ABSTRACTS

INTERNATIONAL UNION FOR THE STUDY OF SOCIAL INSECTS

IUSSI 2010

XVI CONGRESS IN COPENHAGEN DENMARK

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Abstracts

Abstracts for the XVI Congress of the International Union for the Study of Social Insects
Copenhagen, Denmark, 8-13 August 2010.

ISBN
978-87-92100-00-9

Editors
David R. Nash
Susanne P.A. den Boer
Henrik H. De Fine Licht
Jacobus J. Boomsma

Graphic design & Picture credits
Cover design by Leif Bolding
Termite photographs by Pepijn Kooij
Bee, Ant and Wasp photographs by David Nash
Conference logo designed by Claus Lynggaard, Oktan

Printed by
Clausen Offset ApS
Cikorievej 20
5220 Odense
Denmark

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*For multi-authored talks and posters, the presenting author is marked **

Keynote & Plenary lectures

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Keynote address

JUVENILE HORMONE AND DIVISION OF LABOR RESEARCH: A NEW RELATIONSHIP FOR A NEW ERA

Gene E. Robinson

Department of Entomology, University of Illinois at Urbana-Champaign, USA

This lecture will use juvenile hormone (JH) as a lens through which to view research on division of labor, past, present and future. Drawing primarily on studies of division of labor among workers in honey bee colonies, I will show how JH studies also provide deeper insights into both the biology of social insects and the changing nature of biological research. Topics include: evolutionary aspects of JH regulation; connections between JH, insulin signaling and nutrition; and new, systems biology-inspired, views of JH. I will close by using this example of an integrative analysis of organismal biology to consider challenges and opportunities as a new era in biological research begins.

Bumble bees as model systems in behaviour and ecology

Andrew F. G. Bourke

School of Biological Sciences, University of East Anglia, UK

The study of social insects, like other fields of research, advances through a variety of approaches, including model-system approaches, field studies and comparative analyses. The field has its existing and candidate model systems, and each has its particular strengths and weaknesses. In this presentation I describe work of my group using bumble bees as model systems. A major interest of our group, as for the field as a whole, is in understanding the selective basis of within-colony conflicts. The bumble bee *Bombus terrestris* is an excellent model system in this respect because its colonies are relatively small and short-lived, so in principle it should be feasible to calculate the inclusive fitness of all colony members. In addition, the work of other groups both past and present has provided an invaluable knowledge base on the social physiology, host-parasite relationships and, increasingly, the genomics of *B. terrestris*. I describe our current understanding of social conflict in *B. terrestris*, focussing on queen-worker and worker-worker conflict over egg-laying and the behaviour of reproductive 'drifter' workers. Another major research interest of ours concerns the use of genetic methods to estimate the foraging ranges and densities of wild bumble bees. This is to address basic questions on the spatial ecology and population biology of pollinating insects and also to gain a better understanding of the ecology of wild bees of conservation concern. I describe what we and colleagues have learned from our application of such methods to both common and scarce bumble bees. Overall, bumble bees represent outstanding model systems with which to address many pressing questions in the study of social insects and beyond.

How shortsighted is mother nature, and does it matter?

Hanna Kokko[1,2]

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2. Research School of Biology, Australian National University, Australia

Group adaptation is a much debated concept in the study of animal societies. While recent work has highlighted that kin and group arguments are interchangeable to some extent at least, group adaptations do not evolve very easily. This means that populations are often expected to grow or persist less well than they could if individual-level didn't lead to 'selfish' behaviours or traits. While there is nothing new in stating that, explicit quantifications of the discrepancy between what is good for populations and what is good for individuals remain rare. This discrepancy is modulated by the degree of conflict between individuals, and its relevance extends far wider than cooperative traits (the usual targets of studies that investigate this principle). I will examine it in contexts such as dispersal, territorial fights, and mate choice. Using a bird example, I will argue that even conservation biology could benefit from explicitly acknowledging that individuals are not expected to behave in species-beneficial ways. I will also encourage more explicit modelling of evolutionary timescales when debating levels of selection.

GENOMICS OF PHEROMONE COMMUNICATION IN HONEY BEES

Christina M. Grozinger

Department of Entomology, Pennsylvania State University, USA

Chemical communication plays an important role in many species, and is particularly critical for regulating behavior and colony organization in social insects. Pheromone production and response are highly variable, and can be altered by an individual's physiological state, behavioral state, or the environmental context. How modulation of pheromone signaling is orchestrated at the molecular level and the role it may play in regulating individual behavior and colony organization remain largely uncharacterized. To begin to address these questions, we are studying the pheromones that regulate queen-worker and worker-worker interactions in honey bees. The pheromone released by honey bee queens regulates many aspects of worker bee physiology and behavior, including behavioral maturation and division of labor. We have demonstrated that queen pheromone production is strongly linked to the mating quality and reproductive state of the queen, and this can have consequences on worker-queen interactions, worker physiology, and queen longevity. Furthermore, we have found that worker responses to queen pheromone are highly variable, and are associated with differences in worker physiology, reproductive potential, and brain gene expression patterns. Additionally, the chemical signals that mediate worker-worker interactions can be modulated by social context and physiological state, resulting in altered social behavior among workers, which may play a role in kin recognition and disease transmission. We are now extending these studies to other social insect species, to determine if the genes associated with pheromone response are conserved, and to begin to elucidate the molecular mechanisms underlying the evolution of pheromonal regulation of social behavior.

INTERACTION NETWORKS, FORAGING ECOLOGY, AND THE EVOLUTION OF COLLECTIVE BEHAVIOR IN HARVESTER ANTS

Deborah M. Gordon

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Interaction networks regulate the behavior of social insect colonies. A 25-year study of a population of the red harvester ant, *Pogonomyrmex barbatus*, in the southwest US, links colony behavior, and its development, to the ecology of the population. An ant's task decisions are based on its experience of its brief antennal contacts, in which one ant asseses the cuticular hydrocarbon profile of another. These interactions provide no information other than the encounter itself. Experiments with artificial ant mimics coated with cuticular hydrocarbon extract show that the onset of foraging is stimulated by interactions between returning patrollers, who first explore the foraging area, and foragers. Once foraging begins, the moment-to-moment intensity of foraging is regulated by interactions between foragers returning to the nest with food, and inactive foragers inside the nest,. The rate of forager return is a measure of current food availability because the duration of a forager's trip depends mostly on search time; the more food is currently available, the more quickly successful foragers return. Colonies regulate foraging to manage a trade-off between water loss when foraging and loss of foraging area to neighbors when not foraging, in the context of changing conditions that determine food abundance. Colonies differ in how they regulate foraging behavior, because of differences among colonies in the algorithm that links foraging intensity to forager return rate. Examining variation among colonies in collective behavior, and the ecological consequences of this variation, opens the way to the empirical study of the evolution of collective behavior.

SOCIAL EVOLUTION IN MICROBES

Kevin R. Foster

Center for Systems Biology, Harvard University, USA

"If it could be proved that any part of the structure of any one species had been formed for the exclusive good of another species, it would annihilate my theory, for such could not have been produced through natural selection." Darwin (1859)

Since Darwin, evolutionary biologists have been troubled by cooperation. Many insect workers labor their whole life without reproducing, birds make alarm calls, and humans often help one another. Why do organisms frequently evolve behaviors that help others at an apparent cost to their own reproduction? This fundamental question has received considerable attention over the last 50 years. The result is a solid base of theory, centered on principles like inclusive-fitness, and a myriad of empirical tests; many of which have made use of the amazing sociality seen in the insects. It is now widely accepted that cooperative behaviors evolve because they directly help the actor alongside recipients, or they help individuals who share more alleles with the actor than predicted by chance (genetic relatedness), or both. One major group that remains relatively unexplored, however, is the microbes, whose full spectrum of sociality only recently came to light. We study how social environment and relatedness affects microbial behavior using a combination of theory and a number of model systems, including pathogenic bacteria, slime molds and budding yeast. I will argue that genetic relatedness is likely to be as important for microbes as it is for the insects. However, we also find evidence for novel mechanisms that can stabilize the evolution of cooperative traits. These include pleiotropic genes that link cooperation to a selfish benefit and the evolution of prudent regulation by which individuals cooperate only when it costs them little to do so. I hope to convince you that the microbes, like the insects, provide a wealth of fascinating systems with which to study and understand social behavior.

INFORMATION TRANSFER AND THE EVOLUTION OF COLLECTIVE ANIMAL BEHAVIOUR

Iain D. Couzin

Department of Ecology and Evolutionary Biology, Princeton University, USA

A fundamental problem in a wide range of biological disciplines is understanding how functional complexity at a macroscopic scale results from the actions and interactions among individual components. Animal groups such as insect swarms and fish schools frequently exhibit complex and coordinated collective behaviors and present unrivalled opportunities to link the behavior of individuals with the functioning and efficiency of group-level properties.Using an integrated experimental and theoretical approach involving both insects and vertebrates I will address both how, and why, animals coordinate behavior. In some animal groups individual decision-making is so integrated that it has been associated with the concept of a "collective mind". Since each organism has relatively local sensing ability, such animal groups have evolved collective strategies that allow individuals to access higher-order computational abilities at the group level. I investigate the coupling between spatial and information dynamics in groups and reveal the critical role uninformed individuals play in effective consensus decision-making.Understanding mechanisms that drive specialization within initially homogeneous populations is a fundamental challenge for evolutionary theory. It is an issue of relevance for significant open questions in biology concerning the generation of biodiversity, the origins of reciprocal cooperation, and the efficient division of labor in social or colonial organisms. I will explore the relationship between collective behavior and game theoretic dynamics using a framework generally applicable to situations where information can be considered a public good. Finally, an experiment will be discussed in which real predators are used to exert selection pressure on virtual prey populations, allowing us to estimate both within-group and between-group selection. Our results provide insights into the proximate and ultimate factors that underlie evolved collective behavior.

THE EVOLUTION OF TERMITE-EGG MIMICRY BY TERMITE-BALL FUNGI: HOW THE FUNGI CONTROL TERMITE SOCIAL BEHAVIOR

Kenji Matsuura

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Among the great diversity of insect-fungus associations, fungal mimicry of termite eggs is a particularly fascinating consequence of evolution. Along with their eggs, *Reticulitermes* termites often harbour sclerotia of the fungus *Fibularhizoctonia sp.*, called 'termite balls', giving the fungus competitor-free habitat within termite nests. The fungus has evolved sophisticated morphological and chemical camouflage to mimic termite eggs. In this talk, I would like to show how the fungus mimics termite eggs so as to control egg protection behavior of termites and discuss the evolutionary process of this interaction. We found that the termite egg-recognition pheromone consists of the enzyme beta-glucosidase and the antibacterial protein lysozyme. Both enzymes are major salivary compounds in termites and are also produced in termite eggs. Termite balls were tended by termites only when the fungus produced beta-glucosidase, indicating that the fungus mimics termite eggs chemically by producing beta-glucosidase. Our results demonstrate that termites produce beta-glucosidase for cellulose digestion as a primary function and also use it for egg recognition as a secondary function, indicating that the overlap of the cellulose digestion niche between termites and the fungus sharing the same chemicals provided the opportunity for the origin of termite egg mimicry by the fungus. Recently, we found a novel type of termite ball 'Z-type' in the subtropical termite, *Nasutitermes takasagoensis*. Phylogenetic analysis indicated that Z-type is an undescribed Trechispoloid fungus, *Trechispora* sp., which is phylogenetically distant from *Fibularhizoctonia*, indicating at least two independent origins of termite-egg mimicry in sclerotium-forming fungi. Egg mimicry, by which the fungus can easily gain access to the centre of the nest, seems to be an evolutionary loophole around anti-parasite defense in termites. Termite-egg mimicry by these fungi offers a model case of parallel evolution.

Oral Presentations

From colonies to communities: the consequences of behaviour for communities

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COMPETITION IN ANT COMMUNITIES: NOT ENOUGH THEORY?

Donald H. Feener Jr.

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Competition among ant species in local communities typically show three common features: 1) remarkable diversity in the behavioral form of these interactions, 2) asymmetric outcomes with the advantage often going to the colony with the larger worker force, and 3) linear dominance hierarchies among competing species. Linear dominance hierarchies should lead to competitive exclusion and reduced local diversity in the absence of compensatory mechanisms that prevent competitive dominants from eliminating competitive subordinates. I argue that interspecific trade-offs can often serve as compensatory mechanisms that facilitate coexistence in ant communities and that one major goal of ant community ecology should be to categorize these trade-offs and understand how they emerge from the behavior of individual workers and colonies. Here I focus on four distinct interspecific trade-offs that are likely function in ant communities: 1) the trade-off between resource dominance and resource discovery, 2) the trade-off between resource dominance and vulnerability to natural enemies, 3) the trade-off between resource dominance and vulnerability to abiotic extremes, and 4) the trade-off between resource dominance and colonization ability. I show how each of these trade-offs may arise naturally from individual or colony-level behavior and argue that the development of mechanistic models that integrate these trade-offs into a general framework will help us predict how they interact with one another to influence the patterns of species coexistence at different spatial scales.

VENOM TOXICITY, AGGRESSION AND COEXISTENCE PATTERNS BETWEEN *MONOMORIUM* AND ARGENTINE ANTS.

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The ability of native ants to coexist with invasive species varies considerably. Ant species in the genus *Monomorium* are known to coexist with invasive species such as the Argentine ant (*Linepithema humile*). This coexistence is thought to be related to the use and toxicity of *Monomorium* venom. We have been examining patterns of aggression, venom composition and toxicity, and patterns of co-occurrence of Argentine ants with four *Monomorium* species in New Zealand. The venom of all four *Monomorium* species was observed to be chemically different, although the toxicity to Argentine ants was similar. Short-term experiments showed major differences in the survival of co-occurring *Monomorium* and Argentine ants, which behavioural assays showed to be a result of varying levels of aggression. Two *Monomorium* species were highly aggressive and quickly killed Argentine ants. Nevertheless, coexistence could be achieved between these aggressive *Monomorium* species and Argentine ants depending on the colony size of each species. The use of venom by a third *Monomorium* species resulted in the Argentine ants attacking and killing each other. A fourth *Monomorium* species showed no aggression or use of venom in the presence of Argentine ants. Clearly the use of venom by *Monomorium* can be important, but a variety of behavioural factors contribute to the coexistence of such species with Argentine ants.

THE EVOLUTIONARY DYNAMICS OF SOCIAL FORAGING IN ANTS: THE INFLUENCE OF COMPETITION AND RESOURCE DISTRIBUTION.

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Ants use species-specific foraging strategies, either searching for food individually or collectively, by recruiting other workers. What are the evolutionary and ecological conditions that promote the sympatric emergence and coexistence, in the same community, of such diversity of strategies? By means of mathematical modelling, we analyse the consequences of both inter-specific competition and resource distribution for the evolutionary dynamics of social foraging in ants. The evolution of social foraging behaviour is modelled by a stochastic mutation-selection processes relying on the interactions between colonies. Colonies interactions within the community depend on the specific foraging strategies, the distribution of resource, and the degree of asymmetry in competition. We first show how the foraging strategy (the degree of social foraging) is involved in a double trade-off between resource discovery, and both resource exploitation and dominance, and we propose a model of foraging processes that reflects the mechanisms underlying these trade-offs. Then, at the evolutionary timescale, we identify the conditions of competition asymmetry and resource distribution that lead to the emergence and coexistence of both collective and individual foraging strategies. We predict that niches partitioning is not essential in the emergence and maintain of foraging strategies diversity in a sympatric ants community.

EXPLORING THE DISCOVERY-DOMINANCE TRADE-OFF AND THE ROLE OF HABITAT COMPLEXITY

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2. Department of Zoology, LaTrobe University, Australia

Interspecific trade-offs are thought to facilitate co-existence between species at small spatial scales (e.g. Kneital & Chase 2004). One trade-off that is widely accepted in ant ecology is the `discovery-dominance' trade-off. This trade-off between a species' ability to discover food resources and to dominate and gain control of them is thought to explain how so many species apparently dependent on similar resources can co-exist. While several authors have found support for this trade-off, it is thought to be broken by invasive or territorial species that are able to both locate and capture resources more efficiently than other species (e.g. the Argentine ant). Other potential mediators of this relationship include parasitoids, temperature and habitat structure. Here we investigate the generality and form of the discovery-dominance relationship in habitats of contrasting complexity across three continents (Europe, Australia and Africa). In addition we review empirical studies to assess whether the discovery-dominance trade-off tends to be broken by invasive and territorial species. From our own fieldwork and a review of the literature we conclude that the discovery-dominance trade-off is more often the exception rather than the rule. Using experimental data from simple and complex habitats within each continent we explore the role of habitat complexity in mediating the discovery-dominance relationship and in facilitating co-existence between species.

FAST FOOD IN ANT COMMUNITIES: HOW COMPETING SPECIES FIND RESOURCES

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Omnivorous woodland ant species trade-off between the ability to find and control food resources. While resource dominance by ant species has been well-characterized, the mechanisms underlying discovery ability are largely unexplored. The effect of forager number and forager discovery capacity on resource discovery was examined in two disparate temperate ant communities, located in Texas and Arizona. Forager discovery capacity was defined as the per capita rate of resource discovery, or how quickly an individual forager arrived at a resource. Both within communities and within species, more foragers meant that resources were discovered more quickly, suggesting that resource discovery is a matter of random processes, with ants essentially bumping into resources at a rate mediated by their abundance. Species-specific discovery ability, defined as the proportion of resources a species discovered first, was not determined by mean forager number. Instead, both forager number and forager discovery capacity determined discovery success. Texas species used both forager number and capacity, whereas Arizona species used only forager capacity. There was a negative correlation between a species' prevalence in the environment and the discovery capacity of its foragers, suggesting that a given species cannot exploit both high numbers and high discovery capacity as a strategy. The two aims of future work should be a) to determine how such characteristics as the sensory detection of resources, search patterns, and biomechanics interact to define forager discovery capacity and b) to contrast discovery speed and discovery mechanisms across a broader range of communities.

EFFECTS OF A GEOGRAPHIC MOSAIC OF ANT AGRESSIVENESS ON HERBIVORE COMMUNITIES

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Interactions between species are usually context-dependent. We examined how the functional responses of herbivores to plant-defending ants depend on community context. We compared the behavior of a phytoecious ant, *Azteca pittieri,* which is a symbiotic mutualist of the myrmecophytic plant, *Cordia alliodora*, across several Neotropical dry forest communities in Mexico and Costa Rica. *Azteca pittieri* can aggressively defend the leaves of *C. alliodora* against leaf-eating herbivores. We found that *A. pittieri* ants are, on average, better defenders of plant leaves at some sites than others. We asked whether these consistent differences in ant defense of *C. alliodora* could lead to differences in herbivore abundances among sites. Two measures of the effectiveness of ant defense, the number of ants patrolling leaves and how quickly the ants were able to evict caterpillars, were significantly different across sites and were positively correlated with ant-colony size. Levels of foliar herbivory were negatively correlated with the effectiveness of ant defense, and were also significantly different across sites. A 32-year database of caterpillar host records from Costa Rica indicates that most of the *C. alliodora* herbivores that occur at all of the sites have a specialized diet primarily restricted to *Cordia* or the family Boraginaceae. We hypothesized that greater ant aggressiveness would lead to a reduced food source and lower abundances of these herbivores. Surveys of caterpillar abundances on *C. alliodora* trees supported this hypothesis: caterpillar abundances were higher at sites where ant defense is less effective. Interestingly, the observed diversity of caterpillar species was also higher at sites with less effective ant defense. The unknown factors that cause the differences in effectiveness of ant defense and ant-colony size across sites may therefore indirectly drive the functional responses of at least 16 common herbivore species in this system.

Poster Presentations

From colonies to communities: the consequences of behaviour for communities

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1-1 DOES FOREST FRAGMENTATION AFFECT PATTERNS OF ANT-DIASPORE INTERACTION? A STUDY CASE USING SYNTHETIC DIASPORES

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Ants are frequently encountered interacting with non-myrmecochoric fleshy diaspores on the ground of tropical forests. While ants benefit by using the fleshy parts of the diaspores to complement their diets, diaspores can benefit from this interaction in two ways: (1) ants often consume the fleshy part on the spot, enhancing the germination chances of cleaned seeds; and (2) ants may carry the diaspore to the nest, offering a directed dispersal benefit. As forest fragmentation affects many attributes of the ecosystem such as species abundance, species composition, and interaction among species, we asked whether fragmentation affects the chance of a given diaspore being found or removed by ants. We controlled diaspore type and quantity by offering synthetic fruits (a red plastic "seed" covered by a whitish lipid-based "pulp") at 30 stations (five diaspores per station) in each of eight areas (four continuous forests and four fragments). We recorded (1) the attracted ant species; (2) their behavior (recruitment of nestmates, dominance, seed-cleaning and seed-carrying behavior); and (3) diaspore removal distance. Fragmented and continuous forests differed in all three aspects investigated. Ant species frequency differed between the two habitats. For instance, while the frequency of big ponerine ants (*Pachycondyla* and *Odontomachus*) was higher in the preserved forests, a *Megalomyrmex* species was more frequently seen in the fragments. As species frequency at stations differed between habitats so did their behavior toward the diaspores. Removal was higher in continuous forests than in fragments (3.88 vs. 1.44 diaspores removed per station), whereas seed-cleaning behavior on spot was more common in fragments. Moreover, removal distance was greater in continuous than in fragmented forests. Our data suggest that fallen diaspores interacting with ground-dwelling ants face different fates depending on whether the forest is continuous or fragmented. (Funds: FAPESP, CNPq)

1-2 CONSUMPTION OF NON-MYRMECOCHOROUS DIASPORES POSITIVELY AFFECTS LARVAL DEVELOPMENT IN *ODONTOMACHUS CHELIFER* ANTS

Claudia Bottcher*[1], Paulo S. Oliveira[2]

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Fleshy diaspores comprise a large portion of the litter on the floor of tropical forests, and interactions involving litter-dwelling ants and diaspores are common in these areas. A diaspore is defined as any seed, fruit, or infructescence that constitutes the unit of dispersal of the plant. Although primarily carnivorous, *Odontomachus chelifer* ants (Ponerinae) commonly collect fallen fleshy diaspores of many tree species in the Atlantic forest of Brazil, and are considered important secondary seed dispersers on the ground of tropical humid forests. Although ant-derived benefits to non-myrmecochorous plants are well-documented, so far the consequences for the ants have not been demonstrated. To test the hypothesis that consumption of fleshy diaspores would benefit ant colonies, we examined the effect of lipid-rich seeds of *Cabralea canjerana* on colonies of *O. chelifer* under controlled conditions in captivity. Colony size and larval production did not differ between treatment and control groups. However, ant larvae fed with *C. canjerana* diaspores were on average heavier and developed better than control larvae. Because *O. chelifer* improves seed germination in *C. canjerana* through removal of the lipid-rich seed aril, demonstration of a reciprocal benefit to ant larvae confirms that this ant-seed interaction is a true facultative mutualism. (Funds: FAPESP, CNPq)

1-3 EFFECTS OF FIRE ON INTERACTIONS BETWEEN ANTS AND TWO SPECIES OF MYRMECOCHOROUS PLANTS

Kieren Beaumont, Duncan Mackay*, Molly Whalen

School of Biological Sciences, Flinders University, Australia.

Myrmecochory is a common seed dispersal strategy of plants in fire-prone sclerophyll vegetation in Australia. We investigated the initial fate of seeds of two myrmecochorous plant species, *Pultenaea daphnoides* and *Acacia pycnantha*, in burnt (3.25 years since fire) and unburnt (53 years since fire) forest at three sites in the Mount Lofty Ranges near Adelaide, South Australia. Specifically, we measured 1) seed removal rates, 2) the frequency of three ant-seed interactions (seed removal, elaiosome robbery and seed ignoring), 3) the relative contribution of different ant species to ant-seed interactions and 4) the abundance of common interacting ant species. Rates of seed removal from depots, and the proportion of seeds removed, were higher in burnt vegetation and the magnitude of these effects was greater for the smaller seeds of *P. daphnoides*. The proportion of seeds with elaiosomes robbed was higher in unburnt vegetation; however, the decrease in elaiosome robbery in burnt vegetation was greater for *P. daphnoides* than for *A. pycnantha*. Ants ignored seed more frequently in burnt vegetation and at similar rates for both seed species. In total, twenty ant species were observed interacting with seeds, however, three common ant species accounted for 66% of ant-seed interactions. *Monomorium sydneyense* almost exclusively robbed elaiosomes, *Rhytidoponera metallica* typically removed seeds, and *Anonychomyrma nr. nitidiceps* showed a mix of three behaviours towards seeds. Differences in the frequency of seed removal, elaiosome robbery and seed ignoring appeared to be largely driven by the increased abundance of *A. nr. nitidiceps* and *M. sydneyense* in burnt and unburnt vegetation, respectively. Understanding how these fire-driven changes in the initial fates of myrmecochorous seeds affect plant fitness requires further investigations.

