functionalization using *O*-methyltransferases to increase the chemical

diversity of secondary metabolites, this work examines how different methyltransferases can modify the methylation pattern of a molecule. This involves the replacement of the native methyltransferase with a library of methyltransferase candidates, and the analysis of the resulting methylation pattern. BGC engineering and secondary metabolite profiles of the mutant strains will be presented.

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886F *Aspergillus nidulans* Inhibitor of Apoptosis-like protein, AnBir1, is essential for survival and regulates fungal development *Meareg Amare*[1], Sachin Jain[1], Mehdi Kabbage[1] 1) University of Wisconsin - Madison.

All organisms balance the decision of life or death at the cellular level. Programmed cell death (PCD) is the coordinated and organized mechanism of cell death. Studies in mammalian, insects and viruses have shown that PCD is important in regulating early development, immune system maturation, host-pathogen interactions and more. Although PCD is largely conserved across eukaryotic kingdoms, there is significant divergence in the regulatory mechanisms. Inhibitors of apoptosis proteins (IAPs) are negative regulators of PCD and are amongst the most highly conserved regulatory proteins across eukaryotic kingdoms. IAPs are defined by the presence of 1-3 *Baculovirus* IAP Repeat (BIR) domain(s) on the N-terminus end of the protein. Studies of IAPs in mammalian, insect, and viral systems show that they are crucial in regulating cell death and other fundamental processes, including cell division, inflammation and more. Although IAPs are conserved in fungi, there is very limited research into the processes that IAPs regulate and the mechanism through which they regulate fungal processes. In this study, we identified an IAP-like protein in the model filamentous fungal organism *Aspergillus nidulans* (AnBir1) and investigated fungal processes it regulates. Bioinformatic analyses revealed that AnBir1 contains two BIR domains, a conserved feature among fungal species. We found that *AnBIR1* is an essential gene. Gene deletion is lethal and when *AnBIR1* is placed under the control of a conditional promoter, fungal growth is only observed when the promoter is turned on. Moreover, we found that AnBir1 is critical in regulating fungal development. Constitutive expression of *AnBIR1* resulted in a strong push towards sexual reproduction with asexual reproduction almost completely lost. Despite this apparent push towards sexual reproduction, the strain overexpressing *AnBIR1* is initially delayed whereby the wild type matures earlier. We are investigating the role that AnBir1 plays in regulating *A. nidulans* PCD and the biochemical context within which it operates to regulate fungal processes.

887W Circadian Clock Control tRNA Synthetases in *Neurospora crassa* *Griffin Best*[1], Kathrina Castillo[1] 1) Texas A&M University.

About half of proteins synthesized in eukaryotic cells under control of the endogenous circadian clock arise from mRNAs that are not rhythmic, supporting a role for clock control of posttranscriptional mechanisms. In *Neurospora crassa*, the circadian clock controls rhythmic mRNA translation through regulation of the eIF2α kinase CPC-3 (the homolog of yeast and mammalian GCN2). Active CPC-3 phosphorylates and inactivates eIF2α, leading to reduced translation initiation. In yeast, GCN2 is activated by binding to uncharged tRNA, which accumulates in cells during amino acid starvation. Consistent with these data, we found that clock control of CPC-3 activity requires the rhythmic accumulation of the valyl-tRNA synthetase (ValRS) and hypothesized that clock control of tRNA synthetases (RS's) promotes the rhythmic accumulation of uncharged tRNAs to activate CPC-3 during the day and coordinate rhythms in translation. We selected the methionyl-tRNA synthetase (MetRS) due to its function in charging initiator methionyl-tRNAs, and because it forms a part of the multisynthetase complex in other model organisms, which regulates secondary functions of its tRNA synthetase constituents. We discovered that MetRS::LUC fusion protein to identify rhythms in MetRS expression. We also designed a MetRS is rhythmic by utilizing a LUC reporter, and we are using a MetRS::V5 in an immunoprecipitation assay to identify the composition of the *N. crassa* multisynthetase complex at different times of the day. This work will provide key insights into the mechanisms of protein production by the circadian clock, and because RS's have roles outside of mRNA translation, may also provide a mechanism to connect clock control of protein synthesis to other clock regulated cellular processes, including nutrient metabolism, cell division, and development.

888T Spatio-temporal dynamics of the *Podospora anserina* fungus using a geomatic-based approach *Cecilia Bobee*[1], Eva Cabet[1], Florence Chapeland-Leclerc[1], Pascal David[1], Frederic Filaine[1], Eric Herbert[1], Christophe Lalanne[1], Clara Ledoux[1], Gwenael Ruprich-Robert[1] 1) Université de Paris, CNRS, Laboratoire Interdisciplinaire des Energies de Demain (LIED - UMR 8236), F-75013 Paris, France.

An innovative approach for analyzing the dynamics of the fungus *Podospora anserina* has been set up by the DREAMS research members (LIED laboratory, University of Paris, France), allowing to follow the growth of the thallus at high spatial and time resolutions [1].
A GIS based approach (Geographic Information System) coupled with classical methods of image analyses revealed to be efficient to follow the spatio-temporal *P. anserina* network growth. Series of very high-resolution grayscale images (~μm) of the thallus, so-called panoramas, allow us to extract different metrics such total length of the thallus, position of nodes and apexes [1]. In this work, we more particularly present three main results, each of them characterizing in different ways the spatio-temporal growth of *P. anserina* and its local dynamics:
i/ through a skeletonization of the thallus, and the detection of nodes and apexes;
ii/ the dynamic evolution of "closed intrathallus areas", defined as surfaces completely surrounded by the filamentous fungus. It reveals to be another way to study spatial differences in densification processes. Results are completely in accordance with maps of linear density, the latter highlighting spatial distribution of the fungus matter;
iii/ Maps of the age formation of intrathallus areas and the temporal evolution of the densification processes inside them, showing the local growth dynamics.
[1] Scientific Reports 10:3131 (2020). https://doi.org/10.1038/s41598-020-57808-y

889F Genetic and genomic analysis of *Malassezia* reveals pseudobipolar-tetrapolar mating-type locus transitions and early steps in sexual reproduction *Marco A. Coelho*[1,4], Giuseppe Ianiri[2,4], Márcia David-Palma[1], Bart Theelen[3], Teun Boekhout[3], Jo-

seph Heitman[1] 1) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina, USA; 2) Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy; 3) Westerkdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; 4) contributed equally.