1-4 EFFECTS OF FIRE AND ELAIOSOME CONDITION ON DISPERSAL BY ANTS OF SEEDS OF A MYRMECOCHOROUS PLANT, *PULTENAEA DAPHNOIDES*

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The fates of seeds dispersed by ants may be influenced by the relative contribution of different ant species and this may change in relation to fire history. A second determinant is variation in the proportion of seeds discarded outside nests following consumption of the elaiosome. This study describes how the frequency, rate and distance of seed dispersal are altered by fire and elaiosome condition. Seed removal rate trials were conducted at four sites in South Australia, each with a recently burnt and long unburnt plot, with seeds of *Pultenaea daphnoides*, and the effects of four elaiosome treatments (“Intact elaiosome”; “Handled elaiosome” (Seeds previously manipulated in nests of *Rhytidoponera*); “Cut elaiosome” (ca. 50% of the elaiosomes removed), and “No elaiosome” (elaiosomes removed)) on seed removal were investigated. Fire altered removal rates and the relative contribution of different ant species to dispersal. Seed removal was significantly faster in burnt plots than in unburnt plots. Seeds previously handled by ants were not removed faster or slower than seeds with intact or cut elaiosomes. Seeds without elaiosomes were removed significantly more slowly than seeds from all other treatments. *Rhytidoponera metallica* was the main seed-dispersing ant in both burnt and unburnt plots. Seeds were dispersed an average of 51 cm and 71 cm in burnt and unburnt habitats, respectively. This difference is due to the greater contribution to dispersal by *Pheidole* ants in burnt plots. In an experiment involving feeding seeds to nests, individual colonies discarded between 0% and 100% of the seeds fed to them. *R. metallica* consumed approximately 50% of the elaiosomes of discarded seeds; only 3% had their elaiosomes completely removed. *Pheidole* colonies did not discard any seeds. Multiple dispersal pathways are facilitated by *R. metallica* which often discards seeds that have some elaiosome attached and thus may be secondarily dispersed.

1-5 Ant diversity in Croatian peat bogs

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Peat bogs are rare and exceedingly localised habitats in Croatia. In terms of their biogeography, their presence in Croatia represents a southern enclave of their northern continuous distribution. These ecosystems are characterised with a specific set of conditions that lead to the development of specialised flora and fauna. Due to their small size, isolation and drainage, and especially to progressive vegetation succession following the abandonment of traditional human activities, these habitats are among the critically endangered habitats in Croatia. In 2008, the ant fauna was studied by pitfall trapping during a seven month period at two acidophilous bogs and their surrounding habitats. A total of 24 ant species and 16 806 individuals were caught in the studied sites. Peat bogs were strongly dominated by *Myrmica* species - *M. rubra* and *M. scabrinodis*. The bog specific species *M. scabrinodis* was found in Croatian largest peat bog, while it was absent from small bog. These species were also present in surrounding habitats though in a much smaller extent. Other true bog specialists, such as *Formica uralensis* and *F. picea*, were not found. Forest habitats were primarily dominated with *Lasius* species. Difference in ant abundance and species richness amongst acidophilous peat bogs is most likely due to the varying bog size, water table and vegetation composition. Like the flora and carabid beetle fauna of peat bogs, it appears that the ant fauna on Croatian bogs is impoverished.

1-6 Density dependent effect of a *Formica exsecta* supercolony on ant community composition and foraging success of rivals

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Territorial ant species, situated on the highest level of the competitive hierarchy, act like community organizing centres. They influence the presence of sub-dominant species, regulate their abundance and can alter their foraging behaviour. Those territorial ant species which develop large polydomous systems (so-called supercolonies) are expected to have even greater impact on local insect (especially ant) communities. We studied the spatial distribution of the territorial *Formica exsecta* within a large supercolony, and the nature and strength of its influence on other co-existing and and other insect species in Romania. Two different areas were selected within the supercolony: a high nest density site (HD - 0,05 nests/m^2), and a low nest density site (LD - 0,006 nests/m^2), the HD site being one of the most dense, the LD site one of the most dispersed parts of the supercolony. The daily activity of ants was studied on randomly selected plots in the absence and in the presence of baits during summer 2007, 2008 and 2009. We assessed the insect community's structure using pitfall traps on the same plots in 2009. The two parts of the supercolony were different in every aspect. While the presence of *F. exsecta* was more or less even in time and space on the HD site, and subdominant species were almost lacking, the reverse was valid for the more dispersed part of the supercolony. Differences were observed in the foraging strategies of subdominant ants as well. Patches with low or zero *F. exsecta* abundance within the supercolony let other species survive and even prosper to a certain extent.

1-7 REVEALING LITTER ANT COMMUNITY ASSEMBLY RULES AT DIFFERENT SCALES THROUGH ECOLOGICAL TRAIT AND PHYLOGENETIC TESTS

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There is a lack of consensus about the underlying mechanisms structuring litter ant communities in tropical forests. These communities are species rich and functionally diverse. Mechanisms that may structure ant communities include habitat filtering (e.g. predation, invasion), disturbance and inter-specific competition. Both inter-specific competition and habitat filtering make clear predictions on the patterns of species co-occurrence they generate when acting upon a community. We used tests based on phylogeny, worker size (an important ecological trait) and litter depth (an environmental gradient) to infer the relative importance of competition and habitat filtering in structuring litter ant assemblages over three nested scales ($0.25m^2$, $1m^2$, $9m^2$) across six forest sites in Barro Colorado Island, Panama. Neither phylogeny- nor trait-based tests indicated competition was the dominant mechanism of community assembly at the smallest scale ($0.25m^2$). At this scale, ant communities tended to be phylogenetically clustered and worker size unevenly spaced: a pattern consistent with habitat filtering. At larger spatial scales, ant communities at different sites were phylogenetically clustered, evenly dispersed (consistent with competition) or random. Deeper litter supported more species, but species in these assemblages were also more evenly dispersed. These results suggest that litter depth, which is variable at the smallest - $0.25m^2$ - scales, can generate community structure. We next scaled up taxonomically, exploring the distribution of genera, and decreasing the influence of locally species-rich and morphologically invariant ant genera (e.g. *Pheidole*, *Solenopsis*, *Pyramica*). Genera were more evenly dispersed than species among our plots suggesting dependency of our results on the taxonomic resolution of the data. Together, our results support the idea of numerous possible mechanisms, subject to ongoing tests, generating litter ant community structure.

1-8 FORAGING MICROHABITAT AND FOOD RESOURCE USE IN TEMPERATE FOREST ANT COMMUNITIES

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Most studies of coexistence in ant communities have focused solely on the ground-foraging fauna, typically omitting species operating in vertical microhabitats such as standing vegetation. In this study, we investigated patterns of ant community structure and mechanisms of coexistence by focusing on the foraging behavior in three microhabitats - leaf litter, shrubs, and trees - within the Great Smoky Mountains National Park, USA. First, we examined variation in patterns of ant species richness and abundance among microhabitats and on four different food resources. We found some evidence that the foraging behavior of species varied among food resources and microhabitats. In addition, we found that distinct sets of species tended to occur more frequently on particular food resources and microhabitats. Taken together, our results suggest that ant foraging activity on vertical vegetative microhabitats in temperate forests is substantial and may help account for variation in species richness among communities.

1-9 SOCIALITY AND RESOURCE USE: INSIGHTS FROM A COMMUNITY OF SOCIAL SPIDERS IN BRAZIL

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Behavioural differences among closely related species, in addition to morphological ones, can play a significant role in species coexistence and thus the assemblage of natural communities. Even though closely related species are expected to share many niche dimensions, they may differ in ways that allow them to utilize different resources and thus alleviate competitive interactions. Body size differences, for instance, often allow exploitation of food of different sizes. Analogous to body size, for species living in groups, group size differences and level of sociality may also contribute to resource partitioning, a possibility that has hardly been considered. We tested this idea in a subtropical forest site in Brazil where five spider species (*Anelosimus*), with levels of sociality ranging from almost solitary to highly social, coexist. We found that the range of insect sizes captured by each species reflected their nest and colony size so that species with larger colonies and webs captured larger insects than less social species. Yet, among those species whose webs did not differ significantly in size - the two with the largest and the two with the smallest webs - one captured significantly larger insects than the other. This difference in prey size was apparently due to differences in the extent to which nest mates cooperated in the capture of prey, as in only one of the species in each pair did the size of the insects captured increase with colony size. The four species were thus packed along the spectrum of available insect sizes from least to most social. This pattern of resource use was more over-dispersed than expected by chance, suggesting limited overlap between contiguous species as would be expected if the species had been assembled to avoid extensive dietary overlap. We suggest that social evolution can potentially create large differences in resource use, thus contributing to the coexistence of a greater number of similar species in ecological communities.

1-10 ARBOREAL ANTS AND TERMITES IN A NEW GUINEAN LOWLAND FOREST: DIVERSITY, COEXISTENCE PATTERNS AND NESTING HABITS

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Ants and termites often represent the majority of arthropod biomass in tropical forest canopies. Nevertheless arboreal species are rarely studied due to the limited access to the canopy. We surveyed arboreal ants and termites in two 1-hectare plots of primary and secondary lowland rainforest in Madang province, Papua New Guinea. Both plots were undergoing complete forest removal for traditional slash-and-burn agriculture. All woody stems over 5 cm in the diameter at breast height (DBH) were inventoried and every felled tree was extensively searched for ants and termites. Here we present results from a 0.25-ha area in each plot. We recorded >1000 nests on ~500 trees and 15 different nesting microhabitats. Ant nests occurred on over 70 % of sampled trees compared to only 5 % of trees with termites. The number of nests per tree was best predicted by tree DBH. We found a rather low average number of nests and nesting species per tree (3 nests, SD=2.5 and 2.2 spp., SD=1.6). These values did not differ between the primary and secondary plot, despite the occurrence of more microhabitats in the primary forest. The termite assemblage was very species poor compared to the ants (5 versus 87 spp.) and both plots were dominated by the same termite species *Microcerotermes biroi*. In contrast, we observed large differences in ant species composition and richness between primary and secondary forest plot (69 versus 32 spp., 14 spp. shared). Additional bait-trapping revealed high behavioral dominance of an invasive species *Anoplolepis gracilipes* in the secondary forest. This study represents one of the largest datasets of its kind for the tropics and challenges the traditional view of exceptionally species-rich assemblages of arboreal ants and termites. Our results stress the importance of primary vegetation for native ant communities and the vulnerability of secondary forest to invasive species.

1-11 THE IMPORTANCE OF COOPERATION AT SMALL COLONY SIZES: THE ALLEE EFFECT IN ANTS

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The Allee Effects (AE) include phenomena in which a population shows a positive relationship between a component of individual fitness and population size. AE are a direct consequence of individual aggregation and cooperation. Eusocial insects are strong candidates for many AE mechanisms due to the nature of their social structure, but empirical or theoretical confirmation of AE in this group is lacking. In the Argentine ant, *Linepithema humile*, small-sized propagules (i.e. queens with as few as 10 workers) have been shown to grow quickly in laboratory colonies, suggesting either the absence of AE in this species or the existence of various mechanisms that counteract it. We studied the relation between the success of colony survivorship and the number of queens and workers that cooperate to found a new colony. We set up lab colonies of two species that present similar colony structure, *L. humile* and *Tapinoma nigerrimum*. We studied the relationship between reproductive colony fitness and the initial size of the colonies (number of queens and workers), by monitoring colony survivorship and other components of fitness (such as reproductive output), for both species. We also set up experimental colonies of different sizes in the field and follow colony survival after one month in order to investigate to what extent the results in the lab correlate with those in nature.

1-12 THE RELATIONSHIP BETWEEN ANT DENSITY, PRODUCTIVITY AND AGGRESSION

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Division of labor has been recognized as a key factor for the ecological success of social insect societies. However, how exactly behavioral variation influences colony fitness is largely unknown. We investigated in the ant *Temnothorax longispinosus* whether aggressive and explorative behavior and/or variation among nestmates in these behavioral traits are associated with an important fitness component, i.e. offspring production per worker. We found strong behavioral differences in aggression and exploration among colonies. Importantly, intracolonial variance in aggression was associated with productivity, suggesting a selective advantage of colonies with a higher variability in worker aggression. Furthermore, ant colonies in dense patches were both more aggressive and more productive. Hence, crowding at the population level may have lead to increased colony aggression. In addition, the high colony productivity in dense sites indicates small scale variation in habitat quality and that competition is of minor importance.

1-13 TRADEOFFS, COMPETITION, AND COEXISTENCE IN EASTERN DECIDUOUS FOREST ANT COMMUNITIES

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What factors promote coexistence in local communities? Two ideas have received much attention: tradeoffs and resource partitioning. Tradeoffs with the potential to promote coexistence among ant species include the dominance-discovery tradeoff and the dominance-thermal tolerance tradeoff, both of which suggest that behaviorally dominant ants tend to be poor at other aspects of food acquisition, including discovering food resources and foraging under a wide range of conditions. Niche partitioning, including partitioning of space and foraging conditions, is another potential mechanism which might promote coexistence. We examined the evidence for 1) the dominance-discovery tradeoff, 2) the dominance-thermal tolerance tradeoff, 3) spatial niche segregation, 4) temporal niche partitioning, and 5) temperature-based niche partitioning in structuring an ant community at a forest site in the eastern US. Many of these mechanisms have been tested in a single system, but never, to our knowledge, have all of these mechanisms been examined simultaneously in a single system. We found no evidence for tradeoffs or spatial niche partitioning. However, we observed significant segregation of foraging times on a daily time scale, with behaviorally dominant ants most frequently observed on baits in the middle of the night while subdominant ants were observed most frequently on baits during the day. Together, these results suggest that the coexistence of ants in the study system may be promoted by the ability of species to forage at different times, thus minimizing direct encounters.

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GLOBAL INVASION HISTORY OF THE FIRE ANT *SOLENOPSIS INVICTA*

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The fire ant *Solenopsis invicta* is a serious agricultural, ecological, and public health pest that was inadvertently introduced into the southern USA almost a century ago and into California and other regions of the world more recently. An assessment of genetic variation at a diverse set of molecular markers in 2,165 individuals from 75 geographic sites worldwide revealed that at least nine separate introductions of *S. invicta* have occurred into newly invaded areas (NIAs) and that the main southern USA population is the immediate source of all but one of these introductions. The sole exception involves a putative serial invasion event from the southern USA to California to Taiwan. Given the invasive potential of *S. invicta*, our study raises concerns about the role of the USA as a conduit for ongoing exportation of this pest ant, as well as other invasive species, throughout large areas of the world.

ECOLOGICAL IMPACT OF INVASIVE ALIEN INSECTS: DOES SOCIALITY MATTER?

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Although alien insects are better known for their economic impact, as pests of agriculture, horticulture, stored products and forestry, there are numerous examples of insects causing serious environmental damage. These can occur at three organizational levels -genetic, species and ecosystem- and through various mechanisms. Genetic impact through hybridization between invasive and native species is only occasionally observed in insects. In contrast, cases of impact on native species populations are much more numerous. Invasive insects may feed on, and kill native plants, which, by cascading effects, may cause substantial changes in natural ecosystems. Invasive insects may also directly affect native species and ecosystems through predation, parasitism, or competition for space or resources. Some are important vectors of animal and plant diseases. Another mechanism through which an invasive species may affect native species is apparent competition through shared natural enemies. A recent literature survey identified 403 primary research publications that investigated the ecological effects of invasive alien insects. A quantified negative effect on native biodiversity or ecosystem processes was identified for 54 alien insect species, 24% of which were eusocial insects. However, these represented over 50% of the primary research publications. *Solenopsis invicta*, *Linepithema humile* and *Apis mellifera* were the three most studied alien insects, with 18%, 14% and 7% of the publications, respectively. Among alien insects, ants clearly show the highest and best documented records of damage on native species and ecosystems. A comparison of mechanisms underlying the ecological effects of social and non-social insects will be provided, as well as tentative explanations for the success of social insects as invaders compared to non-social species.

THE ANTS ARE COMING: THE IMPACT OF CLIMATE CHANGE ON ANT INVASIONS

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Ant invaders have a huge impact on ecosystem functioning and biodiversity as well as on human estates and agroecosytems, where they incur high economic costs. Because of their small size, ants are easily transported all over the world by humans and have increasing opportunities to become invasive. Consequently, several ant species are among the 100 worst invasive species list issued by the IUCN. However, the successful establishment of a species in a new habitat and its potential to become invasive depend on many factors, including local climatic conditions. Our goal is to explore the effect of climate change on invasiveness because global warming is likely to favour invasions over the next century by enabling species from lower latitudes to colonize higher latitudes. For example, the Argentine ant is invasive all around the Mediterranean sea, but has so far failed to invade further north, which could change as the European climate warms. In order to characterise the factors limiting their establishment capacity, we carried out field experiments with this species, setting up secure nests of different sizes (different numbers of both workers and queens) in northern France, where the climatic conditions are hypothesized as unfavourable for establishment. As a comparison, we performed the same experiments in Spain, where they are invasive and already established, i.e. the climatic conditions there are appropriate. We investigated the effects of colony size and of queen numbers on survival, growth rates and ultimately the potential for successful colony establishment in both climatic conditions.

GENETIC AND BEHAVIOURAL EVIDENCE FOR MULTIPLE INTRODUCTIONS OF THE INVASIVE ANT *PHEIDOLE MEGACEPHALA* ON RÉUNION ISLAND

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Introductions of ant species into new areas are often accompanied by population-level behavioural and genetic changes (multi-colonial structure to unicoloniality), thought to contribute to their success as invasive species. The invasive big headed ant *Pheidole megacephala*, native to tropical Africa, was introduced into Reunion Island (South West Indian Ocean) about a century ago. We investigated mitochondrial genetic structure and nestmate recognition in introduced populations of *P. megacephala* on Reunion island, in order to determine whether multiple introduction occurred. To cover the different types of habitats colonized (sugar cane and natural habitats) and climate conditions (very dry to humid) experienced by *P. megacephala*, colonies were collected from twelve sites (20 km apart on average) all around the island. Behavioural assays showed high levels of aggression among workers from most of the sites tested. Aggression scores were not correlated with geographic distances among colonies and nests from the same habitat type or experiencing the same climate conditions did not exhibit lower levels of aggression. Genetic analyses revealed high haplotype diversity, suggesting that different colonies likely resulted from multiple invasion events. Altogether, these results indicate that on Reunion island *P. megacephala* does not form a large unicolonial population as observed in introduced populations in Australia or Mexico. We discuss factors, other than unicoloniality, that may have contributed to the success of *P. megacaphala* on Reunion Island.

CARBOHYDRATE AND PROTEIN COMPETITION BETWEEN NATIVE ANTS AND INVASIVE WASPS IN NEW ZEALAND

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Invasive species frequently become highly abundant, competing with native animals and depleting local food resources. In New Zealand, introduced wasps, *Vespula vulgaris* have successfully invaded beech forests where they reach high densities. These wasps consume considerable amounts of prey and honeydew produced by endemic scale insects. We determined if these wasps affected foraging and the protein/carbohydrate intake of the common native ant *Prolasius advenus*. We combined (1) laboratory experiments to assess the influence of nutrient balance on the foraging activities of *P. advenus*, and (2) field behavioral observations and baiting experiments in both a wasp-invaded site and a wasp-excluded area. Our results on carbohydrate foraging demonstrated that a lack of this resource increases the number and aggressiveness of ants in laboratory colonies. In the wasp-invaded site, ants were more abundant in collecting sugar baits and to foraging for honeydew than at the wasp-excluded site. These results suggest that exploitative competition for carbohydrates likely occur at our field sites. In regards to protein foraging, although the effects of competition for proteins remains unclear at the colony and community levels, this resource was clearly the limiting one in beech forests for both ants and wasps. Moreover, interference competition was frequently observed at the individual level, with wasps removing ants from food resources by picking the ant up and dropping it some distance from the food. Overall, our study documents the link between diet and behavior in ants, and highlights interactions hitherto unappreciated between two major social insects living in honeydew beech forests.

COMPARISON OF THE GENETIC STRUCTURE OF FORMOSAN SUBTERRANEAN TERMITE POPULATIONS FROM THE NATIVE AND INTRODUCED RANGE

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The Formosan subterranean termite (FST) has invaded many parts of the world including the Pacific Rim and the United States. Although the invasion history and current distribution of FST is well documented, little is known about the invasion biology, including the origin and number of introductions to specific areas and the relationships among populations from the native and introduced range. Furthermore, the effect of introduction on genetic diversity and colony structure of FST is largely unknown. It is well established that loss of genetic diversity after introduction of some invasive ants leads to a breakdown in nest mate recognition and the formation of large supercolonies that are able to dominate native species. Like these invasive ant species, colonies of FST can vary in their social organization, ranging from simple families headed by a single pair of reproductives to highly inbred families with numerous neotenic reproductives produced within the nest. In this study we employed microsatellite genotyping to analyze the genetic structure of FST populations from the native (Guangdong and Hunan Province, China) and introduced range (Kauai, Maui, HI). We compare the results of this study to reanalyzed data from previously published FST populations to identify potential factors that may facilitate invasion success and identify possible routes of invasion and subsequent spread. In particular we (1) Describe the population structure and relationships among native and introduced populations of FST, (2) Test whether FST populations in the introduced range lack genetic diversity and show signs of recent bottlenecks similar to invasive ant species, (3) Compare the colony breeding structure among native and introduced FST populations to test the hypothesis that introduced populations contain predominantly extended family colonies (in analogy to the large polygynous supercolonies found in introduced ant species).

NEGATING THE 'FIELD OF DREAMS' HYPOTHESIS: NATIVE ANTS DO NOT RECOLONIZE SITES MANAGED FOR PLANTS

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Plant restoration projects often either completely ignore faunal restoration or implicitly follow the 'field of dreams' hypothesis: if you build it, they will come. But does restoration of a plant community automatically result in re-establishment of its native faunal components? In the subtropical island of Mauritius, native plant restoration projects have been ongoing for several decades, but their effects on native invertebrate populations have never been measured. Ants are of particular interest because they play important and varied roles in the ecosystem and because of their usefulness as indicators of community health. I used three methods (pitfall traps, baiting, and direct observations) to survey 10 sites with managed plant communities and 10 paired adjacent unmanaged sites in heath habitats and upland and intermediate forests to determine whether native ants have persisted and whether they are associated with native vegetation. Results from all methods combined indicate that native ants were more likely to be found in unmanaged sites. Managed sites had a higher proportion of introduced ants and a greater overall abundance of ants than unmanaged sites. Native ant abundance did not correlate with managed fragment size, time since first management, or with the abundance of non-native ants but did correlate with the abundance of dead standing wood. Non-native ant abundance was negatively correlated with site complexity. Although native ant and non-native ant abundance did not correlate, there may be a threshold of non-native ant abundance above which native ants cannot persist or re-establish. Disturbance created by weeding and higher levels of human foot traffic may increase the likelihood of non-native ants being introduced and persisting in managed sites.

TOWARDS AN UNDERSTANDING THE HISTORY AND CONSEQUENCES OF A *RETICULITERMES FLAVIPES* INVASION

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Reticulitermes flavipes is a subterranean termite which is presumed to be introduced in Europe from North America during the 17th or 18th century and has become invasive in several French areas. The introduced populations in France possess particular shifts in social structure: permanent presence of numerous active secondary reproductives, a high level of colony fusion and a lack of intraspecific aggression. Phylogeographic and population genetic analyses can provide crucial information into origin and patterns of introduction, and in elucidating the causes and mechanisms by which introduced species became successful invaders. To identify potential native source populations and to evaluate genetic diversity in both native and introduced populations, we performed an extensive phylogeographic study using two genetic markers; the COII region of mtDNA and 15 microsatellite markers, and two chemical markers; the cuticular hydrocarbons and defensive compounds of soldiers. Our findings show that in the native range (USA), northern populations appeared well differentiated from those in the southern part of its range, where nearly all introduced populations appear to come from. Phylogenetic results of both mitochondrial and nuclear markers showed that French populations are likely to have originated from southeastern American populations, and more particularly from Louisiana. All haplotypes shared between the USA and France are found in Louisiana. The Louisiana as potential native source populations was corroborated by the soldier defensive compound phenotype. Compared to native populations in Louisiana, introduced populations in France show reduced genetic diversity at both mtDNA and microsatellites markers, and a reduced chemical diversity at cuticular hydrocarbon profiles. These findings suggest a founder effect during introduction event. Our results add another example of a genetically depauperate founding population that has become successfully established.

ECOLOGICAL AND SOCIOBIOLOGICAL ASPECTS OF THE LITTLE FIRE ANT INVASION IN ISRAEL

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The little fire ant (LFA; *Wasmannia auropunctata*) is a good model species to study the impact of an invasive ant on other arthropods, and to elucidate the mechanisms of invasion. It invaded Israel about a decade ago, and the molecular evidence indicates that the species was introduced from its native range in either South or Central America. The invasive population in Israel is genetically homogenous and comprises descendants of a single male and a single queen that reproduce clonally. Laboratory aggression tests between nests confirmed that the ant is unicolonial in Israel. Despite this genetic homogeneity and the lack of intraspecific aggression, analyses of cuticular hydrocarbons (the presumed nestmate recognition substances) showed nest specificity, suggesting substantial environmental influence. Indeed, laboratory experiments suggested that diet breadth plays an important role in this system.The effect of LFA on local ant populations as well as those of other arthropods is reduction in species diversity as well as population abundance. We studied in the foraging efficiency of the LFA as compared to non-native species which were displaced by it, with and without between-species interference. Although in the short-term laboratory experiments the LFA was less competent than the opponent species, in both settings its survival was higher, implying a possible advantage over time. Indeed, in long-term experiments the LFA took over the opponent ants' nest and killed all workers and queens. Our results also emphasized the importance of long-term competition experiments, which may yield substantially different results than those of short term experiments. We conclude that the ant's high interference competition abilities, combined with its unicolonial social structure, contribute to the species' ability to reach high densities and thus to displace other ant species.