Malassezia is part of the normal skin microbiota of humans and animals, and has also been associated with numerous dermatological disorders, and implicated in exacerbation of Crohn's disease, pancreatic cancer, and cystic fibrosis. Presently, *Malassezia* comprises 18 species, all of which are found on the skin of humans and warm-blooded vertebrates, but they have also been found to inhabit diverse ecosystems, including soil and deep marine environments. Although a sexual cycle remains elusive, the presence of mating and meiotic genes in *Malassezia* genomes suggest the capacity for mating and sexual reproduction. As in other basidiomycetes, two mating type (*MAT*) loci are found in the genome: a mate recognition pheromone/receptor (*P/R*) locus and a homeodomain (*HD*) locus that typically determines post-mating steps. However, in contrast to bipolar or tetrapolar basidiomycetes (i.e., that have genetically linked *P/R* and *HD* loci or, *P/R* and *HD* loci on separate chromosomes), studies in *Malassezia* have shown that the two *MAT* loci lie on the same chromosome but far enough apart such that recombination can still occur (pseudobipolar). Tetrapolarity is thought to be the ancestral state of all basidiomycetes from which extant bipolar states have repeatedly and independently emerged. Here, we analyzed chromosome-level genome assemblies of all *Malassezia* species and present the first report of derived tetrapolarity in fungi, wherein the *P/R* and *HD* loci were secondarily relocated onto separate chromosomes from an ancestral pseudobipolar configuration. Phylogenetic and synteny analyses across *Malassezia* suggest up to 5 independent transitions from pseudobipolar to tetrapolar. Most of these cases involve major chromosomal changes associated with centromeric breaks, or translocations within centromere-flanking regions. Additionally, to attempt to uncover a sexual cycle in *Malassezia*, we genetically engineered an *M. furfur* strain capable of hyphal formation when grown in solo culture. This was achieved by introducing into a recipient strain the *P/R* and *HD* alleles of a presumably compatible strain via *Agrobacterium* transformation. Introduction of the *P/R* allele alone also yielded a strain capable of hyphal formation, whereas introduction of the *HD* allele alone did not. Taken together, this study reveals a novel observation of genomic relocation between mating compatibility factors in fungi and provides a first step towards the discovery of an extant sexual cycle in *Malassezia*.

890W Determining the carbohydrate profile of the *Cryptococcus* spore coat *Eddie Dominguez*[1], Robert Zarnowski[1], Christina Hull[1] 1) University of Wisconsin - Madison.

Among human respiratory fungal pathogens, spores are presumed infectious particles that first encounter and associate with pulmonary immune cells such as alveolar macrophages. This interaction is generally mediated by recognition of a fungal cell wall carbohydrate (e.g. β-(1-3)-glucan) with a carbohydrate binding receptor (e.g. Dectin-1). We showed previously that the major fungal ligands that alveolar macrophages are known to recognize do not appear to play significant roles in host innate immune cell interactions with *Cryptococcus* spores. As such, we hypothesize that there are interactions between alveolar macrophages and *Cryptococcus* spores that are mediated by currently unknown spore coat carbohydrates. To test this hypothesis, we are using a combination of gas chromatography, high pressure liquid chromatography, mass spectrometry, and nuclear magnetic resonance to determine the specific carbohydrate epitopes present at the spore surface. Identifying these potentially antigenic components in the surface topography/composition of fungal spores will facilitate the characterization of novel macrophage-spore interactions that contribute to immune recognition. This line of investigation promises to ultimately contribute insights into spore-specific host immune responses and generate alternative therapeutic approaches for the treatment of fungal diseases.

891T Developing genetic tools to unlock the biotechnological potential of anaerobic gut fungi *Radwa Hanafy*[1], Casey Hooker[1,2], Ethan Hillman[2], Javier Muñoz[2], Kevin Solomon[1,2] 1) University of Delaware, Newark, DE; 2) Purdue University, West Lafayette, IN.

Anaerobic Gut Fungi (AGF) (phylum Neocallimastigomycota) are native to the digestive system of large herbivores and play a prominent role in anaerobic degradation of untreated lignocellulosic plant materials. AGF have evolved into efficient plant biomass colonizers and degraders through secretion of powerful lignocellulolytic enzymes, and production of secondary metabolites to compete with other microbes. Such remarkable degradation capacities and vast enzymatic repertoire make them promising candidates for enzymes and bioactive molecules that may be used in many biotechnological applications. However, AGF are not yet genetically tractable and require major advances in their genetic manipulation protocols. Primarily guided by available genomic and transcriptomic data, we have identified genetic elements regulating gene expression (e.g. promotors and terminators) and are beginning to assemble a nascent genetic toolbox for manipulation. In this study, we have established a transformation protocol leveraging the natural competency of juvenile zoospores. We have developed methods to introduce selectable markers such as hygromycin resistance gene (*hph*) and validated two fluorescent reporters, flavin-based iLOV and heme-dependent iRFP. In addition, heterologous protein expression may be directed to specific cellular compartments such as the nucleus through the use of localization tags. Efforts to expand this genetic toolkit to include alternative selection genes, reporters, and promotors are currently underway. Important elements such as autonomously replicating sequences and centromere binding sequences are being identified to help stabilize expression vectors. Ultimately, the developed genetic toolkit provides a platform for sustainable bioprocess development, enabling us to exploit the full potential of these non-model anaerobic fungi.