Poster Presentations

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2-1 Regional multi-analyses of Argentine ant populations in Southern Europe

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In their introduced range, Argentine ant populations are often comprised of one geographically vast supercolony, genetically and chemically uniform within which intraspecific aggression is absent. Recent studies found that the supercolonies extending over hundreds of kilometres in the USA, Europe and Australia are chemically and genetically similar and exhibit no mutual aggression suggesting that they belong to only one global supercolony. Frequent movements and introductions of Argentine ant propagules among distant parts of the global supercolony may explain genetics and chemical homogeneity among these populations. In Southern Europe, a third European supercolony was found at a regional scale, a new supercolony showing aggressiveness toward the main supercolony but chemically close. Here we present patterns of intraspecific aggression, analyses of recognition cues (cuticular hydrocarbons) and colony genetic using microsatellite loci of 20 nesting sites across Corsica and the mainland. Behavioural results confirm the presence of the new supercolony. Aggressiveness between the Corsican supercolony and the main supercolony varied from moderate to high following the colony pairing whereas it was systematically high with the Catalonian supercolony. Although chemical data support the behavioural results, the two supercolonies were genetically not clearly discriminated. These regional results highlight the nicety of mechanisms involved in unicolonial ant recognition and address questions on the origin and evolution of two supercolonies chemically close and genetically indistinguishable.

2-2 Native "invasive" ants: a novel evolutionary trend in the age of global change?

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Disturbance resulting from urbanization is a leading cause of biotic homogenization worldwide. Native species are replaced with widespread non-native species and ants are among the world's most notorious invaders. I investigated the effect of urbanization on the evolution of invasive characteristics in a native ant species, the odorous house ant, *Tapinoma sessile* (Say). Colony social structure, life history traits, and the spatial pattern of nest distribution were compared by sampling *T. sessile* across a gradient of three distinct habitats: natural, semi-natural, and urban. Results demonstrate a remarkable transition in colony social and spatial structure and life history traits between natural and urban environments. In natural habitats, *T. sessile* colonies are comprised of small, monogyne (single queen), and monodomous (single nest) colonies. In invaded urban areas, *T. sessile* exhibits extreme polygyny and polydomy, forms large supercolonies, and becomes a dominant pest. Results also suggest that urban *T. sessile* colonies may have a negative impact on native ant abundance and diversity. In the natural environment *T. sessile* coexisted with a wide array of other ant species, while very few ant species were present in the urban environment invaded by *T. sessile*. Habitat degradation and urbanization can lead to extreme changes in social and spatial colony structure and life history traits in a native ant species and can promote the evolution of invasive characteristics such as polygyny, polydomy, and supercolonial colony structure.

2-3 PREDICTIVE FACTORS IN THE POTENTIAL LOCATION OF THE ARGENTINE ANT WINTER NESTS

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The Argentine ant, *Linepithema humile*, shows a regrouping pattern in its winter nests which are located in similar sites every year. Since these nests are constructed superficially, soil moisture and extreme temperature could be two of the factors limiting their distribution. The main objective of this work was to characterize the microclimate of the Argentine ant winter nests and to study its link with the environmental pattern of the invaded areas. Temperature and Volumetric Water Content (VWC in %) were measured in 90 nests and 90 control areas and compared to air temperature and humidity. Canopy openness and nest location (distance to the nearest tree) were also assessed from late November 2009 to mid March 2010, at two different areas in the NE of theIberian Peninsula. Nests had a mean temperature of 16.72±9.22ºC s.d, and there was a strong relationship between environmental temperature and temperature from nests and control areas. On the other hand, nest VWC had a daily mean of 6.52±4.39% s.d, and along with the VWC from control areas were independent from air humidity. Hence, winter nest water content could be depending on the nest's physical characteristics rather than on the local climate. Correlation analyses showed a weak direct relationship between nest VWC and both canopy openness and nest location, and canopy openness was lower in nests than in control areas. Then, the temperature of the Argentine ant winter nests seems to be determined by air temperature, probably as a result of their superficiality. Soil moisture, in turn, explains partially the preference of this ant species to locate its winter nests near trees, and away from wide canopy gaps. This might prevent nests from being strongly affected by precipitation, and thus, to have optimal moisture conditions. Therefore, nest location and canopy openness, could be used as predictive factors to spot potentially attractive areas for the foundation of winter nests, and to help target control measures.

2-4 ASSESSING THE EFFECTS OF THE EXTIRPATION OF ARGENTINE ANT WINTER NESTS ON NATIVE ANTS COMPOSITION AND DIVERSITY

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The Argentine ant, *Linepithema humile*, was introduced in Europe a century ago, and has spread very effectively across the continent. In winter, Argentine ants aggregate into large nests on the same locations year after year, which makes them easily detectable, and therefore, potentially controllable. By extirpating those nests, the density of emerging queens at springtime could be decreased, and consequently, the invasion rate reduced. In this work, we studied the effect of the extirpation of Argentine ant winter nests on the composition and diversity of native ant communities in three Mediterranean forests in Catalonia, Spain. This study was carried out between January 2009 and January 2010 on 18 plots, located in three different areas of each forest, categorized in accordance with their level of Argentine ant invasion (non invaded, front of invasion and invaded areas). After nests extirpation, ants were sampled using pitfall traps on extirpated and non-extirpated (control) plots every two months. We found 23 species, belonging to 13 genera and three subfamilies. *L. humile* was the dominant species in both the front and invaded areas, while *Pheidole pallidula* was dominant in the native area. Overall, there is an inverse relationship between the abundance of *L. humile* and the abundance of native ants, which can be explained by the fact that some native species are only present when the abundance of Argentine ant is low. On the extirpated plots, there is an increase in the abundance of native species and an emergence of species that are not found in the control plots. Preliminary results show a trend on the improvement of native ants' diversity and abundance in the extirpated plots, although not statistically significant at the moment. Data for the second year after extirpation are being collected, to assess longer-term changes, which, if positive, would confirm that nest extirpation could be used as a tool to control Argentine ant invasions locally.

2-5 THE EFFECT OF BIOTIC AND ABIOTIC FACTORS ON THE BEHAVIOUR AND ECOLOGY OF *MYRMICA RUBRA* COLONIES

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The field site at Kimmeridge, Dorset contains colonies of *Myrmica rubra* and *Lasius* ant species and has been studied annually for the past 25 years. In the past, colonies flourished, but it has been evident in more recent years that the ecology and population density of ant colonies has changed. This investigation considers the effect of biotic and abiotic factors upon the habitats of ants. The effect of other ant species invading the nests of native species is also considered. Population density was measured in 15 *Myrmica rubra* colonies, chosen at random, which were scattered within the main location of ant colonies in the field site. It was found that there is a significance difference in the caste composition and population density between each colony and between those located on either side of a public footpath. The proximity to food sources was s significant cause of ant colony structure. Further laboratory tests suggested that the make-up of the colony affects the speed at which the ants move nest upon disturbance. The results of this investigation suggest that social and environmental factors are having a significant impact on the caste composition and population density of ant colonies in the study area. Clearly more work needs to be done, not only on this site, but many more to establish the impact of a dynamic environment upon social insects.

2-6 HETEROZYGOSITY-FITNESS ASSOCIATIONS IN THE INVASIVE FIRE ANT *SOLENOPSIS INVICTA*

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Evolution occurs in systems with heritable variation in fitness. It follows that a central goal in evolutionary biology is to elucidate the genetic causes of variation in fitness in populations. Accomplishment of this goal in part entails characterizing the role that within-individual genetic variation (measured as some function of heterozygosity) plays in promoting individual fitness. To characterize such a role is in effect to conduct a heterozygosity-fitness association (HFA) study. HFA studies are carried out by sampling individuals from a population, measuring traits associated with fitness in these individuals (e.g., fecundity, survival), genotyping each individual at a number of marker loci, estimating their levels of heterozygosity based on these marker genotypes, and regressing their fitness measures on these heterozygosity estimates. We are employing this methodology to conduct a heterozygosity-fitness association study on the invasive population of the red imported fire ant, *Solenopsis invicta*, in the United States. To date, we have developed over 150 microsatellite loci in addition to 8 allozyme loci, which we have used to genotype several hundred newly-mated queens from a population in northeastern Georgia. Several indices of multi-locus heterozygosity have been calculated from these genotype data, and numerous measurements of fitness-associated traits were taken for each queen. Our large set of genotype data from numerous, diverse markers and large numbers of individuals will be used to scan the *S. invicta* genome for specific genotypes (heterozygous or otherwise) that associate strongly with fitness in addition to searching for genome-wide trends associated with heterozygosity.

2-7 *PISONIA GRANDIS* MONOCULTURES LIMIT THE SPREAD OF AN INVASIVE ANT - A CASE OF CARBOHYDRATE QUALITY?

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The mechanisms by which invasive species are able to spread into and dominate natural communities are poorly understood. In this quest, studying invasions that are limited by a controlling factor will be more informative than will studies documenting unabated spread and impacts. We report here a study showing the highly invasive African big headed ant *P. megacephala* having a novel distribution on coral cays within Australia's Great Barrier Reef. These patterns displayed a clear limitation of its distribution with monocultures of the tree *Pisonia grandis*. This distribution was contrary to the known environmental limitations of the ant, and the limitation could not be associated with an underlying abiotic determinant of the vegetation type. We present these distributional patterns, and following consideration of all known biotic and abiotic limitations of ant invasions we discuss the potential that the peculiar ecophysiology of *P. grandis* is the causal factor. Specifically, we suggest that the quality of carbohydrate supply to ants is a limitation to invasive spread in much the same way that carbohydrate quantity is known to affect ant population densities in other ecosystems.

2-8 IS THE EVOLUTION OF MALE MORPHOLOGY RELATED TO COLONY BUDDING IN THE INVASIVE ARGENTINE ANT, *LINEPITHMA HUMILE*?

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Ant species that are invasive frequently have a series of traits that enabling their relocation, establishment and population growth in foreign ecosystems. For example, reproductive gynes typically mate within the natal nest and establish new colonies with a subset of workers. This behaviour, called colony budding, greatly enhances survivorship of nest founding queens. While the occurrence of colony budding is well studied, the evolutionary context of this behaviour has not been addressed. We looked at male morphology of all nineteen species of the ant genus *Linepithema*, including the invasive Argentine ant *L. humile*. Phylogentic comparative methods indicated that *L. humile* males deviate from allometric scaling relationships in body size found in other members of the genus. *Linepithema humile* males have enlarged thoracic regions suggesting an increase in musculature for flying. We hypothesize that *L. humile* males have undergone directional selection for enhanced flight, thereby compensating for flightlessness in reproductive females. This may be an example of a preadaptation to invasive success in *L. humile* that is not seen in congeners. Future research to test this hypothesis will be discussed.

2-9 Bacterial communities in *Solenopsis invicta* and *Solenopsis geminata* characterized by 16S-amplicon 454 pyrosequencing

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Social insects harbor diverse communities of microbes, and these microbes may play a crucial role in the success or failure of biological invasions. The invasive fire ant *Solenopsis invicta* has spread widely across the Southeastern United States since its introduction to North America, and because of its impact on native biodiversity, *S. invicta* has become one of the best studied model systems to understand the dynamics of invasive social insects and their biological control. However, little is known about microbes as biotic factors influencing success or failure of the invasion. This study is the first attempt to characterize microbial communities associated with introduced *S. invicta* and native *S. geminata* populations. Using 16S-amplicon 454 pyrosequencing, we compare bacterial communities on workers, on brood, and in the nest chambers in neighboring *S. invicta* and *S. geminata* colonies at Brackenridge Field Laboratory, Austin, Texas, with the aim of identifying potential pathogenic, commensal, and mutualistic microbial associates that may modulate the ecological success of these ant species.

2-10 Comparative toxicity of two fire ant venoms to agricultural pests in Taiwan

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There are two invasive fire ant species in Taiwan, the red imported fire ant (*Solenopsis invicta*) and the tropical fire ant (*S. geminata*). Both species are serious pests affecting humans, wildlife and crops. Fire ant venom contains about 90% to 95% water insoluble alkaloids and a small amount of proteins. The alkaloids exhibit antibacterial, hemolytic, insecticidal and histamine-releasing properties, whereas the protein allergens in the worker venom are responsible for anaphylactic reactions. This study investigated the toxicity of fire ant venom toward the larvae of *Spodoptera litura* and *Plutella xylostella*, two major pests of cruciferous vegetables in Taiwan. To determine the toxicity of fire ant venom, the third to fourth instar larvae of *P. xylostella* and the 8-day-old larvae of *S. litura* were treated with fire ant venom by topical application or directly stung respectively in the dorsal region. The results showed that fire ant venom-induced symptoms in both larvae include contractile, flaccid paralysis, edema and finally become blacken. The tissue of larvae treated with venom exhibited marked structural changes. The susceptibilities of both pests to fire ant venom were as follows: *S. geminata* > *S. invicta* (monogyne form) > *S. invicta* (polygyne form), as evidenced by their LT50 values at 24 h. These results supported the hypothesis that solenopsin A (the main venom in *S. geminata*) is the ancestral venom and has greater insecticidal activity than solenopsins B & C, the derivative venom types found in *S. invicta*.

2-11 POLARIZED AGGRESSION AND CUTICULAR HYDROCARBON DIVERSITY IN NATURAL POPULATIONS OF THE INVASIVE ANT *LASIUS NEGLECTUS*

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Until recently, very few invasive ant species have been able to spread in the temperate climate zone. However, the invasive garden ant *Lasius neglectus* is currently spreading in Europe, raising the need to better understand the evolutionary mechanisms leading to its invasiveness. Previous studies on the invasive Argentine ant (*Linepithema humile*) have shown that laboratory colonies become more aggressive towards conspecifics when genetic diversity, and consequently the diversity of chemical recognition cues such as cuticular hydrocarbons, is reduced. Hence, in an encounter the least diverse colony is more likely to be the aggressor, thus conferring a competitive advantage. This raises the hypothesis that invasive ability mediated by such polarized aggression paradoxically may increase over time in introduced populations when colonies go through more genetic bottlenecks. Here we tested this hypothesis in natural populations of *L. neglectus* representing four independent introductions into Europe (Rostock, Budapest, and two in Barcelona). At each location we collected live ants from the oldest, largest and most genetically diverse colony and from young, small and less genetic diverse colonies. Behavioural tests were performed to detect polarized aggression among colonies originating from the same introduction, and to detect differences in aggression towards other local ant species that are likely competitors. Furthermore, we analysed how the diversity of worker cuticular hydrocarbons (chemicals recognition cues) may be related to the presumed size, age and genetic diversity of the colonies. Overall, the results of this study will show whether (1) polarized aggression also occurs in *L. neglectus* and, if so, (2) this can potentially increase future invasiveness of introduced colonies.

2-12 UNICOLONIALITY OF *WASMANNIA AUROPUNCTATA* (HYMENOPTERA, FORMICIDAE) IN GABON: BEHAVIOURAL AND CHEMICAL ANALYSES

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The little fire ant *Wasmannia auropunctata* has been invading Gabon since the beginning of the 20th century. Behavioural and chemical studies were performed on different populations up to 130km distant from Libreville, along the main road from Libreville to Lambaréné, to evaluate the colonial structure of this species. We tested the hypothesis that the patchy distribution of *W. auropunctata* populations in Gabon is correlated with both interpopulation aggressiveness and a variability of their cuticular hydrocarbon profiles. We did not find a high level of intraspecific aggressiveness among the different populations. Nevertheless, ants of the different populations significantly discriminate nestmates from non-nestmates. Their behaviour is also highly correlated with the chemical data, which appear to be more similar to chemical profiles of native populations than other invasive ones. Our results showed for the first time that the distribution of *W. auropunctata* in Gabon is the result of multiple local widespreads along the roads rather than a continuous front of invasion. They may also suggest a possible evolution from the unicolonial structure to the native multicolonial organisation.

2-13 IMPACT OF *WASMANNIA AUROPUNCTATA* (ROGER) ON PLANT ANT *TETRAPONERA AETHIOPS* (SMITH) ON ITS HOST PLANT *BARTERIA FISTULOSA*

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The mutualistic association between the ant *Tetraponera aethiops* Smith (Pseudomyrmecinae) and *Barteria fistulosa* allows the ant to continuously clean the plant leaves and attack herbivorous insects and even vertebrates, while the plant provides *T. aethiops* with permanent housing in its hollow branches (domatia). In this study, we analysed the impact of the little fire ant *Wasmannia. auropunctata* on the mutualistic association *T. aethiops* - *B. fistulosa*. In two different areas in Gabon, invaded or not by *W. auropunctata*, we compared presence of both species of ants i) on the trunks of *B. fistulosa*, ii) in the domatia and iii) the health of the trees. Thus 381 domatia samples were taken from 18 *B. fistulosa* trees in invaded areas by *W. auropunctata* and 229 domatia from 20 *B. fistulosa* trees in non invaded areas. In the invaded areas, *W. auropunctata* occurred in 81% (Ekouk) and in 50% (Lopé) of *B. fistulosa domatia*. Whereas the native ant *T. aethiops* was present in only 1% (Ekouk) and 20% (Lopé) of them. Furthermore, in the whole 331 *B. fistulosa* trunk sampled recorded at Lopé invaded area, *W. auropunctata* was present in 100% on *Barteria* trees less down 1m, in 95.5% on trees having height between 1m to 5m and 89.29% on *Barteria* having 5m than taller. These results suggest that *W. auropunctata*, already known to displace litter ants, displace also the aggressive arboreal ant, *T. aethiops* from its host plant.

2-14 *MYRMICA RUBRA* IN CANADA: NEST DENSITY AND RELATIVE ABUNDANCE AND THEIR RELATIONSHIP TO SOIL STRUCTURE AT AN URBAN PARK

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Invasive species frequently disrupt the structure and function of ecosystems, and are a main contributor to global biodiversity loss. One of the most successful invasive species are ants, due to their ability to travel long distances and establish in high population densities. The European fire ant (*Myrmica rubra*) has invaded Tommy Thompson Park (TTP), a man-made peninsula extending 5km from the northwest shore of Lake Ontario in Toronto, Canada. These aggressive ants have spread to eastern United States and Canada from their native regions in Europe and Asia. We compared nest densities at TTP with another non-native range in Maine USA, where *M. rubra* has been established for over 50 years, and with their native range nest densities. In July 2009, nest densities at TTP ranged from 0 to 5.0 nests/m2, with an average of 1.0±1.65 nest/m2. We attributed this range of nest density to a habitat gradient of vegetation cover. The ant nest density within TTP is comparable to nest densities in Maine, USA (1.24 nests/m2) despite being established for a much shorter period of time. Both of these non-native ranges are higher than their native range (0.02 to 0.13 nests/m2). From data collected in 2010, we will consider a hypothesis that soil type predicts abundance of *M. rubra*. This work constitutes the first descriptions of *M. rubra* in Canada. The managing authority, the Toronto Regional Conservation Authority, is investing hundreds of thousands of dollars annually in the habitat enhancement of this urban wilderness yet *M. rubra* could seriously undermine these efforts. Understanding their impact to TTP is a crucial to the success of maintaining it as a valued public green space.

2-15 RAPID PREDATION OF JAPANESE MYRMECOPHAGOUS JUMPING SPIDER *SILER VITTATUS* AGAINST THE ARGENTINE ANT *LINEPITHEMA HUMILE*

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Argentine ant *Linepithema humile* has invaded many parts of the world and caused serious damage to native ecosystems. Therefore, it is needed to achieve successful control of this species. In Japan, increased density of Japanese myrmecophagous jumping spider *Siler vittatus* was reported on the infected area of the Argentine ant. It suggests the potential value of *S. vittatus* as a natural enemy. Moreover, the population of *S.vittatus* living in the infected area of the Argentine ant may be specialized to hunt Argentine ants, because Argentine ants eliminate native ant species very quickly and almost no native Japanese ant species can exist in the infected area. We collected *S.vittatus* from both infected (over ten years after invasion) and uninfected areas and observed the predatory behavior against the Argentine ants. Both from infected and uninfected area, *S.vittatus* could paralyze the Argentine ant very quickly and no significant difference was existent. Furthermore, the predatory behavior had small difference between each other. We discuss about the predatory behavior of this species.

2-16 PREDICTING THE OUTCOME OF COMBATS BETWEEN THE INVASIVE *L. NEGLECTUS* AND NATIVE ANT SPECIES: WHO IS THE WINNER?

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Lasius neglectus is an invasive ant which recently spread its range throughout Europe, causing concerns for the effects on native ants. In this study we analysed the outcomes of combats between groups of *L. neglectus*, and three native species: *L. paralienus*, *L. emarginatus* and *Crematogaster scutellaris*. First individual duels between *L. neglectus* and opponents were staged, to estimate individual fighting ability. Second we staged encounters between opposing groups of native and invader ants species. Group size was varied to provide a range of ratios of natives to invaders. Encounters were followed for up to 7 hours and hourly attrition rates were fitted to three different models: 1) Lanchester's linear law, where battles consists in of a series of individual duels, 2) Lanchester's square law, where ants from the more numerous group 'gangs up' together on individual opponents and, 3) a 'constrained Lanchester law'. The latter is a Lanchester's square law with an upper limit to the number of ants that may be simultaneously engaged against a single opponent. *L. neglectus* had the lower fighting ability in individual duels and suffered high mortality rates during group battles. The model receiving the least support was Lanchester linear. Battles between *L. neglectus* and *L. paralienus* were well described by the constrained Lanchester law. Battles against *L. emarginatus* were better described by Lanchester square law, but none of the models was able to describe fights against *C. scutellaris*. The final outcome of a battle depends however on the ratio of initial group sizes. Threshold values were shown to be 2.35 and 2.87, respectively for *L. neglectus*/*L. paralienus* and *L. neglectus*/*L. emarginatus* battles. Entering a fight with more than twice the number of opponents may thus allow *L. neglectus* to win. However, the rapid increase in time needed to overcome the opponent around these thresholds and the presence of stochastic components, may make the outcome less predictable.

2-17 LOCAL SCALE POPULATION GENETICS OF PHARAOH ANTS IN CENTRAL- AND SOUTHERN THAILAND

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Pharaoh ants (*Monomorium pharaonis*) are widespread introduced ants associated with human habitation and as such well-known domestic pests. We will present the results of a currently ongoing study examining the population genetic structure and phylogeography of pharaoh ants on a small geographical scale in Central- and Southern Thailand. Pharaoh ants are extraordinarily common in this region, allowing investigations of their population genetics at different geographical scales within Thailand, as well as inferences regarding the local history and mode of spread of the species. Preliminary data using six species-specific microsatellite markers indicate a structure of multiple introductions and isolation even on local scales. Data will be presented on a total of 1100 individuals from 55 colonies, genotyped using 17 polymorphic species-specific microsatellite markers.

2-18 COMPARISON OF COLONY BREEDING STRUCTURE IN NATIVE AND INTRODUCED POPULATIONS OF TWO INVASIVE SUBTERRANEAN TERMITES

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A major question concerning the biology of invasive species concerns the factors that make some species successful invaders. Ants are among the most successful social insect invaders. The success of many invasive ants is thought to involve a breakdown in colony boundaries resulting in the formation of large unicolonial populations that are ecologically dominant in introduced areas. In this study we used microsatellite markers to compare colony breeding structure and genetic diversity in native and introduced populations of two invasive termite species: *Coptotermes formosanus*, native to southeastern China, and *Reticulitermes flavipes*, native to the eastern U.S. Introduced populations of both species showed reduced genetic variability compared to native populations. Colonies of introduced populations of *R. flavipes* in France had much higher numbers of reproductives, showed signs of frequent colony fusion, and were spatially more expansive than colonies in the native range. Thus, introduced populations of this species exhibited strong similarities to the unicolonial populations of invasive ants. However, introduced populations of *C. formosanus* showed no consistent tendency toward higher numbers of reproductives, did not exhibit evidence of colony fusion, or show greater spatial expansiveness. These results suggest that a breakdown in colony boundaries leading to large unicolonial populations may be a factor in the invasion success of some but not all invasive termites.

2-19 CLIMATE AND ITS EFFECT ON THE INVASION OF *P. DOMINULUS* TO NORTH AMERICA

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Social insects are frequently particularly successful invaders. Many invasive species, such as the Argentine ant and the Africanized honeybee come from different climates than the ones they invade. For these species, their different thermal adaptations may be important for their ability to invade, but may also limit their invasion range. *Polistes dominulus* is a paper wasp that is native to the Mediterranean that has successfully invaded North America, but seems to be limited in both its northern and southern spread. The native congener, *P. fuscatus* is found throughout Eastern North America, from the southern US up through southern Canada. We studied the differences in flight and thermoregulatory abilities between *P. dominulus* and the native *P. fuscatus* at a range of temperatures. We found that *P. dominulus*, coming from the Mediterranean, seems to be very well optimized for a limited range of temperatures, while *P. fuscatus* is able to deal with a wider range of temperatures, but may pay an energy cost for doing so. *P. fuscatus* is better able to defend a particular thorax temperature against high or low ambient temperatures and is also more able to fly at very low and very high temperatures. However, *P. fuscatus* has a higher energetic cost of flight overall, and some of this cost may be due to defending a thorax temperature. The increased temperature range specialization of *P. dominulus* may help explain both the success and the limitations of the *P. dominulus* invasion, and help us understand what to predict with changing climate.