892F Dynamic expanding fungal networks: characterization of the spatio-temporal hyphal growth in the filamentous fungus *Podospora anserina* *Clara Ledoux*[1], Florence Chapeland-Leclerc[1], Gwenael Ruprich-Robert[1], Cecilia Bobee[1], Christophe Lalanne[1], Eric Herbert[1], Pascal David[1] 1) Universite de Paris, UMR CNRS 8236, Paris, France.

The success of filamentous fungi in colonizing most natural environments can largely be attributed to their ability to form an expanding interconnected network of hyphae, the mycelium. The hyphae are growing filaments which are able to branch and fuse, then allowing an efficient spatial exploration and exploitation of the nutritive resources. This mycelium exists between the initial spore and the stage at which the organism can reproduce. Thus the hyphal network must ensure an optimal growth for survival until reproduction.

In the present work, we accurately analyzed the development of the network of *Podospora anserina* under controlled conditions through specific quantitative parameters. To do so, a temporal series pictures of the growing mycelium from a germinating ascospore was

produced.

The high resolution of the images allows to study the network at the hypha scale (about 5 μm), while keeping the possibility to work on the complete thallus. It is then possible to extract characteristic quantities of the fungal growth and complexity (quantity of living matter, number of hyphal tips and intersections, intra-thallus areas). This approach allows to quantify the growth profiles of the network submitted to different constraints, such as nutrient limitation, intense lighting, or low temperature.

In addition, using manual image processing, the analysis focuses on the branching process, that reveals two new hyphae coming from a node. In particular, we studied the branching frequency, as well as the relative growth rate of each emerging tips. It was also suggested that the branching angle values allow for an optimal exploration and exploitation of the environment.

Finally, a fluorescent approach provides additional information on the branching process through the visualization of intracellular structures.

893W ***Candida albicans*** **and IL-17A stimulate cytokine production by oral epithelial cells via different mechanisms** *Jianfeng LIN*[1], Quynh Trang[1], Hong Liu[1], Sarah Gaffen[2], Scott Filler[1,3] 1) The Lundquist Institute, Torrance, CA; 2) University of Pittsburgh, Pittsburgh, PA; 3) University of California- Los Angeles, Los Angeles, CA.

IL-17 signaling components (IL-17A, IL-17RA, IL-17RC, ACT1, etc.) are critical for the host defense against oropharyngeal candidiasis (OPC). Both IL-17A and *Candida albicans* stimulate oral epithelial cells to secrete pro-inflammatory cytokines. We investigated how these two different stimuli induced this pro-inflammatory response. Using indirect immunofluorescence, we found that the IL17RA and IL17RC co-localize with the epidermal growth factor receptor (EGFR) around the *C. albicans* hyphae on the OKF6/Tert-2 oral epithelial cell line. Although IL-17A and *C. albicans* cells both stimulated epithelial cells to secrete pro-inflammatory cytokines such as IL-8 and GM-CSF, they do so through distinct mechanisms. Inhibition of EGFR with either gefitinib or an anti-EGFR antibody reduced IL-8 production in response to both *C. albicans* and IL-17A. However, knockdown of *IL-17RA* or *ACT1* with siRNA or knockout via CRISPR abolished IL-8 production induced by IL-17A, but not *C. albicans*. RNA-seq analysis revealed that IL17A and *C. albicans* induce the expression of distinct sets of genes. Strikingly, only *C. albicans* infection caused significant upregulation of genes encoding transcription factors encoding such as *c-FOS*, *c-JUN*, *NF-IL6*, *NFκB1*, and *NFκB2*, which in-turn resulted in massive up-regulation of pro-inflammatory cytokine gene mRNAs including *IL-6*, *IL-8*, *CSF2*, and *CSF3*. Although IL-17A induced low levels of pro-inflammatory cytokine transcripts relative to *C. albicans*, both stimuli induced similar levels of cytokine proteins. Intriguingly, transcripts of the mRNA binding proteins TTP and BRF1 were significantly upregulated in response to *C. albicans,* but not IL-17A. Knockdown of *BRF1,* dramatically increased *C. albicans*-induced IL-8 production without changing the *IL-8* transcript levels. By contrast, *BRF1* knockdown did not affect *IL-8* transcript or IL-8 protein levels in response to IL-17A. Single-molecule fluorescent in-situ hybridization (sm-FISH) analysis in oral epithelial cells infected with *C. albicans* demonstrated that *IL-8* mRNA does not associate with BRF1, indicating a yet unknown role of BRF1 in *C. albicans*-induced production of IL-8. Thus, *C. albicans* stimulates proinflammatory cytokine production in oral epithelial cells by inducing a strong transcriptional response that is modulated by BRF1 whereas IL-17A induces a weaker transcriptional response that is not affected by BRF1.

894T **Nanoparticles and pathogenic fungi: a non-uptake delivery** *Thomas Orasch*[1], Gauri Gangapurwala[2,3], Katherine Gonzalez[1,4], Julien Alex[2,3], Alicia De San Luis[2,3,5], Antje Vollrath[2,3], Christine Weber[2,3], Stephanie Hoeppener[2,3], Zoltan Cseresnyes[6], Marc Thilo Figge[4,6], Ulrich S. Schubert[2,3], Axel A. Brakhage[1,4] 1) Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute; 2) Laboratory of Organic and Macromolecular Chemistry (IOMC), Friedrich Schiller University Jena; 3) Jena Center for Soft Matter (JCSM), Friedrich Schiller University Jena; 4) Institute of Microbiology, Friedrich Schiller University Jena; 5) POLYMAT and Kimika Aplikatua Saila, Kimika Fakultatea, University of the Basque Country UPV/EHU; 6) Applied Systems Biology, Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute.

Newly discovered antifungal substances often display pharmacological problems, like low solubility or high toxicity. Several studies showed that nanoparticles (NPs) can be used to overcome these problems of antimicrobials. Liposomal Amphotericin B is an example for such a nanoformulated antifungal drug already being on the market. The mechanism, how polymeric NPs deliver encapsulated substances into pathogenic fungi, was suggested to be either *via* endocytosis or an endocytosis-independent uptake of the whole NP. However, the mechanism of uptake remains to be understood.
Therefore, we investigated the interaction of different NPs with several human pathogenic fungi to elucidate the uptake mechanism irrespective of the polymer or fungal species.
NPs were prepared by utilizing 4 different polymers and were labelled with 3 different covalently attached fluorescent dyes and/or a fluorescent dye or antifungal drug encapsulated. The interaction of the fluorescently labelled NPs with the filamentous fungi *Aspergillus fumigatus*, *A. nidulans*, *A. terreus*, and *A. oryzae*, and the yeasts *Cryptococcus neoformans* and *Candida albicans* was investigated by confocal laser scanning microscopy and transmission electron microscopy. The efficacy of itraconazole-loaded NPs on these species was determined by MIC-testing following the respective EUCAST methodology.
Irrespective of the applied conditions (such as pH value or salt concentration in the medium, polymers used, size of the NPs, incubation time up to 24h, culture medium used), none of the used NPs reached the fungal cytosol, but adhered to the fungal surface. Investigations on the exact localization of NPs revealed their appearance in the interspace between cell wall and membrane of the fungi. Nevertheless, encapsulation of a fluorescent dye or itraconazole led to an accumulation of the fluorescent dye in the fungal lumen or a lower MIC compared to the pristine drug, respectively.
In conclusion, polymeric NPs are not taken up by pathogenic fungi. Nevertheless, the delivery of hydrophobic substances like antifungals into these fungi with the help of NPs is possible and effective, making NPs a promising tool for antifungal treatment.

895F **Mechanisms of circadian clock control of CPC-3 activity in** ***Neurospora crassa*** *Ebimobowei Preh*[1], Deborah Bell-Pedersen[1] 1) Texas A&M University, College Station, TX.

The circadian clock in *Neurospora crassa* regulates rhythms in the phosphorylation and activity of the conserved translation initiation factor eIF2α, which promotes rhythmic mRNA translation (Karki et al., 2020). Cycling phosphorylated eIF2α levels requires rhythmic activation of the eIF2α kinase CPC-3 (the homolog of yeast and mammalian GCN2). However, the mechanisms controlling rhythmic CPC-3 activation are not fully understood. Studies in *Saccharomyces cerevisiae* suggested that activation of GCN2 requires direct interaction of GCN1 and GCN2 with ribosomes. Based on these data, I hypothesized that *N. crassa* GCN1 and CPC-3 rhythmically interact with the ribosome, and that this interaction is necessary for rhythmic CPC-3 activity. To test this hypothesis, I examined the interaction of CPC-3::V5 and GCN1::HA with ribosomes. The tagged strains were confirmed to be functional by their ability to promote eIF2α phosphorylation. Ribosomes were purified by sucrose density gradient ultracentrifugation from cultures grown in constant dark (DD) and harvested during the subjective day (DD18), corresponding to the time of peak CPC-3 activity. While CPC-3::V5 and GCN1::HA were found to co-sediment with monosomes and polysomes, the interaction of CPC-3::V5 with ribosomes was arrhythmic in DD. Data will be presented on clock control of the interaction of GCN1::HA with the ribosome, and the role of CPC-3 ribosome binding in controlling the levels and/or rhythmic accumulation of phosphorylated eIF2α.

896W Validating pantothenate kinase as a novel target for antifungal development *Jessica Regan*[1], Parker Reitler[2], Stacey Barnett[3], Tracy Peters[4], Glen Palmer[4] 1) Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, Memphis, TN; 2) Department of Molecular Immunology and Biochemistry, University of Tennessee Health Science Center, Memphis, TN; 3) Regional Biocontainment Laboratory, University of Tennessee Health Science Center, Memphis, TN; 4) Department of Clinical Pharmacy and Translational Science, University of Tennessee Health Science Center, Memphis, TN.

The synthesis of coenzyme A from pantothenate is essential in all living organisms. Pantothenate kinase (Cab1p) catalyzes the first and rate limiting step in this pathway and is essential for viability in *Saccharomyces cerevisiae* and several bacterial species. However, it has yet to be studied in any pathogenic fungal species. We sought to investigate the physiological importance of Cab1p in *Candida albicans* and determine if it can provide a viable target for antifungal development. To accomplish this, we constructed a strain with doxycycline repressible expression of *CAB1* (P_{TETO}-*PANK*) and compared its growth in the presence and absence of doxycycline. P_{TETO}-*PANK* grew to the same density as the wild-type strain in the absence of doxycycline, however, in the presence of doxycycline, growth was substantially reduced. We also tested the virulence of the P_{TETO}-*PANK* strain using a mouse model of disseminated infection. All mock treated mice succumbed to infection by day 7. In stark contrast, all mice treated with doxycycline survived until the end of the experiment. These results confirm that *CAB1* is essential for fungal viability and virulence and is therefore a valid target for antifungal development. To facilitate the discovery of inhibitors that specifically target Cab1p, we have developed and validated high-throughput compatible screening assays. *C. albicans* pantothenate kinase was purified with a his6-tag and its biological activity confirmed using a commercially available assay based on ATP hydrolysis. A target-based whole-cell screen based on competitive fitness was also developed to identify specific chemical-target interactions. We have initiated screening with several small molecule libraries and identified potential Cab1p inhibitors.