2-20 SPREADING OF INFESTATION OF THE INVASIVE DRY-WOOD TERMITE *INCISITERMES MINOR* IN JAPAN - JAPANESE PERSPECTIVES

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Infestation of the invasive dry-wood termite, *Incisitermes minor* (Hagen) in Japan was first reported in 1976 from a residential house in Tokyo. Since then, the records of *I. minor* have steadily increased, and some regions have turned to infestation areas at the present. Although the economic impact of *I. minor* is still very small, it would be a big issue in the near future not only in Japan, but also in Pacific-Rim Asian countries. We have just organized the research group to know the in-house ecology of *I. minor* and to manage it by IPM approaches in Japan. Our research targets are as follows: better understanding of the gallery system in attacked timbers for the effective spot treatment; figuring out the relationship among multiple colonies in an attacked house to know the mode of spreading; developing the standard laboratory test methods to evaluate the anti-*I. minor* performance of wood and wood-based materials; improving the monitoring system against *I. minor*. A state-of-the-art X-ray CT Scanning Machine was used to examine structural timbers collected from an infested warehouse in Wakayama Prefecture, and galleries, fecal pellets and living insects in the timbers could be well distinguished from the intact wood portion. Three colonies collected from the warehouse were used for the microsatellite analysis, resulting in finding strong independency of each colony. Feasibility of several laboratory test methods is under evaluation in association with organizations regarding termite control. An acoustic emission (AE) monitoring device and a microwave detector were used to examine the structural timbers in the warehouse, and, in addition, a detailed laboratory survey was conducted to know their accuracy. Approximately 80 % accuracy was obtained when using these devices with the proper sensitivity.The authors acknowledge the Japan Wood Preserving Association, the Japan Termite Control Association and the Japan Forestry Agency for their support.

Oral Presentations

Going big: large scale spatial and temporal patterns in social insect communities

3

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REGIONAL PROCESSES AS DRIVERS OF ANT COMMUNITY STRUCTURE

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Ant community ecology has traditionally highlighted the importance of local processes such as competitive dynamics and niche partitioning. However, there is increasing evidence that local species richness is not strongly niche-limited, and that competitive exclusion often does not limit species richness. The strong relationship between local and regional diversity is strong evidence that most ant communities are not saturated with species, and that the regional species pool is a major determinant of local richness. We illustrate the importance of regional process by comparing ant communities of the climatically well-matched savannas of Australia and Brazilia, whose ant faunas have contrasting biogeographic origins that reflecting the proximity of the two savanna regions to vast arid and humid tropical zones respectively. The composition of the two savanna ant faunas is broadly similar at the sub-family level, except that behaviorally dominant dolichoderines (mostly species of *Iridomyrmex*) were proportionally far more abundant in Australia, with their relative importance assumed by species of *Pheidole* and *Solenopsis* (Myrmicinae) in Brazil. *Iridomyrmex* is a strongly arid-adapted genus, with the generalised myrmicines *Pheidole* and *Monomorium* (closely related to *Solenopsis*) being sub-dominant to them in Australia. Overall ant abundance was almost three times higher in Australia than in Brazil, which is consistent with the remarkably high productivity of ants in arid Australia. However, local species richness is considerably higher in Brazil than Australia. This difference is due to a remarkable diversity of arboreal species in Brazil. With standardised sampling we recorded 48 species with arboreal nests in Brazil, compared with only five in Australia. These arboreal species are overwhelmingly tropical forest taxa. Regional processes can therefore account for the marked differences in ant diversity and community structure in ecologically matched habitats.

MACROECOLOGY OF THE MYRMECOFAUNA

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Have ant ecologists learned what governs broad-scale variation in the diversity and abundance of ants in the 100 years since WM Wheeler's 1910 statement that "The great importance of ants in the study of geographical distribution has not been overlooked by students of this fascinating subject"? In this talk, my aim will be twofold. First, I will (quickly) review what ant ecologists have learned about the biogeography of ants over the past 100 years. Second, I will use two case studies, one at a global scale, and one at a more regional scale, to examine a suite of factors that determine the distribution and abundance of ant species. At global scales, the interplay between contemporary climate and climatic history seem drive global patterns of diversity. At more local scales, the interplay between contemporary climate and interspecific competition shapes the distribution of ant species along an elevational gradient. Together, these results suggest a strong role for deterministic, niche-based processes on spatial variation in the structure of ant communities. Replicated experiments along climatic, disturbance, and resource gradients throughout the world might whether there are general rules that govern the assembly of ant communities and determine the distribution and abundance of ants at larger spatial scales.

BIOGEOGRAPHY OF ANTS IN THE AUSTRALIAN WET TROPICS

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The Australian Wet Tropics (AWT) is the largest surviving tract of Gondwanan rainforest, but modeling and previous research on montane taxa has shown that the region contains multiple, disconnected refugia. By contrast, the lowlands of AWT are thought to have responded differently to the Last Glacial Maximum, Holocene and older climate changes, perhaps undergoing extreme contraction, and, for many groups, large-scale extinction. Ants provide an ideal system to address questions of lowland historical biogeography. They are a dominant component of the fauna in almost every terrestrial ecosystem, and given their high species richness in AWT, and low to mid elevation distributions, offer an interesting contrast to other insects and vertebrates, which are more diverse and endemic at high elevations. By focusing on the ant genus, *Polyrhachis* (spiny ants), with several subgenera with differing nesting habits and microhabitat affiliations found in the AWT, I have been able to investigate the affect of these historical events on sister groups of ants. Preliminary analyses suggest that past drying events did impact the low elevation species and some of the known genetic barriers to populations appear to be older and have affected invertebrates including ants. In addition, some ant species were more impacted than others due to their ability to climatically buffer suggesting that arboreal nesting ants may have been more impacted than ground living species.

HOW DOES HABITAT COMPLEXITY AFFECT RESOURCE USE? A TEST WITH PHYLOGENETICALLY DISTINCT ANT ASSEMBLAGES

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Habitat complexity can mediate key processes that structure local assemblages including competition, predation and parasitism. Interspecific competition is considered to be an important force shaping ant assemblages. While most studies address assemblage responses to habitat complexity within one locality, a more global approach allows conclusions independent of the phylogenetic constraints of the target assemblages, thus allowing greater generality. We tested the effects of natural and manipulated habitat complexities in phylogenetically distinct ant assemblages from South Africa, Australia and Sweden, in order to determine if there were globally consistent responses in competition for food resources. Specifically, we considered the speed of discovery, rate of monopolisation and the size of ants occupying the resources. In all regions, ants responded to habitat complexity treatments, where other factors were controlled for, in accordance with predictions. Ants had smaller body sizes, were slower to discover baits and monopolised fewer baits in the more complex treatments. The response of monopolising ant size suggested that the outcome of competitive interactions was consistent within a habitat only until complexity reached a critical threshold, possibly as a result of a bimodal body size distribution. Responses to baits in natural habitats were inconsistent between regions, possibly due to interactions with other abiotic and biotic factors. Consistent with our predictions, habitat complexity thus clearly affected the outcome of competitive interactions, both in terms of size and discovery and monopolisation rates, but only when other factors were controlled for.

THE STRUCTURE OF BIODIVERSITY IN THE MELANESIAN ANT FAUNA: SCALING UP FROM POPULATIONS TO CONTINENTS.

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The Melanesian ant fauna has been described as an intersection of Asian and Australian elements with an exceptionally high proportion of endemic species. However, any quantitative biogeographic assessment of Melanesian ant fauna is missing. Here we present an overview of diversity patterns based on recent intensive studies of ant communities in the rainforest of New Guinea combined with a recently compiled comprehensive dataset of ant distribution records across Southeast Asia and the Pacific. We surveyed ground-foraging ants at seven localities distributed across a 500 km transect of lowland forest in New Guinea and investigated diversity on the population and community levels. Within the local community we found more than 200 species from 52 genera recorded in the 11 study plots (400m2 each). We investigated species turnover (beta-diversity) of five ant genera across the localities and compared the results with other insect groups. Furthermore, we analysed phylogeographic and population relationships of selected species (*Oecophylla smaragdina*, *Acropyga spp*., *Philidris spp*.) across the island and compared it with species distribution data. The impact of different dispersal barriers on population genetic structure will be discussed. More broadly across Melanesia, different patterns of faunal relationships can be observed at the species and generic levels. Species distribution and population genetic data suggest close affinities between New Guinea and northern Australia, which both show more separation from the Asian region. Interestingly, genus-level data suggest certain faunal similarity of Melanesia with Southeast Asia region and existence of several dispersal routes between New Guinea and Southeast Asia.

EFFECTS OF EXPERIMENTAL RAINFALL EXCLUSION ON A DIVERSE ANT ASSEMBLAGE FROM ECUADORIAN MOUNTAIN RAINFOREST

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For a majority of taxa, species richness increases with rainfall at macroecological scales. Ants usually do not follow this trend and tend to be either non-correlated or negatively correlated with rainfall. Reasons why ants are such an exception remain speculative. One hypothesis is that availability of favourable nesting microhabitats decrease with moisture. We conducted a field experiment aiming to study the effects of rainfall exclusion on a diverse ant assemblage. We made two predictions. (1) Ant species richness will increase because of a higher number of favourable nesting sites. (2) Soil-nesting species will be less affected than species nesting in dead wood or in leaf litter because the moisture decrease will be more marked aboveground than underground. The study was carried out at 1000m a.s.l. in a mountain rainforest of Ecuador, where mean annual rainfall was 2300 mm. Three 3*3 m experimental tents and three controls were installed in March 2009. Ants were collected six months after the experiment started. At that time, dead wood, leaf litter and soil samples were ~45%, ~50% and ~20% drier underneath tents than controls, respectively. Preliminary results indicated that total species richness was not significantly affected by the experiment. Assemblage composition was however modified. Changes differed according to microhabitat. *Camponotus* and *Solenopsis* in dead wood, and *Dacetini* in leaf litter, were more frequent underneath tents. At the opposite, *Pheidole* species seemed to prefer moist conditions. Dramatic changes in soil-nesting species were observed as almost half of the tent soil samples were empty (opposed to less than 10% for control ones). It thus appears that (1) the rainfall exclusion did not cause physiological distress to a large number of ant species, excepted soil-nesting ants and (2) at least at the local scale under study, a moisture decrease may be linked with a higher availability of some ant species nesting sites.

CLIMATIC CHANGES IMPACT ON TWO SUBSPECIES OF *MELIPONA BICOLOR* (APIDAE, HYMENOPTERA) THROUGH ECOLOGICAL NICHE MODELING

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Melipona bicolor is a representative Neotropical stingless bee species that is facultatively polygynic, with two subspecies: *M. bicolor schencki* and *M. bicolor bicolor*. There are co-occurrence areas in southeast Brazil, in the states of São Paulo and Rio de Janeiro. The geographical distribution of *M. b. bicolor* (hereafter bicolor) extends to northern Brazil (to Bahia), while that of *M. b. schencki* (hereafter schencki) to southern Brazil, Paraguay and northern Argentina. The preference of bicolor for areas with higher temperatures and schencki for lower temperatures has been well documented. Ecological niche modeling was performed in order to investigate the impact of climatic changes on these two subspecies. This procedure was conducted using 25 occurrence locations for bicolor and 21 for schencki, obtained from bee collection data and literature reports, using 36 environmental layers with 5 min-arc resolution (annual precipitation, and minimum and maximum annual temperatures). For the future climate scenario evaluation, we used climate change projections based on the Canadian Center for Climate Modeling and Analysis (CCCMA) with less optimist scenarios (A2a) for 2080, with a 3.65ºC increase in average temperature (see Worldclim website for details). The algorithms that were applied were GARP with best subsets (Desktop GARP implementation - available on openModeller 1.0.9) and Maxent 3.3.2. The resulting maps pointed to an occurrence area reduction for both subspecies; GARP indicated a more severe reduction than Maxent. The percentage remaining areas, when compared to the areas under current climate conditions, were: bicolor - GARP=15%; Maxent=91%; schencki - GARP=24%; Maxent=50%. The co-occurrence area moved south of its current position, to Paraná State (according to GARP) or Paraná and Santa Catarina states (according to Maxent), with unknown consequences for the survivorship of these two subspecies.

CHARACTERIZING TERMITE ASSEMBLAGES FROM 15 RAINFOREST SITES 6 SEMIDECIDUOUS AND 3 DECIDUOUS SITES IN ATLANTIC DOMINIUM.

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We studied 15 sites between 7º and 27º S covered by rainforest in the Atlantic Dominium. Termite assemblages were investigated by standardized surveys and species classified in 5 feeding groups, 7 nest-site types and 4 defense categories. Multiple regressions were used to identify which environmental variables were significant predictors of species richness and relative abundance. In total, 727 encounters of 87 species were identified, and we estimate that about 50% of all species are new to science; the wood-feeders and soil-feeders are dominant in all localities. Species richness and relative abundance were negatively correlated with latitude and minimum temperature was the primary predictor for its richness and relative abundance. The relation among sites was evaluated using indirect ordanation analysis. We classified roughly the latitudinal gradient in three regions: the "North Sector" (7°S - 13°S), the "Costal Bahia +ES", and the "Serra do Mar", including the southernmost localities. Within the Costal Bahia sector, a detailed survey was realized to evaluate the effects of habitat and season on termite beta-diversity. We surveyed 6 semideciduous, 3 deciduous and 2 rainforest sites to quantify ensemble turnover, considering habitat and season related effects on their composition. We applied additive partitioning of diversity, species indicator analyses, and hierarchical partitioning of diversity applied to functional trees to understand the organization of assemblages. In all, 708 encounters of 65 species were captured in 600 samples. Diversity partitioning models demonstrated that turnover in species richness is not equal across all spatial scales. Season affects the spatial partitioning of alpha and beta-diversity across sampling scales. We suggested that studies realized at large spatial scales combined with multiple data analysis approaches contributes to our understanding of the community organization.

3. *Going big: large scale spatial and temporal patterns in social insect communities.* Oral

DISTRIBUTION OF TRAITS AND ASSEMBLY RULES OF LEAF-LITTER ANT COMMUNITIES

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Increase attention has been paid to factors accounting for the functional diversity of species assemblages and its association with species richness, providing general principles about community structure that can be used to create predictive models in Ecology. We analyzed the pairwise interactions between leaf-litter ant species richness, functional structure and fundamental niche within the context of latitudinal gradients in eastern Brazil, one of the world's most species-rich ecosystems representing 3,000 km of tropical Atlantic rainforest. We use data from 26 regularly spaced local ant communities to examine latitudinal patterns of species richness, functional diversity, guild classification, and niche-based leaf-litter ant community assembly. We combined morphological traits associated with habits with species co-occurrence data to develop estimates on the community-trait distribution and guild proportionality tests to assess guild structure at the 1-by-1-m scale. We tested for niche-based alternatives (the principle of limiting similarity and environmental filtering) and neutral models. Further, we examined the effects of climatic variables (temperature, precipitation and temperature range), site characteristics (elevation and area), and spatial data (latitude, longitude) on local ant species richness and functional diversity. To reach our models, we collected estimated 300,000 specimens of which 120,000 were pin-mounted. Our results suggest that both a habitat filter and a limit to the similarity of coexisting species can simultaneously shape the distribution of traits and determine the assembly rules of local leaf-litter ant communities. We claim that habitat fragmentation along Atlantic Forest history may have strongly affected species density, species interactions, and the rate of ecosystem processes. The model can be applied to other animal groups and biomes.

Poster Presentations

Going big: large scale spatial and temporal patterns in social insect communities

3

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3-1 Application of ant species distribution data to conservation efforts in Papua New Guinea and Fiji

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The conservation of biodiversity and habitats requires a solid foundation of species distribution data to determine the sites and species in greatest need of conservation action and to determine and guide appropriate actions. Data on the species richness, composition, distribution and ecology of ants are especially valuable because ants are ecologically important, numerically dominant, easy to survey and increasingly readily identifiable. This poster will illustrate how ant data from the highlands of Papua New Guinea and Fiji are being used to identify key sites for conservation action based on high levels of species richness, presence of endemic and/or threatened species, and an evaluation of habitat quality based on ant species composition.

3-2 From micro-landscape to landscape: a study of ant distribution in two Pyrenean valleys

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As in other mountain ranges in the world, the insect fauna of the Pyrenees (SW France) presents interesting cases of endemism, making it an area of great interest for entomologists. Surprisingly however, relatively few studies have been devoted to the ant fauna of these mountains. Here we present a survey of the distribution of ant species along an altitudinal gradient in two Pyrenean valleys located in the central part of the ridge and on the opposite sides of the mountain range (France and Andorra).Habitat characteristics are known to be important drivers of local species richness. Differences in local species richness however may be caused by ecological processes that operate simultaneously across a range of different spatial scales. Although it is commonly assumed that ecological patterns are affected by processes acting at different spatial scales, empirical knowledge of the effect of scale on animal communities is still scarce. The main goal of our study was to explore the effect of scale and habitat heterogeneity on ant species richness and communities. We simultaneously examined the distribution of ants at four different scales: regional (valley), large (transect - 2000 m^2), intermediate (100m^2, centered on the sample point) and small (sample point - 1m^2). We investigated the relationship between the spatial distribution of ants and the factors considered at these four spatial scales. The results show that both the factors considered at a large scale (such as altitude) and those considered at intermediate and small scales (land cover) contribute to explaining the differences observed in the distribution of ant species.

3-3 Analysis of ant community structure in urban green areas of Parma (Italy)

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According to the World Urbanization Prospects of United Nations, in 2007 half of worldwide population lived in urban areas, whereas in 2050 the threshold of 70% will be reached. Global urbanization is leading to an intense transformation of natural ecosystems that are consumed, degraded and fragmented in small natural islands entrenched in a matrix of urbanized areas. Biodiversity loss is associated with this process and it can be expected that urban green areas will play a fundamental role in species conservation. For this reason, the knowledge of green areas characteristics will be the first step in order to protect and manage them efficiently. In this work, we focused on ant fauna in order to study the effects of urbanization in the community structure and to find the features that favour ant biodiversity. Ants have an important role in almost all the ecosystems where they live, including the urban areas. These insects move and enrich the soil, favour plant dissemination and are consistent food source for many animals as well as they are predators themselves. Ants are good indicators of environmental management practices because they are worldwide spread, easy to sample and sensitive to environmental variations. In this research, we collected basic data on ant biodiversity and on their community structure in urban green areas that differ in size and management. We studied the urban areas of Parma (Italy) where pitfall traps associated with food baits were used to achieve information of species richness, abundance and hierarchies of interspecific food dominance. Results and their interpretation through Functional Group approach show that differences in ant communities are linked to differences in management of urban green areas.

3-4 Termite diversity and abundance across habitat variability in a tropical moist savanna (Lamto, Côte d'Ivoire)

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At Lamto, little is known about animal community responses to habitat variability resulting from fires and the mosaic pattern of the vegetation in general and in particular about that of termites which play key roles in this ecosystem. With a standardized method, data were collected on termites from four habitats differing in their vegetation cover and fire-history: annually burned savanna, savanna woodland, forest island and gallery forest. A range of environmental variables was measured and correlated with species abundances. The number of termite species collected in the savanna woodland was very close to that found in the gallery forest while the forest island was the richest habitat. The species richness of the savanna woodland and forest island seemed partly due to their heterogeneous and transitional vegetation structures and variable food resources. With regard to the fire-history of habitats, Connell's intermediate disturbance hypothesis offers an explanation for differences in the patterns of habitat-specific species richness. Variation in species abundances was significantly correlated with only two environmental variables (soil pH and woody plant species richness). The pH appeared as the most influential factor for fungus-growers while tree invasion in the savanna strongly reduces the abundance of grass-feeding species (e.g. *Trinervitermes geminatus*). Although not significantly correlated with species abundances, soil carbon showed a positive correlation with the dominant soil-feeder *Basidentitermes potens*. As for wood-feeders, they were not strongly correlated with woody plant species richness; this fact might be linked to their use for other sources of nourishment. Overall, it appears that habitat variability in the Lamto reserve contributes to the maintenance of different subsets of the termite community.

3-5 ASSEMBLAGES OF ANTS ALONG ALTITUDINAL GRADIENTS IN THE BRAZILIAN ATLANTIC FOREST

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To understand patterns of richness is crucial in ecology of communities. The description of species richness patterns along altitudinal gradients has contributed significantly to understand the distribution of organisms. For ants, one question not yet investigated is how the different segments of the fauna behave along altitudinal gradients. We analyzed the richness and distribution of the ant assemblages that forage in the upper layers of soil, surface soil and vegetation along two altitudinal gradients located in the Brazilian Atlantic Forest. Further, we examined the effects of environmental variables (air humidity and temperature; pH, temperature and humidity of the soil; leaf litter depth; and density of plants) on distribution of fauna and species composition in those scenarios. To reach our objectives we use data from six regularly spaced altitudinal zones in each mountain, collecting in total 166 species. Partial results analysis suggests that species richness didn't follow a pattern along the gradients in any fauna segment, but in other hand it was possible to see the structure of fauna: firstly a strong difference in fauna composition between arboreal and soil communities, and within these groups the ant fauna was structured in two other subsets, a community of the lowest and another of the higher altitudes. This information should help to increase scientific understanding of the factors that determine the distribution of the ant fauna along environmental gradients.

3-6 THIRTY YEARS' TRENDS IN ANT COLONY DENSITY IN SANDY GRASSLAND

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In order to follow the long-term changes of ant colonies with minimal disturbance, eighty slates of 40x40 cm size were placed onto the soil surface, 50 cm apart. 40-40 slates were put on the drier, warmer sand ridges with poorer vegetation cover and in the deeper wind-furrows with higher and dense vegetation, respectively. The ant colonies moved under the warm slates in early spring (March or April) and they could be counted very easily with a minimal probability of missing any. The observations have lasted from 1981 to 2010. The small-scale colony density of the following species could be estimated in this way: *Solenopsis fugax*, *Tetramorium caespitum*, *Tapinoma madeirense*, *Plagiolepis taurica*, *Lasius niger*, *L. prammophilus*, *L. carniolicus*, *Formica sanguinea* and *F. cunicularia*. The ants occupied 82 ± 9.9 p.c. of the slates. Basing on former researches, the ants of the studied site could be grouped into three: (1) thermophilous-xerotolerant, (2) transitional and (3) hygrophilous coalitions. During the study period the density of the populations belonging to the third group significantly decreased, whereas the others show an increasing tendency. This trend is especially clear in the case of *P. taurica*, one of the most thermophilous and xerotolerant species in the region. These changes could be brought about by the climatic change observable also on the vegetation composition and the intensive decrease of the water table in the soil.

3-7 Ant assemblages at flood plains: Regional-scale convergence, artifact, or only random patterns?

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Enlarging and reducing the researches' traditional local spatial scales to regional or to microcosm levels were one of the main ecological paradigm changes in the last quarter of the 20th century. We employ macroecological approach in an attempt to compare the ant faunas at flood plains in the Carpathian Basin, Central Europe (river Tisza and tributaries) and southern Siberia (Irtish river system). In Siberia 70 ant species were found from 90 localities of 18 landscape level sites from Irtish flood plains and in the Carpathian Basin ants belonging to 58 species were collected from 145 localities of 21 landscape level sites, respectively. The Jaccard similarity of the two faunas is 0,24 for species and 0,57 for genera. The regional rank-frequency curves of the two ant faunas are astonishingly well fitting: $r = 0.92$, $p = 2{,}06 \times 10\text{-}29$ and they do not fit significantly to the corresponding null models ($p = 0.54$ at the Irtish and $p = 0.31$ at the Tisza, respectively). The frequency of the predominant species is lower then predicted by the random null model ($p < 0.02$), whereas the numerically subordinant ones have higher frequency ($p < 0.02$). The mean overlap in the distribution of the Siberian species is lower and its CV is higher then by the null model ($p < 0.001$ in both cases), whereas at river Tisza only the CV values indicate the habitat-level aggregation of species populations ($p < 0.001$). The correlation between the within-landscape and between-landscape frequencies is close in both cases ($p < 0.0001$). Also there is a congruence in the Cornel-Lawton's model on local against landscape level frequency functions: they show a week saturation tendency at both regions. In the NMDS ordination scattergrams of the habitats basing on their ant fauna, a stronger segregation is observed by regional ("zones") than habitat level in the Irtish ant fauna, while there is a more apparent segregation by habitat types in the ant faunas by the river Tisza.

3-8 Where are the last geographic frontiers for ant new discoveries?

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During the past two decades, distribution maps of global diversity have been made for several clades of vertebrates and plants. For terrestrial invertebrates, only a limited number of attempts have, however, been realized. In order to answer biogeographical questions and to develop conservation plans for insect diversity, at least some of the groups studied need to be relatively diverse and, ideally, ecologically consequential, as is the case for several social insect groups. Here we focus on ants, with which we examine the regions of the Earth where we remain most ignorant of the genera (and likely species) which are present. We developed a global database that includes all extant ant genera and their presence or absence in each of 370 political regions. Our results show that several regions of Africa, north east Brazil, southern Mexico or south-east Asia remain frontiers for ant biologists. In addition, for given latitude, regions of Africa seem to be less diverse when compared with other regions from Asia or New world (In contrast to patterns for termites, but similar to those for, for example, birds). In the long term, we expect that the individual maps we have generated for ant genera will provide an interesting tool for understanding ant biogeography and evolution and contribute to increases in the use of insects in general and ants in particular in conservation decisions.

3-9 Rapid assessment protocol for surveying dominant arboreal-nesting ant distribution in tropical forests.

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Numerically dominant arboreal-nesting ants are known to structure the distribution of other ant species and of other arthropods, such as hemiptera providing them with energy-rich resources that sustain their large colonies. The diversity and distribution of arboreal-nesting ants are difficult to study in tropical forests due to tree heights reaching up to 45m. Commonly used techniques (canopy fogging, pitfall trapping, baiting) involve climbing trees which is time-consuming. The aim of the current study was to evaluate the efficacy of an alternative protocol based on baits spread every 5m along a rope. One end of the rope is tied around the trunk and, with the help of a sling-shot, the other is slung over a branch in the canopy, forming a loop that enables the baits to be easily brought back down for inspection. So, no climbing is required. Baits were composed of a mixture of proteins, lipids and carbohydrates, and were left for 24 hours before being collected. The protocol was tested in two very different regions: a dry forest in Mozambique (n= 55 trees sampled; tree height <20m) and an Amazonian forest in French Guiana (n= 15; h<45m). Arboreal-nesting ants were also collected by hand from branch-clippings and by beating the vegetation. The protocol allows the presence of dominant arboreal-nesting ants (*Dolichoderus*, *Azteca* and *Crematogaster* in Amazonia, *Crematogaster* in Mozambique) to be easily detected. A stratified ant distribution at the baits was observed on tall Amazonian trees. On-site confrontations between dominant ants colonizing baits allowed us to identify a supercolony of *Crematogaster* colonizing three-fourths of the trees along the 500m Mozambique transect. The protocol offers a tool for rapidly investigating spatial and temporal patterns in dominant ant distribution. Considering the structuring effect of these ants such a protocol has potential for monitoring functional processes in tropical forest ecosystems.

3-10 Comparative phylogeography of the ants *Myrmica rubra* and *M. ruginodis*

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Phylogeography studies geographic distributions of genealogical lineages. One scope of phylogeography is to examine how past climatic fluctuations such as the alternation between Pleistocene ice ages and interglacial periods have affected species and their genetic constitution. We conducted a comparative phylogeographic analysis of the ants *Myrmica rubra* and *M. ruginodis*, which both have a wide Palearctic distribution. Queens of both species are size-dimorphic with small microgynes and large macrogynes. The microgynes of *M. rubra* are obligate social parasites of the macrogynes, whereas those of *M. ruginodis* are able to establish their colonies independently. Our aim was to investigate the phylogeographic structure of the species, localise their glacial refugia and postglacial colonisation routes, and to compare genetic differentiation of the queen morphs within both species. We collected samples from the Atlantic coast to Japan and sequenced two mitochondrial gene fragments (COI and Cytb). We found within both species a similar west-east phylogeographic structure, which has probably been affected by several Pleistocene ice ages. The species probably survived the last ice age in several refugia and recolonised the northern areas along both western and eastern routes. The queen morphs differed genetically from each other in *M. rubra* but not in *M. ruginodis*.

3-11 ASSESSING DETERMINISM IN THE ASSEMBLY OF ANT COMMUNITIES USING PHYLOGENIES AND CLIMATIC GRADIENTS

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While examining the determinants of community structure, macroecologists tend to focus on the effects of climatic variation over broad geographic provinces. Alternatively, community ecologists often focus on the role of species interactions in a single locality or on a set of geographically clustered localities. When combined, these approaches can help determine the relative contribution of ecological and evolutionary processes in shaping contemporary community structure. Here, I examine how phylogenetic structure (i.e. mean phylogenetic relatedness) of local ant communities varies along climatic gradients. I assess whether the degree to which species composition is deterministic depends on climatic conditions. In addition, I evaluate how the scale of analysis affects the relationship between community phylogenetic structure and climate. My results support the hypothesis that extreme climatic conditions lead to higher determinism in the assembly of ant communities than mild climatic conditions.

3-12 IS THERE AN ANT MOSAIC IN THE HIGH CANOPY OF LOWLAND DIPTEROCARP RAINFOREST?

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Ants are the ecologically dominant animals in most tropical rainforests, and it is therefore vital that we understand what determines their community structure in this highly biodiverse yet vulnerable ecosystem. It has been suggested that the ant communities in tropical forest canopies are organized as mosaics: that is, with one or two species acting as dominants over a relatively large area, with a small number of other species as sub-dominants. Ant mosaics are typical of tropical plantations, but there is little evidence that they occur in primary forests, although previous studies have looked mainly in the understory.We studied the structure of ant communities in the high canopy of lowland dipterocarp rainforest at Danum Valley, Sabah, Malaysia, over a period of 14 months. We developed novel methods of fogging and baiting, which proved very effective at sampling the ants. We sampled 185 species of ants in 20 trees of Parashorea tomentella and P. malaanonan. We also developed videoingtechniques to study ant behaviour in the canopy over a 24-hour period.Our results show that there is a clear temporal partitioning of ant species, with different species appearing consistently at different times of day. Spatially, ant species co-occur in a deterministic pattern in a crown of a tree and across all trees: the ant communities are not randomly structured.There is also obvious aggression between different ant species and some dominant species are able to dominate and exclude others at the baits. There is therefore clear evidence for the existence of ant mosaics, but these mosaics are quite fluid, with a changeover of different dominant species between day and night and between different trees within the forest.

3-13 ANTS AND PLANTS ON OLD-FIELDS IN ROMANIA: DIFFERENCES IN SUCCESSIONAL PATHWAYS

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Ants are one of the most abundant insect groups, which makes them perfect candidates for monitoring studies. As significant proportions of agricultural lands are abandoned nowadays in Central Eastern Europe the study of insect versus plant community succession could help us understand how these lands transform and eventually return to a semi-natural state. We studied epigeic ant communities of abandoned old-fields in order to elucidate how these communities change structurally in time. Pitfall traps were used on four different old-fields (1-, 8-, 16- and 30-years old) as well as on two control sites (a reference grassland and a shrub-land) in Transylvania, Romania. Collections were carried out for 10 days in spring, summer and autumn 2006, and autumn 2008. The structure and composition of vegetation was also analyzed. The most abundant ant species were the disturbance-tolerant *Lasius paralienus*, *L. niger* and *Tetramorium cf. caespitum*, but *Myrmica sabuleti*, *M. specioides* and *Tapinoma ambiguum* were also quite frequent. Contrary to any expectations the ant species number does not show any abrupt changes during the aging of the fields, whereas the number of plant species grows. Parallel to this the diversity of the vegetation also increases in time, but the diversity of ant communities shows a slight decrease. Although the vegetation of the studied fields clearly differ in structure and composition, these differences are not in the least mirrored by ant communities. The dominance of disturbance-tolerant ant species stays unaltered during succession, and they also dominate in the possible climax stages (grassland and shrub-land). Seemingly, while a return to natural state can be observed in plants, such clear-cut changes do not occur in ants.

3-14 DETERMINANTS OF ANT COMMUNITY COMPOSITION ALONG A 1200 KM LATITUDINAL GRADIENT IN ARID AND SEMI-ARID REGIONS OF IRAN

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Understanding the mechanisms that cause the patterns of biodiversity in communities at large scales is a key task in current macroecology. We assessed the influence of environmental parameters on ant communities along a 1200 km north-south transect through desert and steppe regions of Iran and evaluated whether World Wildlife Fund's ecoregions units are the appropriate scale for conserving ant diversity in that area. Using pitfall trap methods for sampling ants, we applied canonical analysis to disentangle the relative importance of environmental and spatial processes on ant communities. In addition we partitioned species richness and Shannon diversity to its alpha and beta components, to find variations of them across four hierarchical levels. Hierarchical cluster analysis was applied to evaluate the similarity of ant communities among ecoregions. Our results show that environmental and spatial models jointly explained 62% of variation in community composition of ants. Ant assemblages were dominantly controlled by the spatial-dependent environment component (39%), including the variables annual rainfall, summer rainfall, and temperature range. The significant contribution of spatial processes suggested that ant community composition was also influenced by dispersal of sexuals, especially at shortest distance where environmental conditions were suitable. Partitioning of the regional diversity demonstrated that the highest value of diversity was generated by beta diversity among ecoregions, while the alpha component contributed less to total diversity. Hierarchical partitioning of diversity indicated that ecoregions are the proper scale for conserving ant diversity. However, the boundaries of the respective ecoregions remain the subject of further studies.

3-15 ANALYSIS OF ITALIAN ANT-FAUNA COMMUNITY STRUCTURE AS A TOOL FOR THE ASSESSMENT OF ENVIRONMENTAL QUALITY

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Ants are informative ecological indicators since they are key elements in several ecosystems: they are present at different trophic levels, they are keystone species and they act as real "ecological engineers" since they are responsible of deep physical and chemical changes of the soil profile, variations in nutrient and energy fluxes as well as vegetation changes. Ants are routinely used for biological monitoring worldwide mainly in Australia, but in Europe and especially in Italy, the potential of ant-fauna as bioindicator has been poorly studied. This research is the follow-on of a previous work aimed to study the ecological structure of ant communities in typical Mediterranean ecosystems and it is an attempt to deepen the acquirements in order to provide a more detailed representation of these communities. Sampling areas were selected inside the Natural Reserve of the Castelporziano Presidential Estate (Roma, Italy). The Estate is an ideal model to develop and test biological indicators of environmental quality, because it offers an elevated variety of habitats and diverse ecological conditions, hosting about 20% of the whole Italian ant species. In this research we used two different sampling techniques: pitfall trapping (already tested in the former study) in association with baiting. Pitfall traps allowed the assessment of species richness and abundances, while baits gave information about the degree of behavioural dominance among species in relation to food competition. Data interpretation using the Functional Groups Approach showed differences in ant community structures in terms of species richness, abundances and behavioural dominance hierarchies. This can be explained considering differences in both ecological conditions and levels of human exploitation.

3-16 ASSESSMENT OF ANT ALPHA AND BETA DIVERSITY WITH REDUCED SAMPLING EFFORT

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Representative sampling of ant communities is time-consuming and laborious. Because of their manifold habitat requirements and sociobiological attributes usually a suite of sampling methods is necessary to detect nearly all ant species that thrive in one habitat. We analysed how well different combinations of sampling methods and intensities are suited to assess ant species richness (alpha diversity) and to identify ant community patterns (beta diversity). Sampling occurred at 24 sites at mountain and floodplain habitats in eastern Austria using pitfall traps, Winkler litter extraction, hand sampling of foragers, and colony sampling. We drew diverse reduced subsets of sampling approaches and compared these to the complete data. To show alpha diversity effects, mean relative coverage of ant species were calculated for each reduced subset. Mantel matrix correlation test between each subset and the complete dataset was used to compare beta diversity changes. Many reduced subsets that allowed for substantial reduction of working effort still well reflected the beta diversity patterns of the whole dataset, whereas alpha diversity was more sensitive to reduced sampling effort. Pitfall traps turned out to be indispensable to collect ant species for biodiversity studies in temperate regions. On the other hand, using only pitfall traps resulted in an undersampling of ants. Winkler extraction was found to be highly dependent on habitat structures. It emerged as important method for temperate lowland deciduous forests where leaf litter is abundant. Generally, the optimal selection of sampling methods varied between habitat types. In rugged montane habitats pitfall trapping should be complemented by colony sampling, whereas in flat floodplain forests additional Winkler extraction samples are advisable.

Oral Presentations

Ecological and evolutionary implications of inter-specific, multipartite interactions

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NETWORKS BETWEEN SOCIAL INSECTS AND PLANTS: PATTERNS AND PROCESSES

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Ecological interaction networks describe who interacts with whom in multispecies communities. Network analysis can help to understand biodiversity and community stability as well as evolved traits that determine relationships across species. However, the interpretation of network patterns has to be taken with caution. Sampling intensity varies across species and networks, which may strongly bias some of the conclusions, e.g. on specialization and niche partitioning. Based on a quantitative network metric which corrects for this sampling bias, we compare the degree of niche partitioning (complementary specialization) across different insect-plant associations, focusing on social insects. Ants show a particularly generalised relationship with plants bearing extrafloral nectaries (EFNs), but a strong species partitioning across myrmecophytic plants. Whereas EFNs are easily accessible, various morphological features such as trichomes, waxes and domatia structure may constrain the spectrum of symbiotic ant associates on each myrmecophyte. Tropical stingless bees are highly generalised in their choices of plant species when foraging for pollen and nectar, but also for resin sources. Other social bees in higher latitudes show a higher extent of flower partitioning, albeit not as strong as solitary bees or the remaining pollinator community. Ants only occur on a small subset of flowers due to morphological and volatile defences. Consequences of higher specialization include a higher vulnerability of populations to changes in plant communities due to intensive land use.

BEHAVIORAL ECOLOGY AND MULTITROPHIC INTERACTIONS: FOLIAGE-DWELLING ANTS MEDIATE PLANT COLONIZATION BY INSECT HERBIVORES

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Natural enemies represent a constant threat to insect herbivores, which in turn face a major dilemma: the need of an enemy-free space on foliage. Consequently, the ability to make appropriate decisions during host plant selection is an important behavioral trait in phytophagous insects. Information about predation risks is critical before oviposition, and natural selection may favor the capacity of females to detect predators and to select less-risky foliage to improve offspring survival. Ants are major predators on foliage and many aggressive liquid-feeding species complement their diets by actively hunting on herbivores. Thus detection of predacious ants upon plant colonization may allow females to shift egg-laying to less risky, enemy-free foliage. Certain phytophagous insects, however, not only circumvent ant predation but even attract ants for their own benefit: by producing liquid rewards trophobiont herbivores attract aggressive tending ants that create an enemy-free space in their vicinity. Due to this important benefit, natural selection on myrmecophilous herbivores may favor the ability to detect ant mutualists before oviposition and select ant-patrolled foliage to enhance offspring survival.Here, we use two contrasting scenarios - antagonism and mutualism with ants - to investigate behavioral decisions by egg-laying butterflies with respect to the risks/benefits associated with encountering ants on foliage.Results show that detection of ants to the benefit of larval offspring - either through avoidance of ant predation, or through protection via ant mutualists - may have represented an important evolutionary step in the process of host plant selection in butterflies living in ant-rich habitats. (Funds: FAPESP, CNPq, CAPES).

SYMBIONT GENETIC DIVERSITY AND REPRODUCTIVE MODE IN AN ANT-APHID MUTUALISM

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Interspecific cooperation (mutualism) is widespread and of major importance to life as we know it. Since the partner species' interests will never be fully aligned and mutualism is often costly, there is considerable potential for conflict. Yet, numerous mutualisms appear to be stable over evolutionary time, so it is important to ask what normally prevents conflict from becoming a major destabilizing force. In mutualisms involving a host and one or several symbionts, conflicts often arise because energy allocation optima differ. For example, it may be in the interest of symbionts to invest in sexual reproduction, dispersal and competition with other symbionts while the host would profit more from enhanced overall productivity. The question is whether the evolutionary predictions of conflict regulation are so general that resolutions can be universally predicted, or whether model systems are so different that every particular system has its own optimal resolution. Ant-aphid interactions are particularly suitable to address questions of evolutionary stability since aphid symbionts vary greatly in reproductive mode, dispersal, and dependency on their hosts. We studied the interactions between the ant *Lasius flavus* and four species of subterranean root-aphids by extensive field sampling and microsatellite genotyping. Genetic analyses of field populations showed that asexual reproduction is more common than previously assumed and that within-nest levels of diversity are generally low. This result provides an interesting analogy with fungus-farming mutualisms that involve social insects, where within-colony symbiont reproduction is also asexual and within-nest symbiont diversity very low. Although this is still work in progress, the first contours of general answers are beginning to emerge.

EVOLUTIONARY CONFLICTS AND SANCTIONS BETWEEN A NEOTROPICAL ANT AND ITS HOST-PLANT

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In theory, mutualisms are intrinsically unstable and every mutualist should become a cheater. However, mutualisms are ubiquitous and of major importance in ecosystems, suggesting the existence of mechanisms enhancing the maintenance of such relationships. We focused on the obligatory myrmecophytic association between the neotropical plant *Hirtella physophora* (Chrysobalanaceae) and the ants *Allomerus decemarticulatus* (Myrmicineae). The plant shelters the ants in leaf pouches in exchange for protection against phytophagous insects. However, *A. decemarticulatus* negatively affects the reproduction of *H. physophora*. We experimentally demonstrated that ants partially castrate their host-plant by destroying almost two thirds of its buds. The presence of ants on blooming flowers also lowers fruit production, probably because of negative interactions between ants and pollinators. Consequently, *H. physophora* resources initially intended for its reproduction are reallocated to its vegetative growth. This leads to more available domatia, which the ants use as living space. However, the volume of these domatia is smaller than the volume of domatia from non-castrated *H. physophora*, indicating that plants retaliate against the sterilizing ants.Altogether, these results highlight evolutionary conflict between *H. physophora* reproduction and the growth of *A. decemarticulatus* colonies. However, this conflict does not result in complete castration of the plant, contrary to what theory predicts, since the resources that the plant invests in reproduction are completely unavailable to the ants. This contradiction probably results from sanctions imposed by the plant on too virulent ants thus enhancing the stability of the mutualism.

THE EFFECT OF GEOGRAPHIC VARIATION IN PARASITE AND HOST RANGE ON CO-EVOLUTIONARY TRAJECTORIES

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The number of host and parasite species within a community network can have significant effects on co-evolutionary outcomes as more enemy (parasite) options typically temper exposure to selection and multiple enemies force trade-offs by their host targets. These relationships are also affected by and affect intra-specific interactions. Here I examine tri-partite evolutionary dynamics with respect to intra-specific competition of two social parasites (slave-makers) in Formicidae that conduct `brood-raids' on a shared host species and use the captured brood as a functional work force. Slave-makers from two disparate populations were challenged with either a heterospecific and/or conspecific slave-maker and the impact on hosts and slave-makers was evaluated. In populations with competition between slave-maker species for host access, the less virulent slave-maker had an attenuated impact on the host population compared to populations in which host availability was exclusive. In all, these results suggest that a strong competitor in a tri-partite system can produce significant ecological shifts in interacting species and modify the nature of the co-evolutionary host-parasite relationship of the rival. Furthermore, these results indicate the importance of embedding the coevolutionary arms race paradigm within a community-level context to understand the resulting geographic mosaic of outcomes.

INTERACTIONS BETWEEN MICROBES AND *NASUTITERMES ACAJUTLAE* (INSECTA:ISOPTERA): ARE THERE ADVANTAGES TO ARBOREAL NESTING?

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Arboreal nesting termites play key roles in tropical ecosystems. Previous studies have revealed a lower prevalence of parasites or pathogens associated with arboreal nesting species as compared to ground nesting insects and mammals. The current study quantified culturable bacterial and fungal loads associated with *Nasutitermes acajutlae*. Substrate and cuticular microbial loads were measured from the nest cores, trails, and surrounding soils in five habitats. Abiotic factors were measured to determine their effects on the microbial communities. In addition, cultured fungal isolates were identified using environmental PCR. Overall, a lower number of microbes, and a lower diversity of fungi were cultured from core samples when compared to trail and soil samples. Linear regression models found year, habitat, sample, temperature and light to be significant predictors of microbial numbers. Termites carried relatively few microbes on their cuticles as compared to the substrates. Bacterial numbers were positively correlated with temperature and rainfall while inversely correlated with fungal loads. Fungi also decreased with increasing light. These dynamic interactions emphasize the importance of longitudinal studies when studying the microbial communities associated with termites, and support the growing evidence that lower microbial pressures occur in arboreal microhabitats.

Poster Presentations

Ecological and evolutionary implications of inter-specific, multipartite interactions

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4-1 BEHAVIORAL COMPLEXITY AND CHEMICAL ECOLOGY IN ANT-TERMITE INTERACTIONS

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A great number of species live together, sometimes for reciprocal benefits, as mutualism, sometimes for benefit of only one, as predation. Ants are generally described as the main termite predator. Usually they don't live together, but in some cases they are living closely. In these conditions, we wonder what kind of association they can form. In this preliminary study, we used two ant species (*Monomorium carbonarium* and *Hypoponera eduardi*) and two termite species (*Reticulitermes flavipes* and *R. grassei*), living in sympatry on the island of Oléron (France). In literature, *M. carbonarium* is described as living in very populous colonies of small individuals, next to termite colonies, and known for being an aggressive predator. Conversely, *H. eduardi* is a bigger ant, forming small colonies, and is suspected to be a commensal parasite. Our first aim was to see if ants had a different behavior if they were previously in contact or not with termites. To test this, we performed different dyadic ant-termite encounters*: M. carbonarium vs*. *R. flavipes* or *R. grassei*, and *H. eduardi vs*. *R. flavipes* or *R. grassei*. Our first results showed that, unlike the literature, *M. carbonarium* was not aggressive towards termite workers; just waiting in a defensive posture for the termite to adsorb the poison drop. *H. eduardi* was more aggressive, looking for the termites to bite and sting them. For both ant species, it seems no significant difference occurred in behavior whether they were living nearby termites or not. Our results may probably be different with group confrontations, to be done next, notably because of the use of cooperative foraging strategy in *M. carbonarium*. One of the hypotheses explaining how termites and ants can live together could be a change in the chemical signatures (i.e. cuticular hydrocarbons, well known to be involved in recognition) when ants are living in or near termites' nests. For that we are analyzing the signatures of the different ants.

4-2 HOW IMPORTANT IS THE ANT-TERMITE INTERACTION IN TROPICAL RAIN FORESTS?

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Termites and ants make up the single largest animal biomass contribution in tropical rain forests and perform vital ecosystem functions. Given that many species of ant are at least partly predatory and termites are a highly abundant potential prey, it has always been assumed that ants eat large numbers of termites and that, therefore, the ant-termite trophic interaction is a key one in tropical rain forests. Although a small number of studies have confirmed the existence of this interaction between pairs of species, direct evidence for the general strength of these relationships is scarce. This is mainly because the majority of ant-termite predation events take place underground, making them difficult to observe. We use both classical ecological and molecular approaches to assess the importance of the ant-termite interaction in tropical rain forest. In order to quantify spatial co-variation in ant and termite distributions we sampled ants and termites from grids of soil pits at sites in Gabon and Malaysia. We found that while non-predatory ants were distributed independently of termites, predatory ants were more often found at locations with termites than would be expected by chance. We then sequenced termite cytochrome oxidase subunit II sequences from the guts of the ants collected from the soil pits and compared these sequences to a database of sequences for different termite genera. This enabled us to distinguish between three alternative dietary hypotheses for each ant species: 1. That the species does not feed on termites; 2. That the species does feed on termites, but feeds on different species in relation to their availability i.e. it is a generalist; 3. That the species is specialised on a particular species, clade or feeding group of termitesHere we present some preliminary data for a selection of common ant species in Gabonese rain forest indicating overall reliance on termites as a food source and levels of dietary specificity.

4-3 INVASIVE ANTS, MEALYBUGS AND COCCIDOPHAGOUS BEETLES: A CASE STUDY ON ORNAMENTAL PLANTS WITH ECO-ETHOLOGICAL IMPLICATIONS

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The Argentine ant, *Linepithema humile*, is considered a major pest among invasive insects. In fact, colonies of this species may strongly affect the arrangement of natural as well as of agricultural and ornamental ecosystems, being often associated with outbreaks of honeydew-producing homopterans. Mutualistic interactions between ants and these pest insects imply a direct nutritional advantage for the ants (honeydew), and a series of benefits for the homopterans (protection). Here we present preliminary data on the relationship among Argentine ants, mealybugs growing over ornamental greenhouse plants, and the coccidophagous ladybird *Cryptolaemus montrouzieri*. In particular, we tried to verify if the presence-absence of the ants may affect the predatory effectiveness of the beetle against the homopterans, and eventually to collect information on the strategies allowing this beetle to be a specialized predator also on ant-tended coccids. Evaluations of mealybug and ladybird larvae density on ant-tended and ant-excluded plants showed that: 1) the ants do not seem to negatively affect egg-laying ability of *C. montrouzieri*, as well as eggs and larvae survival; 2) the impact of the ladybird in limiting the mealybug populations was evident in both the treatments, although it was more rapid on the ant-excluded plants. Observations on mealybug infested plants showed that contacts between *L. humile* workers and *C. montrouzieri* larvae are very limited and not aggressive. Moreover, behavioural experiments confirmed that the ants seem to be indifferent to the presence of the ladybird larvae. Possible explanations for this sort of "immunity" to the ants showed by *C. montrouzieri* may include the release of repellent-toxic secretions or the mimicking of superficial mealybugs odour. Our chemical analyses support both these hypotheses, although the ethological data seem to fit better with the second one. Evolutionary and ecological implications of these results are finally discussed.

4-4 DO BIODIVERSITY OF ANT-MIMIC SPIDERS ASSOCIATE WITH ANT BIODIVERSITY?