897T The role of ABC transporters in resistance to SDHI fungicides in the obligate fungal pathogen Erysiphe necator *Anastasios Samaras*[1], Alex Zaccaron[1], Ioannis Stergiopoulos[1] 1) Department of Plant Pathology, University of California Davis, One Shields Avenue, Davis, CA 95616-851, U.S.A..

Erysiphe necator is one of the most important foliar pathogen in grapevines across the world. Sulfur-dusts and synthetic fungicides such as the sterol demethylation inhibitors (DMIs), the quinone-outside inhibitors (QoIs) and the succinate dehydrogenase inhibitors (SDHIs), are commonly used in control of the pathogen. However, field-level resistance has already been observed against DMIs and QoIs, whereas over the last couple years a dramatic increase has been observed in Western U.S. in the number of E. necator isolates with putative SDHI target-site mutations. Interestingly, our studies also revealed the presence of E. necator isolates with increased tolerance to SDHIs but with no SDHI target-site mutations present in them, indicating that an additional mechanism of resistance to SDHIs could be operating in this pathogen. Pleiotropic drug resistance (PDR) is a well-described phenomenon occurring in fungi. In various fungal species, a number of membrane-embedded energy-consuming ATP-binding cassette (ABC) transporters have been implicated in the development of PDR phenotypes. In order to investigate the possibility of ABC transporters being an alternative mechanism of SDHI resistance in E. necator, ABC transporter-encoding genes were cloned from this pathogen and are currently functionally characterized. Specifically, homology searches revealed a total of 22 genes encoding ABC transporters in E. necator, representing seven subfamilies, i.e. ABCI (n=1), ABCB (n=5), ABCC (n=4), ABCD (n=2), ABCE (n=1), ABCG (n=3) and ABCF (n=6). Among the 22 ABC transporters, six ABC transporters from subfamilies ABCB, ABCC and ABCG that represent the multidrug resistance (MDR), the multidrug-resistance-related protein (MRP) and the PDR families, respectively, were predicted based on their structural architecture to act in the detoxification of xenobiotic compounds. Two additional transporters in E. necator homologous to the nucleobase-related transporters AzgA and FcyB involved in SDHI uptake in Aspergillus nidulans were also identified. In order to investigate the role of these transporters in SDHI resistance, they were expressed in a yeast strain with multiple knock-outs in native ABC transporter genes and the transformed yeast strains are currently tested for their sensitivity to SDHIs and other fungicides. The results of these experiments will be presented.

898F Import and export of mannosylerythritol lipids by *Ustilago maydis* Fabienne Becker[1], Uwe Linne[2], Michael Bölker[1,3], Johannes Freitag[1], *Björn Sandrock*[1] 1) Philipps-University Marburg, Department of Biology; 2) Philipps-University Marburg, Department of Chemistry; 3) SYNMICRO - Center for Synthetic Microbiology, Philipps-University Marburg.

Upon nitrogen starvation the basidiomycete *Ustilago maydis* secretes amphipathic glycolipids including **mannosylerythritol lipids** (MELs). MELs consist of a carbohydrate core whose mannosyl ring is acylated with fatty acids of different length and acetylated at four different positions. While biosynthesis of MELs is well characterized, their biological functions and transport routes are less understood.
We report our recent advances on transport of MELs in and out of the cell dependent on the **transport protein Mmf1**, which belongs to the major facilitator superfamily. Analysis of *mmf1* mutants and mutants lacking the acetyltransferase Mat1 revealed that Mmf1 is necessary for **export of acetylated MELs**, while MELs without acetyl group are secreted independent of this transporter.
With the help of **feeding experiments** we demonstrate that MELs are taken up by *U. maydis* in an *mmf1* independent manner, which

either leads to catabolism or to rearrangement of acetyl- and acyl side groups and subsequent secretion. We provide evidence that catabolism of MELs involves the presence of Mac2, an enzyme required for MEL biosynthesis.
In addition, we developed a **co-cultivation assay** to show that MELs can be exchanged between cells and modified or even degraded by recipient cells.
Finally, we propose a novel function for fungal glycolipids as an **external carbon storage**.

899W The story of a sentinel tree, and the story of its fungal demise *Jonathan Schilling*[1], Molly Moran[1], Lauren Otolski[1], Jackson Traas[1] 1) University of Minnesota.

The science of growing trees is a well-known story. The stories of trees after death, however, have few narratives, and their scripts are often written by those working in the dark - the fungi. We connect with forests as natural spaces for us to enjoy, and we regard trees as a resource to be managed. This focuses our lens to view tree growth as gain and tree death as loss. Lumber is our material - the rest is debris. In the face of climate change, we now place even more value on planting trees as a means to fix and store carbon in a massive woody 'sink' that we hope can store and offset our emissions.

But for every atom of carbon from the atmosphere that is captured and stored in woody tissues, there is another being released when decomposers, primarily fungi, metabolize wood. Wood becomes a carbon 'source' rather than a sink. We know far less about this process of disassembly and carbon release than we do about how wood is assembled. This leaves us unable to accurately predict the true size of this carbon sink when we measure forests or plant trees. We do not fully know the good that trees do for the Earth. We increasingly have tools and techniques, however, to better quantify the 'afterlife' of trees and improve our predictions. We should pursue this science.