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Ant-mimicry has evolved in numerous families of spiders, such as, non-web-weaving spiders, *Salticidae*, *Corinnidae* etc. In the tropics, salticids tend to be dominant spiders and ants tend to be dominant and high-biodiversity insects. The present study examined how ant biodiversity reflects on biodiversity of ant-mimic spider in morphology and species-diversity in Bornean tropical forests. In our previous studies on ground layer of the tropical forests, we found 11 mimic-types in 26 species of 8 genera, two families of ant-mimic spiders. Although high-diversity on ant-mimic spider was found in the ground layer, 60% of ant-mimic spider collected was showed poor ant-mimicry (i.e. some similarity to several different species of ant model). Among vertical structure of tropical forest, the ant assemblage of the ground layer is most diverse, because both ground and arboreal ants appear in the layer. A poorer mimic, in comparison with good mimic (specific resemblance to a model), will achieve a high degree of protection in place where many different model species occur together (multimodel hypothesis). We, therefore, considered that high species and morphological diversity of ant-mimic spiders in the ground layer is reflected to high species-diversity of ant in the layer. Furthermore, we predict that low diversity of species and high proportion of good mimic of ant-mimic spider is found in place with low diversity of ant. In top of canopy in tropical forest, some few ants, such as *Crematogaster* and *Camponotus*, tend to be dominant and ant diversity is lower than that in the ground layer. In the present study, we examine diversity of ant and ant-mimic spider in canopy layer using canopy crane to test our hypothesis. The implications of the results for association with biodiversity of ants and ant-mimic spiders are then discussed.

4-5 EVOLUTION OF CHEMICAL SIGNALLING IN LYCAENID-ANT ASSOCIATIONS

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The majority of lycaenid butterfly associates with ants during their larval stages. These associations are mainly mutualistic interaction, but range from non-ant associated to parasitism. In any associations, lycaenid caterpillars will have evolved a range of chemical signals to communicate with their ant partners. However, little is known about the function and evolution of chemical signalling in the lycaenid-ant associations. Using mutualistic (*Narathura japonica*), parasitic (*Niphanda fusca*), and non-ant associated (*Lycaena phlaeas*) butterfly species, we addressed how chemical signals from caterpillars are processed by the ants, and whether signals to ants are varied according to type of associations. In mutualistic association, we found that ants can learn to associate the nectar secretion with the cuticular chemicals of mutualist caterpillars, and increased attending behavior toward them. Such association via ant learning was not observed in non-ant associated *L. phlaeas* caterpillars. They have no nectar-secreting organ on their surface. In addition, learning experiments using artificial nectar and *L. phlaeas* cuticular chemicals revealed that ants could not recognize their cuticular chemicals. In parasitic association, dummies coated with cuticular chemicals of *N. fusca* caterpillars were enthusiastically tended by host workers like ant sexuals. Chemical analysis showed that cuticular chemicals of mutualist caterpillars were composed by complex mixture of various kinds of own (non-mimetic) hydrocarbons. On the other hand, non-ant associated caterpillars have simple set of hydrocarbons, mainly occupied by linear alkanes, and parasitic caterpillars have host-like hydrocarbons that were particularly close to those of the host sexuals. From these results, we will discuss the evolution of chemical signalling in the diverse lycaenid-ant associations.

4-6 NUTRIENT LIMITATION IN AN ANDEAN BROWN FOOD WEB: EFFECTS OF C, N, P ADDITIONS ON LEAF-LITTER ANTS AND MESOFAUNA

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The effect of nutrient addition (Carbon, Nitrogen, Phosphorus) on leaf-litter mesofauna and ants was investigated in a lower tropical montane forest of the Ecuadorian Andes. We added the equivalent of five-fold the leaf-litter annual input (C = 23 T ha^{-1} yr^{-1} ; N = 420 kg ha^{-1} yr^{-1} ; P = 36 kg ha^{-1} yr^{-1}) in 3 applications, each second month, on 24 2x2m plots: 8 controls, 8 C+N and 8 C+N+P enriched plots. We extracted the leaf-litter mesofauna with Berlese apparatus (2 samples per plot) and ants with Winkler bags. This experiment allowed us:1) to study the effect of nutrient limitation on ant community. We will discuss the effects of enrichment on ant diversity, community composition and shifts of dominance. These effects may be explained by changes in the whole brown food web due to bottom-up effects of CN & CNP additions on decomposing microbes, grazers and finally predators, as ants. 2) to get a finer understanding of ants feeding preferences by using stable isotopes. The use of N stable isotope ratios is a powerful tool for analyzing trophic niche partitioning, and it allowed us to distinguish different trophic levels amongst ants. The use of sugar cane glucose as carbon source allowed us to discriminate root associated carbon flux from added carbon, using stable isotope analysis.

4-7 LINKING BIOLOGICAL DIVERSITY AND CHEMICAL DIVERSITY: RESIN COLLECTION IN A TROPICAL STINGLESS BEE COMMUNITY

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The highly social stingless bees (Apidae: Meliponini) are important pollinators in tropical ecosystems. Besides pollen and nectar they also collect large amounts of plant resins which they use for nest construction and defense. They further transfer resin-derived terpenes to their body surfaces, thereby increasing the diversity of their chemical profiles. We observed resin collection by stingless bees at trees and colony entrances in Borneo, to investigate whether bees collected resin from many different or only few specific trees and whether different bee species differed in or shared their preferred resin trees. At our field site, all stingless bee species collected resin from several tree species, particularly from trees of the resinous dipterocarp family. All colonies collected a large variety of resins, suggesting that stingless bees visit several tree species rather than just a few adjacent resin wounds. Analysis of network-level specialization revealed that stingless bees are highly generalist resin collectors with no specific preferences for specific tree species but a variety of resin sources visited. Although stingless bees collect a large variety of resins, they only transfer a subset of terpenes derived from these resins to their body surface. Some species even exclude whole classes of terpenes, thereby increasing chemical differences between species and revealing a hitherto unknown ability of bees to filter terpenes derived from resins and embed them in their chemical profiles.

4-8 SALTATORY *APIS CERANA* SWARMS ELUDE HORNETS IN NORTHERN THAILAND

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In September-October, 2009, in Northern Thailand, many *Apis cerana* swarms appeared in and around a mango orchard along the Pai River. The swarms were under attack by the hornets *Vespa tropica and V. velutina*, which were at their population peaks. The bees employed a variety of defenses, including previously documented behaviors such as zig-zag protean flight, shaking and 'balling.' Also, swarms formed downward aerial extensions, and extensions along tree branches ('tails' and 'arms', respectively). Over a period of days and even weeks, they engaged in repeated, short (10-150 m), saltatory flights by which they steadily left more and more hornets behind, at the previous resting site. Eventually free of hornets, they took long flights out of the study area, until no swarms remained. The flights were either: 1) short, directly provoked by hornet attacks, with no preceding dances or apparent preparation; 2) short, unprovoked, preceded by short waggle dances which generally indicated the direction and distance to the next nearby resting place; or 3) long, unprovoked, preceded by long waggle dances which may have indicated the direction and distance to a more remote nesting cavity. I hypothesize that arms and tails formed by swarms under attack act as expendable decoys to lure hornets away from the more vital center, and the arriving and departing foragers and scouts of the swarm. Further, I hypothesize that the hopscotching flights have evolved as a means of ridding *A. cerana* colonies of predatory hornet loads.

4-9 Spatial variation in tri-trophic interactions: ants determine host plant use by lepidopteran larvae

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Ants are considered dominant organisms in tropical ecosystems, and frequently act as generalists predators on foliage. Therefore they probably have a marked influence on host-plant use and defense mechanisms in phytophagous insects. For instance, interactions with ants have been demonstrated to be important in determining biological traits of lepidopterans, whose larvae are important consumers of leaves. Many plants possess sources of liquid food available for ants, such as extrafloral nectaries and honeydew-producing Hemiptera. Such plants are intensively visited by ants day and night, especially in ant-rich tropical ecosystems such as the Brazilian cerrado savanna. Previous studies showed that infestation levels of phytophagous insects on plants bearing ant attractants could be reduced by the ants' aggressive or predatory behavior. Consequently one would expect that use of food plants by lepidopteran caterpillars would differ among plants species, in accordance with the level of ant visitation (high or low). In addition, the characteristics of caterpillar fauna (i.e., defense traits) would probably vary among plants as well. In this study we surveyed caterpillars using plants with and without ant attractants in four cerrado sites. We also sampled the ant fauna visiting these plants by using pitfall traps adapted to arboreal surveys. We found that shelter construction is a common trait in caterpillars using highly ant-visited plants. Moreover, the composition of the caterpillar fauna is affected by the presence of ant attractants on plants, but the magnitude and direction of this effect considerably varies on a small geographical scale. The specific composition of ant assemblages at each location could be responsible for such variation. Thus, while caterpillars probably respond negatively to some ant species (through decreased infestation on plants visited by aggressive predators), they apparently show no response to innocuous ant species. (Funds: FAPESP, CNPq).

4-10 Dispersal abilities of a social parasite of *Myrmica* ants

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Many butterfly species in the family Lycaenidae associate with ants during at least part of their caterpillar stage. The associations range from loose and facultative to specific and obligate, and in a very few cases develop into parasitism. This is the case for the large blue butterflies (*Maculinea*), which live as social parasites of *Myrmica* ants during the final larval instar. *Maculinea* caterpillars employ one of two feeding strategies; i) predatory, feeding exclusively on ant grubs or ii) cuckoo, partly feeding on ant regurgitations and trophic eggs. *Maculinea arion* is a predatory species, causing severe fitness reductions in infected *Myrmica* nests (= high virulence) compared to cuckoo species. By estimating dispersal abilities of *M. arion* and comparing it to cuckoo *Maculinea* species and other lycaenids, we test the hypothesis that highly virulent parasites evolve better transmission abilities to avoid overexploitation of their hosts.

4-11 TESTING FOR CHEATER BEHAVIOUR IN AN ANT-APHID MUTUALISM

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One of the best examples of a trophic mutualism in social insects is that between ants and aphids, whereby aphids secrete a sugary-rich food source termed honeydew, and the ants protect the aphids in return against natural enemies (Hölldobler and Wilson 1990 *The Ants*). Conflict, however, can occur when either the ants or the aphids exploit the mutualism by not returning their services (Offenberg 2001 *Behav. Ecol. Sociobiol.* 49, 304-310; Stadler and Dixon 1998 *J. Anim. Ecol.* 67, 454-459). The aphids, for example, could gain a selfish advantage by secreting less honeydew, but still take advantage of the protective services of the ants. We investigate this hypothesis using the native *Lasius niger-Aphis fabae* ant-aphid system as a model. Based on HPLC analysis of honeydew we show that some aphid clones do not produce melezitose, which is normally one of the most attractive sugars in ant-aphid mutualisms. We suggest that these clones might be cheaters, which try to keep more resources for themselves to benefit their own growth, whilst taking advantage of the ant-attracting effect of other melezitose-producing aphids coexisting on the same plant. The latter idea is plausible, given that genetic analyses demonstrate that in general, multiple aphid colonies coexist on the same plant. In subsequent experiments, the possibility that melezitose-deficient aphid clones are cheaters is tested by measuring their intrinsic growth rate as well as via binary choice experiments with ant colonies.

Oral Presentations

Major transitions in termite feeding biology and their consequences

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NITROGENOUS SOIL COMPONENTS AS DIETARY RESOURCE OF SOIL-FEEDING TERMITES

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Most termite species do not feed on sound wood but rather on lignocellulose in advanced stages of humification. This change in diet is most obvious in the true soil feeders, which are an important component of the soil fauna in tropical ecosystems. The talk will focus on the nutritional basis of soil-feeding termites and the putative roles of physicochemical gut conditions and intestinal microbiota in the digestive process. A large proportion of the nitrogen in humus is present in peptides or other nitrogen-rich residues of microbial biomass, which are structurally stabilized by their association with clay minerals and a covalent linkage to polyphenols. Feeding experiments with artificial humic substances showed that soil-feeding *Cubitermes* spp. are able to mobilize and digest the nitrogenous components of soil organic matter. The mineralization of nitrogen-rich substrates during gut passage is evidenced by the enormous accumulation of ammonia in the feces and nest material. Ammonia concentrations in the posterior hindgut (up to 130 mM) are among the highest values ever reported for soil macroinvertebrates. Nitrogenous soil components have been estimated to contribute substantially (20 -40%) to the dietary carbon oxidized by *Cubitermes* species. In lower termites, the bulk of the microbiota is located in a single hindgut dilation. Higher termites show an evolutionary trend towards an increasing gut compartmentalization, which is most apparent in the true soil feeders of the *Cubitermes* clade. Microsensor and radiotracer studies have revealed large dynamics in the physicochemical conditions and microbial activities along the gut, including an extreme alkalinity of the anterior hindgut (> pH 12). This situation is reflected also in the structure of the microbiological communities in the individual gut compartments, although our understanding of the microbial processes is still fragmentary and the functional role of many microbial populations remains unclear.

EVOLUTION OF TERMITE EUSOCIALITY SHIFTED NITROGEN ALLOCATION

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Termites resemble the young, altricial stages of their sister group, the wood-feeding cockroach *Cryptocercus*. This suggests that termites are paedomorphic, i.e., they have the juvenile morphotype of their ancestors, one that is characterized by a small body size and a thin, unpigmented cuticle. One benefit to remaining small in size and thin in skin lies in bypassing the heavy nitrogenous investment required of a substantial melanized cuticle, a cuticle typical of young precocial cockroaches and of older developmental stages of wood-feeding cockroaches. If nitrogen is viewed as the currency of life history trade-offs in the termite ancestor, then the evolution of eusociality can be framed as a shift in the allocation of available nitrogenous resources, giving an explicit ecological context to social evolution in the group.

TERMITE GUT STRUCTURE AND ASSOCIATIONS WITH MUTUALISTS: 33 YEARS IN WONDERLAND

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I review landmarks in the documentation and understanding of termite gut structure and associations with mutualists, now informed by a great increase of data on intestinal microbial diversity made possible in the last decade by molecular genomics and proteomics, and in the light of contemporary theories on the origin, evolution and trophic diversification of the Isoptera. Detailed morphology is not included, but the more modern synoptic literature on anatomy, histology and in situ coiling is noted and discussed in relation to current concepts of the termite gut as a bioreactor system. Knowledge of intestinal microbiology, and of microbial physiology and metabolism, has outstripped progress in understanding secretory and absorptive processes by the gut wall and associated structures, such that the primary substrates fermented in the hindgut and the end products utilised by the termite host are still not precisely identified in many cases. Current perceptions of the specialised digestive processes of fungus-growing and soil-feeding termites are summarised, and an overarching evolutionary thesis is proposed, arguing that social organisation in termites has developed primarily to safeguard the fidelity of symbiont transmission between individuals and generations. Suggestions for future lines of work by the younger generation of researchers will be offered, focusing on physiological regulation of the intestinal microecosystem by the termite host.

THE MAJOR TRANSITION TO TERMITE FUNGICULTURE

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In termites, a single transition has occurred to the cultivation of edible mushrooms, in the subfamily Macrotermitinae, whose ancestor domesticated basidiomycete fungi (genus *Termitomycees*) ca 30 mya. The fungi produce most of the food for the termites, while the termites provide optimal stable growth conditions for the fungi. Despite the obligate nature of this relationship, both partners still reproduce and disperse independently and potentially can form associations with many alternative genotypes. In my talk, I will discuss recent findings on the evolution of this mutualism, focusing on how termites can select fungi during several stages of their life cycle. First, studies on large-scale co-evolutionary patterns have shown that interaction specificity occurs, mainly at the genus level. I will discuss how specificity may arise, and how partners select each other. Second, experimental work shows how colonies succeed in propagating only a single heterokaryon of their *Termitomyces* symbiont, despite initiating cultures from genetically variable sexual spores from the habitat at the start of a colony. This exclusive lifetime association of a host colony with a single fungal symbiont hinders the evolution of cheating. However, I will argue that continuous `artificial' selection of the fungus by termites during a colony's life time is required in order to keep the fungus productive.

EVOLUTION OF TERMITE FEEDING BEHAVIOUR IN A PHYLOGENETIC PERSPECTIVE

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Thanks to their complex social organisation, termites have colonised many new habitats and nowadays are amongst the most abundant animals in tropical ecosystems. This is particularly true in rainforests where they are outstandingly abundant, being one of the principal organic matter decomposers. Whereas all termite families except the Termitidae are mostly xylophagous, food source diversification characterise this latter family whose members can feed on plant material at various stages of decomposition, from living wood to soil organic matter. Here, we investigate (1) the evolutionary shift from wood to soil-feeding habit, through phylogenetic autocorrelograms among the major Termitidae lineages, and (2) the feeding substrate diversification (estimated from δ15N isotopic analyses) among species of the *Anoplotermes*-group. When all Termitidae were considered, related species feed on more similar substrates than unrelated ones. This phylogenetic autocorrelation is mainly marked among species of the same genus. A detailed analysis of the *Anoplotermes*-group also shows that closely related species tend to feed on similar substrate. This suggests that if food source diversification may favour species coexistence, it is not a primary factor of speciation.

LIGNOCELLULOSE DIGESTION AND SIZE REDUCTION OF THE BODY AND THE MIDGUT OF TERMITES

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It seems as if the short and slender fore- and midgut and their body sizes of termites were the degenerated status from their voluminous ancestral-forms such as those of wood-feeding cockroaches. Especially their midgut is considered to be dysfunctioal since the acquisition of symbiotic protozoa freed it from digestion (Dow, 1986, Advan. Insect. Physiol. 19:188-328). On the contrary to this conception, we propose a functional size reduction of the termite midgut and the body of termites through divergence from those of the common ancestor of a cockroach form. Our proposal is based on: 1. To break down lignin and hemicellulose matrix and enlarge enzyme-accessible surface area of cellulose fibers, the particle size of the ingested wood should be minimized. Therefore, the mandible size was reduced as termites reduced their body size. 2. To accomplish efficient and quick recovery of soluble sugars (i.e. glucose) generated by the endogenous cellulase, termites needed to enlarge the inner surface area of the midgut per luminal volume, thus, the midgut volume should be minimized. On the other hand, the volume of the hindgut paunch was developed to maintain anaerobic condition for symbionts. 3. Some species of termites accomplished extremely high cellulase activity per volume with limited nitrogen resources, therefore, the midgut volume would be reduced. 4. As the drastic reduction in the body size from cockroaches to termites, the absolute capacity of wood digestion per temite should be remarkably diminished. Thus, to maintain the required productivity of nutrient and energy for prosperity, termites would have developed highly populated societies. To address our assumption, we will discuss mainly about the digestive systems of a wood-feeding cockroach (*Panesthia angustipennis*) and subterranean termites (*Coptotermes formosanus* and *Reticulitermes speratus*).

Poster Presentations

Major transitions in termite feeding biology and their consequences

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5-1 WHY ARE FLAGELLATE – TERMITE ASSOCIATIONS EVOLUTIONARY UNSTABLE?

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Flagellates are necessary for the survival of lower termites because these gut symbionts are required for the degradation of lignocellulose. On the other hand, flagellates are strictly anaerobic and cannot survive outside their gut habitat. Therefore, one would expect that such inter-dependent symbiotic associations would be evolutionary stable and would persist for long periods of co-evolution. However, previous studies revealed that species composition of flagellate communities considerably vary between termite families and genera, suggesting that strict co-evolution between flagellates and termites have not occurred. In this study, we compare flagellate communities between termite species that belong to the genus *Reticulitermes* (Rhinotermitidae). Our microscopic observations confirm the presence of eleven flagellate genera in *Reticulitermes* species (nine genera of *Parabasalia* and two genera of *Oxymonadida*). Further observations as well as the sequencing of a region of about 1800 base pairs of the small subunit of the rRNA region reveal unexpected species diversity within most of the flagellate genera. Phylogenetic analyses of the flagellates and their hosts suggest that the co-evolutionary histories of these symbiotic associations are complex, suggesting events of co-cladogenesis, but also evidencing recurrent events of symbiotic loss and horizontal transmission. This study reveals that, even during the diversification of a single genus of termite, flagellate – termite associations seem to be evolutionary unstable and in perpetual dynamics. The possible reasons for the evolutionary dynamics of these inter-dependent symbiotic systems will be discussed.

5-2 HOW SUBTERRANEAN TERMITES (ISOPTERA: RHINOTERMITIDAE) BEHAVE WHEN THEY FIND CONSECUTIVE FOOD SOURCES?

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Some researchers have proposed that subterranean termites exhibit fidelity to food sources, while others disagree with this hypothesis. The aim of the present study was to verify the feeding behavior of the subterranean termites *Coptotermes gestroi* and *Heterotermes tenuis* in bioassays with three consecutive food sources of the same size, shape and origin. A nest chamber containing foragers of *C. gestroi* (500 workers + 50 soldiers) or *H. tenuis* (500 workers + 30 soldiers) was connected to a food chamber. The latter was connected to other two consecutive food chambers, and all these chambers contained a wood block of *Pinus elliotti* (27 cm3). During the whole experimental period, the movement of individuals was observed and 7 days after termites reached the last chamber, the percentage of consumption of each wood block was calculated for 10 replicates performed for each species. The statistical results revealed that for *C. gestroi*, the consumption in the first chamber was higher than that in the third chamber ($P = 0.0133$). For *H. tenuis*, the consumption in the first chamber was higher than that in the second ($P = 0.0059$) and third chambers ($P = 0.0006$), indicating a chronological priority in relation to the discovery of food resources. The behavioral data of *C. gestroi* revealed that the movement of individuals among chambers was more intense in the first 72-96 hours, suggesting that after the discovery and choice of a food, termites exhibited fidelity to it, not searching and consuming other food sources. However, for *H. tenuis*, intense movement was observed throughout the whole experimental period, suggesting that in addition to fidelity, there is a labor division among certain groups of the same caste. The two species clearly exhibited distinct feeding patterns, and *C. gestroi* showed higher fidelity to the food resource in comparison with *H. tenuis*.

5-3 SIMILARITIES IN MIDGUT CELLULOLYTIC ENVIRONMENT BETWEEN A HIGHER TERMITE AND A LOWER TERMITE

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Unlike lower termites, xylophagous higher termites thrive on wood without the aid of symbiotic protists. In the higher termite *Nasutitermes takasagoensis*, we found that cellulases were present both in the midgut and the hindgut, while approximately 60% of total cellulolytic activity in the gut was localized to the midgut. We have previously shown that the site in expression of endogenous endo-β-1,4-glucanase was shifted from the salivary glands of lower termites to the midgut of higher termites. Our recent study also revealed that the endogenous β-glucosidases genes were expressed in the salivary glands and the midgut in *N. takasagoensis*, while the apparent expression site of β-glucosidase in lower termites is only the salivary glands. These results lead to the possibility that the production of cellulases in the midgut is related to a loss of protists in higher termites. To further explore the detailed celluloytic system in the midgut of *N. takasagoensis*, we raised antisera against these cellulolytic enzymes. Using immunohistochemistry and digital light microscopy, we determined the spatial distributions of cellulolytic enzymes as well as the volume of the endoperitrophic space of the midgut. In *N. takasagoensis*, the cellulolytic enzymes were uniformly distributed in the midgut epithelium and the concentration of cellulase activity against microcrystalline cellulose in the midgut was comparable to that of the wood-feeding lower termite, *Coptotermes formosanus*. These results suggest that the micro-environment in the midgut of *N. takasagoensis* is very similar in terms of cellulose digestion to that of *C. formosanus*, which digests cellulose with the aid of the hindgut protists. The present data may exclude the possibility that the alteration in the site of cellulose digestion directly triggered a loss of the symbiotic protists in termites.

Oral Presentations

The role of relatedness in social evolution: a critical assessment of when it matters and when not

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Dominance, policing, and manipulation: does variation in relatedness matter?

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Kinship plays a fundamental role in the evolution of insect societies. Given that it affects the inclusive fitness of nestmates, it is also expected to have a strong influence on the outcome of intracolonial conflict about resource allocation and the partitioning of reproduction. Indeed, variation in nestmate relatedness has been shown to affect sex allocation ratios and the division of reproductive labor in some species, but this influence appears to be less universal than often assumed. For example, reproductive skew among nestmate queens in *Leptothorax* ants does not vary with relatedness as expected from skew theory, the production of male ants is usually monopolized by the queen rather than workers regardless of the mating frequency and number of queens, and policing and dominance did not differ between single-clone and multi-clone colonies of the thelytokous ant *Platythyrea punctata*. This does not mean that variation in relatedness does not influence the social structure of insect societies, but in the case of male production it might reflect the importance of costs associated with worker egg laying and, in competition among potential totipotent reproductives, the relative unimportance of kin relationships among locally competing individuals.

Reciprocity: an evolutionary mechanism promoting advanced sociality

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Since Trivers' (1971) influential paper reciprocity has been conceived as one of several evolutionary mechanisms responsible for cooperation in animals. However, despite high expectations regarding the importance of this mechanism in social evolution, empirical evidence for its existence is scant. There is a disturbing discrepancy between the development of theoretical models in search of reciprocity mechanisms generating stable cooperation and the information on reciprocity mechanisms causing cooperation among animals. Is reciprocity a concept providing intellectual challenge but without practical importance, perhaps because competition and exploitation cannot be overcome? Here I aim to show that reciprocity does indeed generate cooperation in animals, and I argue that the apparent discrepancy between theory and data originates from unrealistic expectations of mechanisms underlying animal behaviour.