The goal of the story we present here is both to share a story of fungal decomposition science and to propel others studying it. Specifically, we share a study with only one replicate (n=1) – a single, sentinel white pine tree in northern Minnesota (U.S.A.) - and the science of the fungi that weakened its crown and sent it to the ground in a matter of seconds. Our investigation to determine a culprit fungus blends old and new techniques in wood microbiology, treating decomposition as a crime scene, complete with a lineup of potential offenders. The story also nicely illustrates a scientific link between fungal characters (traits) and real-world consequences (function), and we provide key citations for pursuing deeper inquiry.

900T Flotillin-dependent membrane microdomains are required for functional phagolysosomes against fungal infections *Franziska Schmidt*[1,12], Andreas Thywißen[1,12], Marie Goldmann[1,12], Cristina Cunha[3,4], Zoltán Cseresnyés[2], Hella Schmidt Schmidt[1,12], Muhammad Rafiq[1,12], Silvia Galiani[5], Markus H. Gräler[6], Georgios Chamilos[7], João F. Lacerda[8], António Campos Jr.[9], Christian Eggeling[10], Marc Thilo Figge[2], Thorsten Heinekamp[1], Scott G. Filler[11], Agostinho Carvalho[3,4], Axel A. Brakhage[1,12] 1) Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI), Germany; 2) Research Group Applied Systems Biology, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Germany; 3) Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus de Gualtar, Braga, Portugal; 4) ICVS/3B›s - PT Government Associate Laboratory, Braga/Guimarães, Campus de Gualtar, Braga, Portugal; 5) MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, UK; 6) Department of Anesthesiology and Intensive Care Medicine, Center for Sepsis Control and Care (CSCC), and the Center for Molecular Biomedicine (CMB), University Hospital Jena; 7) Department of Medicine, University of Crete, and Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, Heraklion, Crete, Greece; 8) Serviço de Hematologia e Transplantação de Medula, Hospital de Santa Maria, Lisboa, Portugal, and Instituto de Medicina Molecular, Faculdade de Medicina de Lisboa, Portugal; 9) Serviço de Transplantação de Medula Óssea (STMO), Instituto Português de Oncologia do Porto, Porto, Portugal; 10) Institute of Applied Optics, Friedrich Schiller University Jena, and Department of Biophysical Imaging, Leibniz Institute of Photonic Technology (IPHT), Germany; 11) Division of Infectious Diseases, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance CA, USA, David Geffen School of Medicine at University of California, Los Angeles, Los Angeles, California, USA ; 12) Department of Microbiology and Molecular Biology, Institute of Microbiology, Friedrich Schiller University Jena, Germany .

Aspergillus fumigatus represents the most important air-borne fungal pathogen. In the lung, resident alveolar macrophages belong to the first line of defense against inhaled conidia. Dihydroxynaphthalene (DHN) melanin on the conidial surface is crucial to inhibit phagolysosomal acidification and to cause increased damage to macrophages compared to non-pigmented *pksP* mutant conidia. Here, we discovered the importance of lipid rafts for maturation of functional phagosomes and as target of DHN-melanin that leads to an impaired formation of lipid-raft microdomains in the membrane of phagolysosomes. As a result, the assembly of vATPase is prevented and as a consequence, phagolysosomal acidification is inhibited. We thus report an unprecedented mechanism of the interference of fungal spores with immune cells.
The conidia-containing phagolysosomal membrane is characterized by a high content of cholesterol and sphingolipids. Further analysis showed that flotillin-enriched lipid rafts co-localize with sphingolipids on phagolysosomal membranes containing *pksP* conidia but not wild-type conidia. In human and mouse, two flotillins, Flot-1 and 2, are present. As chaperons, they stabilize lipid-raft microdomains and thus represent marker proteins.
Optimization of a protocol for isolation of phagolysosomes from macrophages allowed us to monitor the colocalization of microdomains and receptor/enzyme complexes by high resolution fluorescence microscopy. In addition, bone marrow-derived macrophages (BMDMs) of Flot-1/2 knockout mice were compared with C57BL7/6 wild-type macrophages. In line with our previous results, in flotillin-1/2 knockout BMDMs acidification of phagolysosomes containing *pksP* conidia was drastically reduced as well as vATPase assembly and phagocytosis. Infection of Flot-1/2 knockout mice with *pksP* conidia resulted in increased cell damage and killing compared to wild-type conidia in a mouse infection model. Furthermore, we identified an SNP for flotillin-1 in human donors of stem cells, which is associated with an increased susceptibility for invasive aspergillosis. Taken together, these data indicate that lipid raft microdomains provide important platforms for signaling and defense of immune cells and can be manipulated by *A. fumigatus*.

Schmidt F. *et al*., *Cell Reports* 2020;
Goldmann M., Schmidt F. *et al*., *STAR Protocols* 2021

901F Signaling through the STRIPAK complex: Functional analysis of putative phosphorylation/ dephosphorylation targets *Maria Shariatnasery*[1], Valentina Stein[1], Ines Teichert[1], Ulrich Kueck[1] 1) Ruhr-University Bochum .