No intracolonial nepotism during colony fissioning in honey bees

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Most species of social insects have singly mated queens, but in some species each queen mates with numerous males to create a colony whose workers belong to multiple patrilines. This colony genetic structure creates a potential for intracolonial nepotism. One context with great potential for such nepotism arises in species, like honey bees, whose colonies reproduce by fissioning. During fissioning, workers might nepotistically choose between serving a young (sister) queen or the old (mother) queen, preferring the former if she is a full sister but the latter if the young queen is only a half sister. We examined three honey bee colonies that swarmed, and performed paternity analyses on the young (immature) queens and samples of workers who either stayed with the young queens in the nest or left with the mother queen in the swarm. For each colony, we checked whether patrilines represented by immature queens had higher proportions of staying workers than patrilines not represented by immature queens. We found no evidence of this. The absence of intracolonial nepotism during colony fissioning could be because the workers cannot discriminate between full-sister and half-sister queens when they are immature, or because the costs of behaving nepotistically outweigh the benefits.

UNRELATED HELPERS IN A PRIMITIVELY EUSOCIAL WASP: IS HELPING TAILORED TOWARDS DIRECT FITNESS?

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The paper wasp *Polistes dominulus* is unique amongst the social insects in that nearly a third of subordinate co-foundresses are completely unrelated to the dominant individual whose offspring they help to rear. These unrelated subordinates stand to gain direct fitness through nest inheritance, raising the question of whether their behaviour is adaptively tailored towards maximizing inheritance prospects. Unusually, in this species a wealth of theory and empirical data allows us to predict how unrelated subordinates should behave. Based on these predictions, here we compare helping in subordinates that are unrelated or related to the dominant wasp across an extensive range of field-based behavioural contexts. Our study provides one of the most comprehensive attempts to clearly seperate the effects of kin selection and direct fitness benefits on individual behaviour.

UNDERSTANDING NEST DRIFTING BEHAVIOUR IN PAPER WASPS USING A SOCIAL NETWORK APPROACH

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Workers in social insect nests are usually faithful to their natal nest where they gain indirect fitness benefits by helping raise relatives. Nest drifting behaviour, where workers, known as drifters, visit nests other than their own, is an exception to this. Explanations for drifting behaviour include errors in nest identity when nests are located close to each other, and social parasitism where drifters infiltrate colonies in order to reproduce. In the Panamanian paper wasps, *Polistes canadensis*, however, it appears that drifting wasps gain indirect fitness benefits by helping raise brood on several different (but related) nests (Sumner *et al.* 2007 *Current Biology* 17, 140-145). Leaving their natal nest to drift and help raise less related brood in other nests is a potential challenge to kin selection theory: workers should be selected to maximize their inclusive fitness by focusing their helping effort on highly related brood on their natal nest. We used radio frequency identification (RFID) tags to uniquely identify drifters and detect drifting patterns amongst nests within aggregations. This allows us to accurately quantify the helping effort of drifters on different nests. We analyse drifting patterns using a social networks approach (Croft *et al.* 2008 *Exploring animal social networks*, Princeton) in order to determine the importance of relatedness relative to productivity benefits, spatial and demographic factors.

ARE THERE BENEFITS TO FORAGING WITH KIN IN AN INBRED SOCIAL SPIDER?

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According to Hamilton (1976) limited dispersal (population viscosity) can generate high degree of relatedness between interacting individuals, allowing for the development of group cooperation by kin selected benefits. However, it also means that closely related individuals stay and compete over common resources. Thus, individuals may trade benefits of kin cooperation for costs of competition for local resources. Given a paucity of empirical studies that investigate this tradeoff, we explored the cost and benefit of kin cooperation in the permanently social spider *Stegodyphus dumicola*. In *S. dumicola* individuals cooperate in prey capture and feed communally on large prey items. There is no division of labor among group members but larger individuals mature first and are able to reproduce, while smaller ones may remain virgin and thus act as workers in the colony. By manipulating relatedness among group members, we investigated the possible cost and benefits of cooperating with kin and non-kin in prey capture. Individuals in kin groups did not forage more efficiently and did not increase in weight more than individuals in non-kin groups. However, in groups of non-kin the number of group members that foraged together decreased as the amount of food remaining decreased over a feeding bout, suggesting greater competition in non-kin groups. This effect declined over a period of two months from the initial formation of the groups. These results suggest that foraging in groups of kin and non-kin produce the same net benefit in spite of weak competition in non-kin groups.

COLONY-SIZE DEPENDENCE OF WORKER POLICING IN THE MONOGYNOUS AND MONANDROUS ANT *DIACAMMA SP.* FROM JAPAN

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A new mathematical model on the evolution of worker policing in the social Hymenoptera has recently been proposed based on an assumption that intracolonial conflicts over sex allocation, male parentage, and reproductive allocation occur simultaneously. A novel prediction of this model is that worker policing will depend on the growth stage of the colony and, a single mating by the queen, effective worker policing will occur only during the ergonomic stage. Our data on the ponerine ant *Diacamma sp.* from Japan, which is a monogynous and monandrous species, clearly supports this prediction. In small colonies, worker-produced eggs were strongly policed by workers during the ergonomic phase, whereas in large colonies during the reproductive stage, these eggs were not strongly policed and consequently a high percentage survived. Our results suggest that resource allocation and colony efficiency rather than relatedness has a major impact on the evolution of worker policing.

NO FACULTATIVE WORKER POLICING IN A DANISH POPULATION OF THE VESPINE WASP *DOLICHOVESPULA SAXONICA*

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In some ants, bees and wasps, workers kill or "police" male eggs laid by other workers in order to maintain the reproductive primacy of the queen. Kin selection theory predicts that multiple mating by the queen is one factor that can selectively favour worker policing. This is because when the queen is mated to multiple males, workers are more closely related to the queen's sons than to the sons of other workers. In what is often considered as one of the strongest tests so far, Foster & Ratnieks (2000) provided evidence that in the Vespine wasp *Dolichovespula saxonica* workers only police worker-laid eggs in colonies headed by a multiple mated queen, but not in those headed by a single mated one. This conclusion, however, was based on a relatively small sample size. Here we reinvestigated the possibility of facultative worker policing in a Danish population of the Saxon wasp *Dolichovespula saxonica*. Significantly, our data show that there is no correlation between worker-worker relatedness and the percentage of adult worker-derived males or the percentage of workers with active ovaries. Hence, our study provides no evidence for facultative worker policing, even though it was based on a larger sample size (14 colonies, 521 males genotyped) than the original one, and had sufficient power to detect facultative policing if it would have been present. The percentage of adult worker-derived males, however, correlated with the seasonal progression of the nest and was also higher among males reared in small-sized cells. Overall, our results suggest that the earlier evidence for facultative worker policing in *D. saxonica* may have been due to correlations with certain confounding variables such as seasonal factors, which were not accounted for, or else that there are large differences between populations.

LARVAL CASTRATION IN THE ANT *HARPEGNATHOS SALTATOR*: AGGRESSION AS A CASE FOR CASTE CONFLICT OR A RED HERRING?

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Aggressive behavior is a conspicuous component of animal groups and is often regarded as a physical expression of conflict. In social insects, aggressive behaviors such as worker policing and dominance interactions are involved in regulating conflict over reproductive rights. Here we investigate a behavior of the ant *Harpegnathos saltator*, where workers attack and bite larvae that have been induced to develop as queens. Because workers of *H. saltator* do not control larval feeding, adult workers may bite larvae as a means to regulate queen development. Larvae that develop as queens have the opportunity to reap high direct-fitness benefits, but overproduction of queens would incur a cost to the colony. While this may present an opportunity for conflict over caste fate, it assumes that queens have an inherent fitness advantage over workers, which may not be true in *H. saltator*. In addition to the reproductive advantages of queens, workers of *H. saltator* have an opportunity to receive direct fitness benefits during the colony's queenless phase, when workers mate and reproduce. This second opportunity to become a reproductive may depress the initial advantage of developing as a queen. We present an alternative hypothesis, that larval-directed biting may be a mechanism to control mistakes in development, and does not necessarily imply a conflict between larvae and adult workers.

Poster Presentations

The role of relatedness in social evolution: a critical assessment of when it matters and when not

6

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6-1 Benefits of helping in the primitively eusocial halictid bee *Halictus scabiosae*

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Eusocial halictid bees are good model systems to study factors promoting sociality, because some of the female offspring remain in the natal nest to act as workers even though all females have the capacity to mate and reproduce. We investigated the benefits provided and received by workers in *Halictus scabiosae*. This species has an annual colony cycle with two consecutive broods. The first brood consists usually only of workers, while the second brood develops into reproductives. We measured worker impact on colony survival and productivity by comparing the number of reproductive offspring produced in colonies where the total worker number (mean ± SE = 2.4 ± 0.19) had either been reduced by one (Treatment: n = 131 nests) or left unchanged (Control: n = 114 nests). The number of workers had a significant effect on colony survival and productivity. The treatment decreased colony productivity significantly, particularly in colonies with less than 3 workers (Cohen's d = 0.76). To study reproductive partitioning, we reconstructed sibship in 66 colonies using 11 microsatellite markers and identified mothers of the reproductive offspring in each colony. Previous studies had shown that nearly half of the colonies show complex colony structure. In our study, 27% of the offspring were produced by nestmate foundresses or workers, the rest was laid by unknown females. Moreover, surprisingly few of the original foundresses (15%) or workers (8%) contributed to the reproductive offspring in the investigated colonies. Low relatedness between reproductive female offspring and foundresses (r ± SE = 0.08 ± 0.05) or workers (0.19 ± 0.03) endorses this finding. Egg-laying workers had significantly fewer offspring (mean ± SE = 1.56 ± 0.22) than foundresses (5.25 ± 1.77). Overall, worker help is valuable in small colonies, but further investigation is necessary to calculate the impact of the surprisingly dynamic colony structure on the inclusive fitness of foundresses and workers.

6-2 Social evolution and relatedness in a sphecid wasp

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Hymenoptera are ideal models to address social evolution, especially questions related to inclusive fitness. Kin selection based models of the evolution of sociality have been empirically tested on eusocial Hymenoptera, particularly ants, social bees and vespid wasps. A large family of Hymenoptera, the Sphecidae, has been rather neglected with respect to social evolution, yet the existing body of literature addressing these primarily ground-nesting wasps, is often descriptive and anecdotal, labels many species as social. Nest sharing, for example, is especially frequent in sphecids of the genus *Cerceris*. Given the phylogenetic distance of sphecid wasps from ants, bees and vespid wasps, it offers new and independent insights into the evolution of social behaviour. *Cerceris rubida* (Jurine) is a social Sphecidae. Nest-mates exhibit a division of labour based on differences in size. Surprisingly, some individuals change nest (and task) during their lifetime (Giovanetti and Jacobi, submitted). This species represents an excellent study system to investigate the inclusive fitness expectations of high relatedness among cooperating nest-mates in social Hymenoptera. We developed polymorphic microsatellites for *C. rubida* and genotyped individuals from two Mediterranean populations. Data resulting from a two-year Marie Curie Individual Grant (to MG; SENSE; FP7-PEOPLE-2007-2-1-IEF; N° 220876) are used to examine the kinship structure of nests and explore the role of inclusive fitness for the evolution of social behaviours in this sphecid wasp.

6-3 Evolutionary flexibility of social organisation in an ant

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Important questions in social insect biology, and more generally in the study of the evolution of social groups, are: how flexible is social organisation over evolutionary time and how does selection mould social organisation? We have studied a fundamental feature of social organisation namely the level of skew among queens within nests. We present genetic evidence showing that social organisation in multiple queen nests of the widespread ant *L. acervorum* varies strikingly among populations. In most populations multiple queens reproduce, leading to low relatedness among colony members - polygyny - but recent work shows that in populations from Spain only one queen reproduces so multiple queen nests are in fact functionally monogynous. Here we provide further genetic evidence showing that functional monogyny is also found in Japanese populations. Furthermore, phylogeographic analysis of mtDNA rejects the hypothesis that populations with different social organisations are in fact cryptic species. Instead there is little genetic differentiation at mtDNA between populations with polygynous or functionally monogynous social organisations. Likewise, there is no evidence that the two documented functionally monogynous populations (in Spain and Japan) are either monophyletic, nor genetically distinct in spite of being separated by more than 10,000 km. These data suggest that the degree of reproductive skew amongst queens in *L. acervorum* has been highly labile over recent evolutionary time. We speculate on what might drive selection for alternative social organisations.

6-4 Social wasp worker behavior is not based on relatedness, but eusociality requires it

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Polistes females that become workers at their natal nest do so with activated reproductive physiology, whereas gynes are in reproductive diapause. All female Aculeata require proteinaceous liquid nourishment to enable ovarian development. Newly-emerged reproductively primed *Polistes* find it in the form of nestmate larvae's saliva. Induced by the saliva to remain at the nest, they are exposed to cues from larvae and the nest itself that, if the larvae and nest were their own, would elicit maternal care. The wasps therefore undertake maternal care, but they do so allomaternally. The energetic cost of the allomaternal workload is so high as to inhibit ovarian development. The newly emerged wasps, although primed for reproduction, become workers. If workers and beneficiaries are related, all benefit. If allomaternal care is expressed to non-relatives, the workers cannot benefit. Workers' behaviors are identical in both cases, showing that the expression of worker behavior does not require relatedness. By logical inference, the expression of worker behavior to relatives does not require relatedness. Worker behavior originates *de novo* as a behavioral exaptation in the convergence of adaptations for maternal care that elicit maternal care in a situation in which maternal care is expressed heterochronically and allomaternally. Allomaternal worker behavior is adaptive for all beneficiaries, but allomaternal worker behavior can only become an adaptation when workers and beneficiaries are relatives. The transition from adaptive worker behavior to worker behavior as an adaptation marks the threshold of eusociality. The transition requires kin and multilevel selection and occurs via genetic assimilation. Thus, the origin of worker behavior precedes the threshold of eusociality and does not require relatedness. Eusociality follows, and relatedness is required.

6-5 ENTRAPPED ANTS ELICIT PRECISE RESCUE BEHAVIOUR FROM RELATIVES

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Although many animals can identify their relatives and deliver altruistic behavior in their favor, studies reporting rescue behavior are rare. Nonetheless, here we report the first experimental evidence that a desert ant, *Cataglyphis cursor*, use precisely directed rescue behaviour to free entrapped victims; equally important, they carefully discriminate between individuals in distress, offering aid only to nestmates. Our experiments are based on a simulation of a natural situation which we often observed in the field when collecting *Cataglyphis* ants, causing sand to collapse in the process. Using a novel technique that binds victims experimentally and partially covers them with sand, we show that ants are able to recognize what, exactly, holds their relative in place and direct their behaviour to that object, the snare, in particular: They begin by excavating sand, which exposes the snare, transporting sand away from it, and then biting at the snare itself, thus engaging in highly precise object-directed behaviour to free their entrapped relatives. Our results demonstrate that rescue behaviour is far more sophisticated, exact and complexly organized than the simple forms of helping behavior already known. Although we predict that rescue behavior should be limited to circumstances in which the victim and the rescuer are highly related to one another, or in which unrelated individuals must cooperate very closely with one another, we also predict that it is likely to be far more common than the current literature would suggest. To address this oversight, we propose a rigorous definition of rescue behavior, one that helps researchers to focus on its necessary and sufficient components, at the same time that it helps to differentiate rescue behavior from cooperation and other forms of helping behavior. In this way we also hope to expand our understanding of altruism in particular and kin selection in general.

6-6 QUEEN FECUNDITY AS A FUNCTION OF WORKER ATTENTION IN POLYGYNOUS *FORMICA FUSCA*

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In polygynous ant species queens complete for limited colony resources to reproduce. Reproduction by several queens dilutes within-colony relatedness which reduces inclusive fitness returns that workers obtain from rearing their full siblings. Far from being helpless though, workers may be in the position to manipulate queen reproductive traits to increase their inclusive fitness. Fecundity (i.e. egg-laying rate) is potentially one of the most important queen intrinsic traits predicting queen's ability to gain a larger share of reproduction. Previous work on *Formica fusca* has shown that queens advertise their fecundity chemically and workers respond to this information by preferentially associating with highly fecund queens. We investigated whether high queen fecundity is the cause or the consequence of worker attention, and by extension, whether workers can manipulate queen fecundity by adjusting the amount of attention they devote to a queen. In a paired-design experiment we matched queens of different fecundities into treatments with different number of workers (high and low worker attention) and monitored their egg-laying over period of 24-days. The amount of worker attention a queen received had a direct impact on her egg-laying rate, so that greater worker attention translated into higher queen fecundity, irrespective of initial queen fecundity.

6-7 Does relatedness matter at the brink of sociality?

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The eastern carpenter bee of North America, *Xylocopa virginica*, is usually found nesting socially, but also may nest solitarily. In southern Ontario, Canada, near the northern edge of its range, most females nest socially, in groups of 2-6. The high frequency of sociality suggests that this nesting strategy is favoured. Moreover, the habit of overwintering with siblings in the natal nest, high rates of inherited nest reuse, and the ability to discriminate between nestmates and non-nestmates, suggest that social nesting occurs in cohesive kin groups. This implies that the high degree of reproductive skew among social females may be explained by kin selection, with subordinates helping to raise the brood of closely related dominants. However, several observations contradict this hypothesis: social nests produce no more brood than solitary nests; adult females frequently relocate to new nests, either temporarily or permanently; dominant foundress mortality and replacement rates are high, so broods often comprise the offspring of several females that may or may not be related; and a few females forego reproduction altogether, overwintering a second time and becoming dominant foragers the following spring. These observations suggest that social nesting is not favoured by benefits of kin-selected, cooperative brood-rearing. An alternative hypothesis is that social nesting is a response to severe competition for nests and nesting substrate, which are costly to construct and reused and refurbished for many generations. Females that move from nest to nest are likely assessing the quality of alternative locations or opportunities for joining existing nesting associations. However, nestmate recognition may sometimes facilitate movements of subordinate females into nests of related or familiar adults. Thus the role of relatedness may be to enhance possibilities for subordinates to inherit breeding opportunities late in the season or the following year.

6-8 Cannibalism and conflict in the ant *Formica aquilonia*

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The polyphenic development into reproductive queens and sterile workers is a fundamental property of social insect colonies. In such systems, conflict over caste-determination can arise because each individual would gain direct fitness benefits from developing into a reproductive queen in the next generation. Selection would therefore favor larvae with selfish traits that increase their own chances of turning into a queen, even at the expense of the colony. Larval egg cannibalism is a prime example of such a selfish trait. In ants, brood is reared in batches and larvae commonly feed on eggs. As caste fate is strongly influenced by nutrition and eggs provide a high-quality food source, cannibalistic larvae can increase their chances of developing into queens, while at the same time removing potential competitors for breeding positions. In mediating this conflict, the relatedness between colony members is crucial. This is because closely-related individuals would suffer severe indirect fitness losses from cannibalizing kin; the level of competition between larvae is therefore predicted to be intense in polygynous (multiple queens) colonies with low levels of relatedness, and decrease in monogynous (single queen) colonies where relatedness is high. Ecology, behavior and genetics thus all play an important role in determining cannibalistic tendencies of larvae. We will study selfish larval egg cannibalism in the highly polygynous ant *Formica aquilonia*. In particular, we will investigate the use of recognition cues underlying cannibalistic behavior by comparing prevalence of cannibalism in broods with experimentally manipulated levels of genetic diversity. If the larvae are able to respond facultatively to the kin structure of their colony, cannibalism should be more prevalent in genetically diverse low relatedness broods than in genetically uniform high relatedness broods.

6-9 Indications for cooperative breeding in *Coccotrypes dactyliperda* (Coleoptera, Scolytidae)

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Actions of group members are defined as cooperation when they benefit both, the carrier and other individuals. Cooperation may occur in foraging, group defense, and offspring care. Researches of social evolution in invertebrates have often focused on relatedness among group members, referring to Hamilton's theory of kin selection. In contrast, studies of sociality in vertebrates emphasized ecological factors that promote cooperation. These approaches were recently bridged in a theory that presents sociality as decisions made both during the organism's life and on an evolutionary scale. The decisions whether to disperse, breed in the natal group or cooperate in offspring care, are key decisions in social strategies. Some characteristics of the haplodiploid beetle, *Coccotrypes dactyliperda* (Scolytidae), suggest cooperation among individuals. We manipulated genetic relatedness in *C. dactyliperda* and examined its effects under high and low availability of breeding sites. The results show that some females delay dispersal even when dispersal opportunities exist. Under high relatedness the proportion of dispersers is lower and the reproductive success of the females and the colony increases. Furthermore, indications for shared parental care were found. The results suggest the existence of cooperative breeding that is affected by relatedness. These results reinforce the theory that combines genetic and ecological approaches to understand evolution of sociality and cooperation.

6-10 The mating system and colony composition of the eusocial beetle *Austroplatypus incompertus*

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The Ambrosia beetle, *Austroplatypus incompertus*, has two unique qualities. First it is the only described beetle species, out of approximately 300,000, known to be eusocial. Second it is one of only a handful of species worldwide which bore into living, healthy trees. As it inhabits living Eucalypt trees, the gallery and its food source is relatively stable and although extensive, possesses a single small entrance that is easily defended. Mature colonies have been shown to possess overlapping generations with morphologically distinct females undertaking tunnel maintenance and defense. Due to its cryptic lifestyle, direct behavioral observations are not possible and molecular approaches are required to learn more about this unique system. We previously determined that the species is diplodiploid; a finding consistent with the idea that collateral kin selection as a result of skewed genetic relatedness (the haplodiploidy hypothesis) has not played a significant role in the evolution of eusociality in this case. By utilizing a set of newly developed molecular markers this project will characterize, for the first time, the mating system and social structure (group composition) of *A. incompertus* colonies within *Eucalyptus pilularis* from the Eastern ranges of Australia. The relatedness data generated in this project will increase our ability to pursue alternative hypotheses for the evolution of eusociality within Coleopterans.

6-11 WHAT DOES IT MEAN TO BE MORE THAN THE SUM OF YOUR PARTS AND HOW DOES THIS AFFECT THE EVOLUTION OF SOCIALITY?

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Many of the structures built by and the tasks performed by insect societies are only possible because of the interaction of thousands or millions of individuals. These observations often lead us to remark that insect societies are more than the sum of their parts or that they act synergistically. But how do we quantify this idea and what consequences does it have for social evolution. I will try to provide an answer to both of these questions. For the first question, I argue that a colony is more than the sum of its parts if, for some range of colony sizes, as we add more members the per capita benefit to an individual grows. There is growing evidence for such synergistic increases in fitness with group size in some aggregative species, such as tent caterpillars (e.g. Allen, Inscetes Sociaux, 2009), as well as in ants and other eusocial insects. One simple evolutionary consequence of a group being more than the sum of their parts is that co-operation can be selected for without relatedness. A more subtle result is that the spread of an allele which encodes for a behaviour that provides synergistic increases in fitness with group size is usually frequency dependent. Moreover, increasing per capita success with colony size removes the potential limitation on the evolution of co-operation between relatives imposed by local competition for resources. These points make experimental testing to establish the origins of co-operation more complicated, since synergy sometimes undermines and other times supports the importance of relatedness in the evolution of sociality. These points relate to the oft repeated but empirically meaningless statements made about the importance of *b* and *c* in Hamilton’s rule. Here I will rather emphasise that measuring how colony fitness increases with colony size, together with measurements of ‘relatedness’, should prove a robust way of investigating the origins of evolution of co-operation.

6-12 WORKER REPRODUCTION AFTER COLONY FISSION IN HONEYBEES

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In honeybees, it is a rule that workers are sterile in the presence of the queen but start laying male-determinate eggs in queenless colonies. However, after colony fission we can expect some selfish behaviour from workers of the original colony towards a new sister-queen. After “election” of a new sister-queen, the sister-workers are obligate to rear their nieces and nephews instead of sisters and brothers like in a colony headed by a mother-queen. This means that the workers are related to the new sister-queen's offspring by half of what is normal in a colony headed by a mother-queen. It is important to remember that during swarming, the mother-queen leaves the original colony with new queens in a preimago stage along with all developing stages of workers. Thus, worker-larvae hatching from eggs laid by the old-queen during last three days (72 h - egg incubation period) before swarming develop in queenless conditions. Our prediction was that the lack of a queen is a proximate factor influencing larval investment into future reproduction. Using three naturally and six artificially swarming colonies, we compared body mass, the number of ovarioles, development of hypopharyngeal and mandibular glands in freshly emerged workers reared as larvae in queenright and queenless conditions. Then, we checked the workers from these two groups to see if they were able to lay eggs in queenright and queenless conditions. Our results clearly showed that workers reared as larvae without a queen were like a “Trojan horse”. They did not differ in body mass, but they had more ovarioles in their ovaries, smaller hypopharyngeal and bigger mandibular glands than workers reared in queenright conditions. After eclosion, these “Trojan” workers were ready to lay eggs both in queenright and queenless colonies. Our findings strongly support the role of kin selection theory in the evolution of eusocial insects.