The striatin-interacting phosphatase and kinase (STRIPAK) multisubunit complex functions as a macromolecular assembly communicating through physical interactions with other conserved signaling protein complexes, such as the septation initiation signaling (SIN) network. STRIPAK is involved in a broad variety of developmental processes in higher and lower eukaryotes. For example, the proliferation of several mammalian cancer cells is correlated with dysfunctional STRIPAK subunits [1], and in fungal microorganisms, the lack of STRIPAK results in sexual infertility, defects in hyphal fusion, reduced secondary metabolite production, and impaired pathogenicity or symbiotic interactions [1].
Recent proteome and phosphoproteome studies, using wild-type and mutant strains from S. macrospora, have identified putative STRIPAK target proteins such as CDC7, SmKIN3, DBF2, which are components of SIN [2,3]. We propose that deletion of STRIPAK subunits results in higher phosphorylation of CDC7, which prevents assembly of SIN. This results in a lower level of phosphorylated SmKIN3, which consequently is unable to phosphorylate the downstream kinase DBF2. Subsequently, signaling of SIN is impaired, affecting the localization of a RHO4/BUD3 GTPase module assembled by its anillin-type scaffold, BUD4.
In order to analyze the function of genes encoding subunits of the SIN signaling pathway, we have constructed deletion and complementation strains. For example, we found that deletion strains, lacking genes for SIN components have a sterile phenotype. The deletion mutants will be used to investigate phosphomimetic and -deficient variants of SIN subunits for identifying conserved (de)phosphorylation residues. The investigation presented here will contribute to the overall mechanistic understanding of how STRIPAK controls cellular development in euascomycetes [4].
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902W *Aspergillus fumigatus* Septation Initiation Network (SIN) kinases contribute to fungal pathogenesis, cell wall construction, and rRNA metabolism. *Xabier Guruceaga*[1], Adela Martin-Vicente[1], Ana Camila Oliveira Souza[2], Ashley V. Nywening[1], Harrison Thorn[1], Jinhong Xie[1], Wenbo Ge[1], Brian M. Peters[1,3], Jarrod R. Fortwendel[1,3] 1) Department of Clinical Pharmacy and Translational Science, College of Pharmacy, University of Tennessee Health Science Center, Memphis, Tennessee, United States of America.; 2) Department of Pharmacy and Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, Tennessee, United States of America.; 3) Department of Microbiology, Immunology, and Biochemistry, College of Medicine,University of Tennessee Health Science Center, Memphis, Tennessee, United States of America..

Aspergillus fumigatus is the main causative agent of invasive aspergillosis and the therapeutic armamentarium to fight against this disease is limited. Therefore, we sought to identify genetic pathways that might serve as novel therapeutic targets through generating and studying an *A. fumigatus* protein kinase disruption mutant library. Our previous work found the SIN kinase genes, *sepH*, *sepL* and *sidB*, to be essential for hyphal septation and survival under cell wall stress. We also found that loss of hyphal septation resulted in almost complete avirulence characterized by lack of tissue invasion. Interestingly, co-culture of mutant and wild type conidia with a macrophage cell line revealed reduced ability of the SIN kinase mutants to elicit pro-inflammatory signaling evidenced by reduced IL-1β and TNFα release. To see if the cell wall hypersusceptibility and reduced immunogenicity phenotypes of the SIN kinase mutants were due to altered cell wall formation or hyphal development, we performed *in vitro* phenotypic characterization of the mutant strains coupled with RNAseq profiling. Interestingly, conidia of the mutant strains were found to initiate germination earlier than wild type and hyphae formed by the mutant germlings were significantly longer than the wild type at the same developmental timepoint. In contrast, the mitotic rate was similar between all the strains, suggesting that loss of SIN activity uncoupled the processes of mitosis polarized morphogenesis. Hyphal staining revealed altered distribution of chitin cell wall deposition and reduced β-glucan levels in the Sin kinase mutants. RNAseq analyses identified 997, 625 and 337 genes from the Δ*sepH*, *sepL-1*, and *sidB-1* mutant strains, respectively, that were differentially expressed when compared to the wild type. Of these genes, only 28 were downregulated in common between the three mutant strains. Gene Ontology enrichment analyses revealed that 45% of these genes were related with transport processes, 34% with RNA metabolic process and 28% with ribosome biogenesis. Our results suggest that SIN kinase activity is essential for normal germination and mitosis and for proper cell wall formation. Although no direct transcriptional link to cell wall biosynthesis was uncovered, RNAseq analyses suggest hyphal septation in *A. fumigatus* is linked to cellular transport, RNA metabolism and ribosome biogenesis.

903V Structure-activity predictions from computational mining of protein databases to assist modular design of antimicrobial peptides Claudia Feurstein[1], Vera Meyer[1], *Sascha Jung*[1] 1) Chair of Applied and Molecular Microbiology, Institute of Biotechnology, Technische Universität Berlin, 10263, Berlin, Germany.

Antimicrobial peptides (AMPs) are promising alternatives to antibiotics to fight multidrug resistant microorganisms. However, despite thousands of AMP entries in respective databases, predictions about their structure-activity relationships are still limited. Similarly, common or dissimilar properties of AMPs that have evolved in different taxonomic groups, e.g., fungi, bacteria, mammals, are nearly unknown.
We leveraged data entries for 10,987 peptides currently listed in the three antimicrobial peptide databases APD, DRAMP and DBAASP to aid structure-activity predictions. However, this number reduced to 3,828 AMPs that we could use for computational analyses, due to our stringent quality control criteria. The analysis uncovered a strong bias towards AMPs isolated from amphibians (1,391), whereas only 35 AMPs originate from fungi (0.9%), hindering evolutionary analyses on the origin and phylogenetic relationship of AMPs. The majority (62%) of the 3,828 AMPs consist of less than 40 amino acids but with a molecular weight higher than 2.5 kDa, have a net positive charge and share a hydrophobic character. They are enriched in glycine, lysine and cysteine but are depleted in glutamate, aspartate

and methionine. Remarkably, the γ-core motif claimed so far as an ancient unifying structural signature in cysteine-stabilised AMPs is absent in nearly 90% of the peptides. However, this motif appears to be of high relevance with respect to fungi. Firstly, antifungal AMPs show an increased presence of the γ-core motif, and, secondly, almost 50% of AMPs originate from fungi contain the γ-core motif. The disclosure of AMPs pattern and their variation in producing organism groups extends our knowledge of the structural diversity of AMPs and will assist future peptide screens in unexplored microorganisms. Structural design of peptide antibiotic drugs will benefit using natural AMPs as lead compounds. However, a reliable and statistically balanced database is missing which leads to a large knowledge gap in the AMP field. Particularly, AMPs from fungi depict a minority in current AMP databases and, furthermore, testing available AMPs for their antifungal activity is significantly neglected compared to antibacterial activities. Thus, thorough evaluation of the available data, mitigation of biases and standardised experimental setups need to be implemented to leverage the full potential of AMPs for drug development programs in the clinics and agriculture.

904V Multiple tolerance mechanisms to the plant saponin α-tomatine in *Botrytis cinerea* *Yaohua You*[1], Suraj Hassan Muralidhar[1], Andrea Lorena Herrera Valderrama[1], Paul Ruigrok[1], Linda Matz[3], Oostlander Anne[3], Xiaoqian Shi[1], Frank Pieterse[1], Edgar A. Chavarro-Carrero[1], Si Qin[1], Iris Kappers[2], André Fleißner[3], Jan van Kan[1] 1) Wageningen University, Laboratory of Phytopathology, Wageningen, The Netherlands; 2) Wageningen University, Laboratory of Plant Physiology, Wageningen, The Netherlands; 3) Technische Universität Braunschweig, Institute of Genetics, Braunschweig, Germany.

α-Tomatine, a steroidal glycoalkaloid saponin, is abundant in vegetative tissues of tomato and has antibiotic activities. Secretion of glycosyl hydrolases (GH) to enzymatically degrade α-tomatine into less toxic products is the only mechanism reported in tomato pathogens so far.
A unique tomatinase activity (β-xylosidase) in *Botrytis cinerea* has been reported more than 20 years ago, however, attempts to clone this gene based on homology to other tomatinase genes failed. In this study, we identified α-tomatine-inducible genes in isolate B05.10 by RNAseq. We observed strong induction of genes encoding a GH from the GH43 family, two glycosyl transferases (GT) from the GT28 family as well as membrane proteins including one ABC transporter and multiple RTA1-like proteins. In addition, the genome sequencing of *B. cinerea* isolate M3a, which is unable to degrade α-tomatine, revealed a missing locus compared with B05.10 comprising the *BcGH43* and one *BcGT28* due to the insertion of a transposable element.
Recombinant BcGH43 protein exhibited *in vitro* tomatinase activity through hydrolysis of the terminal xyloside of α-tomatine. The B05.10 knockout (KO) mutant of BcGH43 lost tomatinase activity, became more sensitive to α-tomatine and less virulent on tomato. Moreover, the overexpression (OE) of either the *BcGH43* or the other two types of tomatinase genes from *Septoria lycopersici* (GH3) and *Cladosporium fulvum* (GH10) separately in M3a conferred tomatinase activities, and restored the virulence on tomato.
The deletion of the *BcGT28* (absent in M3a) in B05.10 reduced its virulence on tomato but did not impact α-tomatine sensitivity. However, OE of *BcGT28* in M3a led to elevated resistance to α-tomatine and increased virulence on tomato. Upon α-tomatine treatment, the GT28-GFP fusion protein was recruited from cytosol to the membranes of hyphal tips (abundant in ergosterol) indicating the importance of membrane modification in resistance to α-tomatine and virulence on tomato. We are in the process of analysing glycosylated fungal membrane compounds in response to α-tomatine in wild-type B05.10 with reduced/abolished glycosylation pattern in the *BcGT28* single and double KO mutants to elucidate the mechanism underlying membrane modification conferred by BcGT28. Besides, KO mutants and OE transformants of other α-tomatine-inducible genes in B05.10 and M3a have been generated and are currently under characterization.
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905V Flower bulb waste material is a natural niche for the sexual cycle in *Aspergillus fumigatus* *Jianhua Zhang*[1], Paul Verweij[2], Antonius Rijs[2], Alfons Debets[1], Eveline Snelders[1] 1) Wageningen University; 2) Radboud University Medical Center, Nijmegen, the Netherlands.

With population genetic evidence of recombination ongoing in the natural *Aspergillus fumigatus* population and a sexual cycle demonstrated in the laboratory the question remained what the natural niche for *A. fumigatus* sex is. Composting plant-waste material is a known substrate of *A. fumigatus* to thrive and withstand temperatures even up to 70°C. Previous studies have shown indirect evidence for sexual reproduction in these heaps but never directly demonstrated the sexual structures due to technical limitations. Here, we show that flower bulb waste material from stockpiles undergoing composting can provide the conditions for sexual reproduction. Direct detection of ascospore structures was shown in agricultural flower bulb waste material by using a grid-based detection assay. Furthermore, we demonstrate that ascospores can germinate after exposure to 70°C for up to several days in contrast to asexual conidia that are unable to survive a two-hour heat shock. This indicates a sufficient time frame for ascospores to survive and escape composting stockpiles. Finally, sexual crosses with cleistothecium and viable ascospore formation could successfully be performed on flower bulb waste material. Recombination of *A. fumigatus* can now be explained by active sexual reproduction in nature as we show in this study that flower bulb waste material provides an environmental niche for sex.