Oral Presentations

Sex and the insect society: focus on unorthodox breeding systems

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CLONE WARS: PERSISTENCE OF THE SINGLE LINEAGE OF CHEATERS IN THE PARTHENOGENETIC ANT *PRISTOMYRMEX PUNCTATUS*

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The Japanese ant *Pristomyrmex punctatus* is known for its obligate parthenogenetic reproduction and extraordinary life history: all nestmates fulfill both reproduction and cooperative tasks. We revealed that most colonies were genetically heterogeneous, most of which should be achieved through drifting of individuals and/or colony fusion. Inclusive fitness theory predicts that the resulting lowered nestmate relatedness allows the cheaters to evolve, and indeed we found that the study population harbors cheaters which selfishly lay more eggs and show negligible participation in colonial tasks, as compared with their nestmate cooperators. These traits result in a negative fitness effect on their nestmates, which we confirmed by laboratory rearing experiments. Although theory predicts that the cheater genotypes are evolutionarily short-lived, population genetic analyses found that the cheaters belong to one monophyletic lineage which persisted for 200 - 9200 generations, which is longer than any comparable example of disruptive cheaters in nature. We also found that the cheaters migrate and are thus horizontally transmitted between colonies. Using computer simulation, we show that the estimated rates of cheating and horizontal transmission are sufficient for the cheater lineage to avoid the immediate extinction. Taking advantage of parthenogenetic reproduction, the cheater lineage serves as a rare example of "transmissible social cancer" in the ant societies.

IMPLICATIONS OF INBREEDING FOR SOCIAL EVOLUTION: INSIGHTS FROM COOPERATIVE SPIDERS

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Kin selection theory predicts that high relatedness was a pre-condition for the evolution of eusociality, such that the inclusive fitness benefits of non-reproducing helpers exceeded the net fitness gain of their individual solitary reproduction. A monandrous mating system would maximize relatedness among group members, and indeed phylogenetic analysis shows ancestral monandry in 8 out of 9 independent origins of eusociality in the Hymenoptera. High relatedness is also considered a key factor for the evolution of sociality in cooperative breeders, where kin selected benefits of cooperation favour helping at the nest. It is assumed that monandrous matings occur between unrelated individuals, and that most social animals are outbreeding. However, alternative breeding systems such as polyandrous mating, parthenogenesis and mating among relatives within social groups may have profound consequences for within-group relatedness asymmetry, genetic diversity and sex-ratio. Inbreeding in particular should increase relatedness among group-members and hence inclusive fitness, but costs arising from inbreeding depression often maintain outbreeding. Although relatively rare, inbreeding is found within several taxa of social insects and is the exclusive breeding system of the social spiders. Sociality in spiders evolves from subsocial ancestors through the elimination of mating dispersal and hence mating occurs among group-members. This has consequences for the sex-ratio which is highly female biased, and for genetic diversity which is reduced through drift and small effective population size. I will discuss the consequences of inbreeding for group dynamics, population genetics and evolutionary potential in the social spiders and social insect taxa.

CRAZY ANT SEX: GENETIC CASTE DETERMINATION, CLONALITY, AND INBREEDING IN A POPULATION OF INVASIVE YELLOW CRAZY ANTS

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Sex and caste determination are key defining features of insect eusociality. Recent discoveries indicate that ant reproductive systems are much more diverse than previously envisioned, with clonality, genetic caste determination and fertility among diploid males challenging conventional views. We investigated the sex and caste determination systems of the invasive yellow crazy ant (*Anoplolepis gracilipes*). We used microsatellite DNA markers to genotype ants introduced to the Northern Territory of Australia, including queens and their spermathecae (i.e. to ascertain the genetic contribution of males), workers and male brood. Our data indicate this is a single population that has apparently experienced a significant genetic bottleneck, which is consistent with its known history. The vast majority of genotyped spermathecae were heterozygous, suggesting males are diploid - an indicator of inbreeding in haplodiploids - likely due to the recent bottleneck. Most queens were homozygous at all loci, suggesting they are produced clonally via automictic thelytoky (meiotic parthenogenesis). In contrast, workers (some of which possessed ovaries) were almost exclusively heterozygous, indicating they are produced through apomictic thelytoky (ameiotic parthenogenesis). We also found that workers could be produced sexually by mated queens, which suggests worker thelytoky is facultative. *Anoplolepis gracilipes* has persisted in Arnhem Land for over 25 years and is now found over an area of 2,500 km2. The potential extinction risk posed by a genetic bottleneck and inbreeding could be mitigated by a conditional use of clonality in this population. This genetic system provides an interesting opportunity to further investigate how reproductive mode contributes to unicoloniality and the invasiveness of *A. gracilipes*. Our study adds to the growing body of evidence for the diversity of ant sex and caste determination systems.

EUSOCIALITY AND SINES IN THE SUBTERRANEAN TERMITE *RETICULITERMES LUCIFUGUS* (ISOPTERA, RHINOTERMITIDAE).

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Subterranean termite eusociality leads to colonies with reduced effective population size (N_e) and high levels of inbreeding, particularly during the first years of colony development. The loss of genetic diversity due to inbreeding has a relevant impact on genome structure/evolution and therefore on organism fitness. Transposable elements (TE) are widespread, self-replicating DNA sequences that proliferate within genomes with either negative or positive effects on gene structure and function. TE dynamics is known to be seriously affected by the breeding system: non-randomly mating populations are prone to accumulate and fix TE insertions owing to the Muller's ratchet effects. We isolated and analyzed four different families of Short INterspersed Elements (SINEs, small non-autonomous retrotransposons) in colonies of the subterranean termites *Reticulitermes lucifugus*. Each TE family showed its own evolutionary history and age. Preliminary data on activity patterns evidenced that insertions occur at high frequency within populations, this observation nicely fitting expectations in self-fertilizing organisms. On the other hand, SINE copy number is well within the range observed in other metazoans. Taken together, these observations suggest an efficient control on TE proliferation by the termite genome, the high frequency of insertions being possibly allowed by a weak selection against them. It can be argued that termite genome may benefit from an equilibrium of TE copy number gain and loss: if avoiding an increase of TE insertions will preserve the host genome functionality, the maintenance of TE copies may serve, for example, in promoting recombination.

SPOTTING THE TOP MALE: SEXUALLY-SELECTED QUALITY SIGNALS IN A PAPER WASP, *POLISTES DOMINULUS*

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Quality signals are widespread in nature, and provide valuable information about the bearer's condition or underlying genetic quality. Typically these signals also serve as ornaments for use in mate-choice, or as traits associated with fighting ability. These sexually-selected quality signals may lower the costs associated with both intra- and inter-sexual selection by enabling females to quickly assess the quality of potential mates or by enabling males to assess the strength of rivals and avoid costly fights. In social insects, little is known regarding the dynamics of mate choice and male-based quality signals have not been identified. Here, using a series of male-male dominance battles and mating trials I demonstrate that coloration patterns of male *Polistes dominulus* function as quality signals used in both inter- and intra sexual selection. This is the first evidence of a sexually-selected visual signal of quality in a social insect. As a lekking species, paper wasps have the potential to become a model system for studies of sexual selection; this work serves as the necessary groundwork and highlights the importance of close examination of mating dynamics in social insects.

DISSECTING THE FACTORS AFFECTING HONEY BEE QUEEN (*APIS MELLIFERA* L.) POST MATING CHANGES AND QUEEN-WORKER INTERACTIONS

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Mating is an intricate process that causes many changes at behavioral, physiological, and molecular levels. Our research strives to elucidate factors causing post-mating changes in honey bee queens. Since queens can be instrumentally inseminated, we can conveniently manipulate the factors affecting these changes. Previous studies suggest that both insemination volume and substance are important factors in causing early post-mating changes in honey bee queens. However, these studies were performed with caged queens and the effects were analyzed shortly after insemination. To further characterize these factors and their possible longer-lasting effects, we studied queens inseminated with low- and high volumes of either saline or semen after they started egg-laying. Our data indicate that insemination factors such as CO_2 anesthesia and physical stimulation also likely affect behavioral changes, while insemination volume is probably involved in triggering ovary activation. Both volume and substance cause distinct changes in gene-expression patterns within the fat body. This suggests that even though queens are in the same physiological state (egg-laying), different factors are responsible for producing distinct molecular changes. This could have implications for colony success, especially if workers can recognize that the queen is “poorly” mated, which is possible since differentially inseminated queens have distinctly different pheromone profiles. Indeed, we observed that low insemination volume negatively affects queen survival, decreases worker retinue response, and increases queen replacement attempts by workers. Thus, multiple factors are involved in regulating short- and longer-term post-mating changes in honey bees. More importantly, in social insects, these factors not only affect the reproductive female, but they may lead to altered social interactions which could affect colony organization.

SEX, SPERM AND PROTEINS: THE STUDY OF EVOLUTIONARY DYNAMICS ON THE MOLECULAR SCALE

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As part of a copulation, males transfer ejaculates to the female that typically consist of sperm and glandular secretions referred to as seminal fluid. Seminal fluid is increasingly recognized as a main driver for male reproductive success in the fields of sexual selection and reproductive biology. Here I provide an overview of what is currently known about the seminal fluid of social insects. I will show some fascinating examples from honeybees, European bumblebees and Panamanian fungus growing ants to demonstrate the multiple functions seminal fluid has on the survival of its own sperm, on sperm viability of a potential competitor and on the female. I will illustrate how proteomics offers possibilities to identify the major proteineous constituents of seminal fluid and the way modern biochemical technology can be used to generate an integral understanding of the macroscopically observed effects on the molecular scale. Social insect queens never remate once they have started to lay eggs and are therefore exceptionally efficient at keeping sperm alive over prolonged periods of time. In *Atta* leaf cutter ants for example queens maintain sperm viable for several decades and use them prudently to sire tens of millions of offspring. I will show that sperm storage is costly for queens and sperm maintenance trades off with other life history traits such as immunity. Females provide stored sperm with spermathecal fluid and I will present results from a recent study in honeybees that identified the proteineous contributions of females towards stored sperm. These insights into the secret of long-term sperm storage provide a first molecular explanation why sperm is costly to store.

FIRST DISCOVERY OF A RARE POLYGYNE COLONY IN THE STINGLESS BEE *MELIPONA QUADRIFASCIATA* (APIDAE, MELIPONINI)

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Stingless bees are highly eusocial bees, and are characterised by having perennial colonies that are typically headed by one single-mated queen. The main exception to this pattern is found in *Melipona bicolor*, which is the only stingless bee species discovered so far to exhibit facultative polygyny, whereby several queens may coexist and share reproduction inside the colony for considerable periods of time. Aside from that, there are, for a few stingless bee species, also some anecdotal reports of temporary, transient episodes of polygyny, which are usually associated with queen replacement events. Here we report on a novel case of occasional polygyny in the stingless bee *Melipona quadrifasciata*, in which an exceptionally high number (8) of egg-laying queens were found to coexist inside the same colony, and provide genetic data about the reproductive partitioning and relatedness among these different queens.

Multiple evolution of gamergates (sexually reproducing workers) in the ant subfamily Ponerinae

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In 99% of ant species, workers never mate and only the queens have stored sperm to fertilize their eggs. It is only in a few taxa (about 200 species in Amblyoponinae, Ponerinae and Ectatomminae) that workers can mate and reproduce sexually ('gamergates' or 'G'). In some species (e.g., *Dinoponera*) gamergates have replaced queens completely, while in others (e.g., *Harpegnathos saltator*) they function as secondary reproductives to extend colony lifespan after the founding (dealate) queens die ('AQ+G' species). Gobin et al. (2008) showed that in Ponerinae species where alate queens monopolize sexual reproduction ('AQ'), workers retain a spermatheca that lacks only a reservoir epithelium rich with organelles. That is unlike higher subfamilies where the spermatheca has completely disappeared in workers. A detailed molecular phylogeny of the Ponerinae revealed that species with gamergates occur in 14 "genera", including seven unrelated lineages of the non-monophyletic genus *Pachycondyla*. These taxa are broadly scattered and are nested within clades of AQ species. Reconstructions of ancestral states indicate that gamergates evolved independently a minimum of seven times within Ponerinae, and probably many more. These analyses strongly suggest that the MRCA (Most Recent Common Ancestor) of extant ants lacked gamergates (BPP = 0.998), and that the MRCA of the ponerine tribe Ponerini (containing 13 genera with gamergates) also lacked gamergates (BPP = 0.962). Hence gamergates are derived both within ants generally, and ponerines (as well as ectatommines) specifically. All these gamergate taxa re-evolved worker sexual ability: their spermathecae regained complete functionality and behavior changed to mate with visiting males. Using available comparative data, we explore the idea that gamergates are selected in species that shift to dependent colony founding. In conclusion, in ant species with a small caste dimorphism, workers can be selected to mate and reproduce as gamergates.

Asexual reproduction and its evolutionary consequences in social insects

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Sex and altruism are central issues in evolutionary biology. Since W. D. Hamilton first explained the evolution of sterile workers in hymenopteran insects by haplodiploidy that involves parthenogenetic production of males (arrhenotoky), the interaction between sex and altruism has been a major focus in insect evolutionary ecology. Recently, the other type of parthenogenesis, thelytoky, i.e. asexual reproduction producing females, has been a hot topic. By thelytoky, females can produce genetically highly related direct offspring. While this feature is likely a key evolutionary factor for aphid soldiers, in social Hymenoptera the body of recent findings indicates that thelytoky is often associated with cheating or intraspecific social parasitism. This is theoretically reasonable, because thelytoky can extend the reproductive options of hymenopteran workers which otherwise can directly produce only male offspring (drones). Furthermore, such a selfish option gives rise to new conflicts with other parties such as males, leading to the evolution of various counter strategies. In this talk I overview thelytoky in social insects and discuss that at least three different consequences of thelytoky can occur. 1. The collapse of the colony or the extinction of the population. 2. The fixation of an asexual genotype, which does not result in population extinction but leads to the resolution of conflict at least in the short term owing to the clonal nature of colonies. 3. The maintenance of eusociality and sex by the power balance of counter strategies. A striking example of the fist category is thelytokous social-parasitic workers in the cape honeybee. The second situation exists in the cooperative societies of *Pristomyrmex punctatus*, *Cerapachys biroi* and other obligately parthenogenetic species/populations. The recently found conditional thelytoky such as in *Wasmannia*, *Cataglyphis*, *Vollenhovia* and *Reticulitermes speratus* might be understood as examples of the last category.

SIB-MATING WITHOUT INBREEDING IN THE CRAZY ANT

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Inbreeding increases the level of homozygosity and, hence, the frequency of detrimental phenotypes caused by recessive deleterious alleles. In Hymenoptera, inbreeding also increases the risk of producing sterile diploid males. We found that both queens and males of an introduced population of the highly invasive ant *Paratrechina longicornis* reproduce clonally. We show that this reproduction system allows reproductives to mate with sibling without leading to any inbreeding. This may be an important feature promoting the success of introduced populations of this and some other invasive species.

LONG LIVE THE QUEEN: ROYAL SUCCESSION THROUGH ASEXUAL REPRODUCTION IN TERMITES

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The major advantage of sexual reproduction (over asexual reproduction) is that it promotes genetic variability, despite the obvious disadvantages of reduced genetic contribution to offspring and the cost of finding mates. The benefits of sexual reproduction can be reduced by inbreeding because inbreeding lowers genetic diversity at both the individual and population levels. Many termite species undergo royal succession, in which the primary reproductives who found colonies (king and queen) are replaced by secondary reproductives from within the nest. Under normal sexual reproduction, such a breeding system is expected to result in inbred colonies with limited genetic diversity. Here, we show that the termite *Reticulitermes speratus* avoids inbreeding by the conditional use of sexual reproduction and parthenogenesis. We sampled mature field colonies and found that in nearly all cases, primary kings were present but primary queens had been replaced by an average of 55.4 secondary queens. Genetic analysis showed that secondary queens were exclusively produced parthenogenetically by the original primary queen, whereas workers and alates (new primary reproductives) were produced by sexual reproduction and therefore retained levels of heterozygosity expected under outbreeding. Moreover, by producing her replacements parthenogenetically, the queen maintains her full genetic contribution to the next generation of primary reproductives. These findings reveal a novel breeding system in which asexually produced replacement reproductives in colonies of a social insect are used to boost sexual reproductive output of the queen to the benefit of both the queen and the colony. Additionally, we also found the same breeding system in *R. virginicus*, raising the possibility that the asexual queen succession system could be widespread in termites.

EXTRAORDINARY REPRODUCTIVE STRATEGIES IN THE ANT *CATAGLYPHIS*

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Sexual reproduction in animal species has long been recognized puzzling because of the inherent cost of transmitting only half the parent's genome to progeny. Recent findings have shown that queens of some social insects can circumvent this cost by taking advantage of the social caste system to benefit from the returns of both sexual and asexual reproduction. New queens are produced asexually by thelytokous parthenogenesis and are almost clones of their mother, while workers are produced by normal sexual reproduction. By selectively using asexual and sexual reproduction for the 'germinal' and 'somatic' castes, respectively, mothers increase the transmission rate of their genes to their reproductive daughters, while maintaining genetic diversity in the worker force. Since its first discovery in the ant *Cataglyphis cursor* (Pearcy *et al.* 2004 *Science* 306, 1780-1783), the conditional use of sexual and asexual reproduction by queens was later reported in a few other species of ants (Fournier *et al.* 2005 *Nature* 435, 1230-1234; Ohkawara *et al.* 2006 *Biology Letters* 2, 359-363) and termites (Matsuura *et al.* 2009 *Science* 323, 1687). DNA microsatellite analyses of reproductive strategies in various species belonging to the genus *Cataglyphis* provide unambiguous evidence for a single and abrupt evolutionary transition from classical sexual reproduction to conditional use of sexual and asexual reproduction by queens. This transition appears to be correlated with two other evolutionary innovations, (1) colony reproduction by budding and (2) asymmetric dispersal of both sexes, which– in some species - results in a complete separation of the male and female gene pools. By contrast, clonality seems not associated with colony queen number, queen mating frequency or even worker reproduction by thelytokous parthenogenesis.

Poster Presentations

Sex and the insect society: focus on unorthodox breeding systems

7

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7-1 MECHANISMS OF GENETICALLY BIASED CASTE DETERMINATION IN A FIRE ANT HYBRID ZONE

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Interspecific hybridization is often associated with reduced hybrid reproductive success, but can be associated with non-reproductive benefits. Eusocial insects may be able to capitalize on non-reproductive benefits while limiting costs if hybrid offspring are constrained to the already sterile worker caste. In a hybrid zone between the polygynous fire ant species *Solenopsis xyloni* and *S. geminata*, hybridizing *S. xyloni* queens produce primarily workers, while intraspecifically-mated *S. xyloni* produce only daughter queens. In allopatric populations workers are pure-species. Three possible mechanisms could generate this association between ancestry and reproductive caste in the hybrid zone: 1) hybrid workers selectively rear brood based on ancestry, 2) queens facultatively bias offspring caste when hybrid workers are present, or 3) offspring caste in the hybrid zone is fixed based on genotype. To distinguish among these hypotheses we conducted cross-fostering experiments in which *S. xyloni* queens from either the hybrid zone or allopatric populations were paired with either F1 hybrid or pure-species *S. xyloni* workers and monitored for worker production. Production of worker offspring was not affected by the type of worker rearing the brood; queens from the hybrid zone were significantly less likely to successfully produce workers than queens from allopatric regions. Within the hybrid zone intraspecifically mated queens were significantly less likely to produce worker offspring than hybridizing queens. These results suggest that the absence of pure-species workers is not due to worker effects or facultative biasing; instead, the majority of *S. xyloni* in the hybrid zone appear to have intrinsically lost the capability to develop into the worker caste. Thus, hybridization to produce workers has become an obligate component of the life-history of *S. xyloni* in the hybrid zone, with important implications for ecological interactions and long-term persistence.

7-2 THE CONSEQUENCES OF POLYANDRY FOR WORKER CASTE DETERMINATION IN ARMY ANT COLONIES

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Army ants are highly eusocial insects that exhibit a complex worker polymorphism. In some species workers vary merely in size, whereas others show highly derived, distinctive worker castes, resulting from excessive allometric growth. Since queens of almost all army ant species are highly polyandrous (Kronauer *et al.* 2007 *Evolution* 61, 413-422), the genetic composition of colonies is also complex, with workers of mixed paternity. Army ants are therefore an excellent system to study genetic effects on worker caste determination. In *Eciton burchellii*, a species with extraordinary worker polymorphism, workers from different patrilines segregated into different castes (Jaffé *et al.* 2007 *Biol. Letters* 3, 513-516). However, comparative studies in other army ants with less pronounced worker polymorphism are lacking. Is the genetic effect on worker castes in these species equally strong? We combine morphometric measurements with patriline analyses of workers in colonies of the two neotropical army ant species *Labidus praedator* (continuous worker size distribution) and *E. mexicanum* (additional distinctive caste of major workers). We extract the genetic variance components for worker size by quantitative genetic analyses, to assess the significance of multiple mating for the evolution of excessive castes in army ants.

7-3 MALE EXTERNAL GENITALIA OF THE ANT GENUS *CARDIOCONDYLA*

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The ant genus *Cardiocondyla* is a model system for studying questions concerning sexual selection in social insects. Several species of this genus exhibit a male dimorphism. Winged, large-eyed males occur, which are typical for most ant species. Furthermore, in some *Cardiocondyla* species, so-called ergatoid males are produced in addition. Ergatoid males are wingless and possess only small eyes. These differences in male morphology are also reflected in male reproductive tactics, which show variation not only between different morphs within a species but also between species. In dimorphic *Cardiocondyla* species, ergatoid males behave aggressively against other ergatoid rivals, but not against winged ones. In other *Cardiocondyla* species only ergatoid males occur. In some of them ergatoid males kill all rival ergatoids. In others, ergatoid males are mutually tolerant. As virgin queens may mate several times, differences in reproductive tactics result in different strength of sperm competition. As in other species, these differences may be reflected in the morphology of male external genitalia. Studying male external genitalia of males of different *Cardiocondyla* species thus provides an opportunity for gaining insight into the processes underlying genital evolution. Preliminary results of the morphology of male external genitalia obtained by scanning electron microscopy are presented. Morphological differences within species, i.e. between different male morphs of single species, as well as between species are shown.

7-4 POST-COPULATORY SEXUAL SELECTION IN SOCIAL HYMENOPTERA

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Social hymenopteran queens (ants, bees and wasps) only mate for a brief period early in life. After mating, large numbers of sperm from a single or multiple males are stored in a queen's spermatheca for prolonged periods of time (in some cases several decades) and these sperm cells are used to fertilize eggs that produce the many workers needed to sustain mature colonies. Here, I discuss several recently discovered adaptations that can influence male and queen reproductive success, because they affect the differential survival of gametes after copulation and affect sperm usage during egg fertilization. I show that a male's seminal fluid is of crucial importance for the production of high quality ejaculates, and that it is also involved in ejaculate competition as it significantly reduces the survival of sperm of rival males in polyandrous bees and ants (Den Boer *et al.* (2010) *Science* 327, 1506-1509). On the other hand, fluid from a queen's spermatheca can stop these negative effects on sperm survival in leafcutter ants, suggesting that eusocial queens are in full control of sperm viability during the years of storage. In addition, I show that leafcutter ant queens are extremely efficient during egg fertilization, as they were only found to use a few sperm per egg (Den Boer *et al.* (2009) *Proc R Soc B* 276, 3945 -3953). These findings generate many interesting questions for future research on ejaculate competition and cryptic female choice in the polyandrous social Hymenoptera.

7-5 Reproductive competition among worker patrilines in the thelytokous ant *Cataglyphis cursor*

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In very few highly eusocial species, workers have recovered the ability to lay diploid eggs by using thelytokous parthenogenesis. Worker production of female eggs allows the replacement of the queen in orphan colonies but generates potential conflicts within the colony. This is especially true when multiple lineages are present. Selection at the individual level might then lead to strong reproduction competition with one or few lineages monopolising reproduction. We studied conflict over reproduction in the thelytokous and highly polyandrous ant *Cataglyphis cursor*, where workers produce queens in orphan colonies and competition may occur among worker patrilines. The reproductive success of each patriline was assessed in 11 orphaned colonies using genetic analysis of 372 workers and 273 queens at the pupal stage. Equal reproductive success of worker patrilines would yield a similar distribution of patrilines in workers and gynes, whereas uneven reproduction of worker patrilines would yield a different distribution of patrilines in workers and gynes. Our results show no evidence for reproductive competition between worker patrilines as we found no differences in the distribution of worker and queen patrilines in nine out of the eleven colonies. This is very different from what occurs in honeybees, where a few patrilines monopolise reproduction, both in species that produce males and in the Cape honeybee that produces females by thelytokous parthenogenesis. The low reproductive competition in *C. cursor* is somewhat surprising and questioned the occurrence of worker reproduction by parthenogenesis in nature.

7-6 Expression profile of sex-determining genes in embryonic and larval development of*Apis mellifera*

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Sex determination is one of the major developmental events in higher metazoans. The initial signals in the sex determination pathway vary, but the terminal signals are conserved throughout the metazoan phyla. In honey bees, the primary sex-determining signal results from an allelic combination of the *complementary sex determiner* (*csd*) gene: males develop from hemi- or homozygous embryos, whereas females develop from heterozygous ones. *csd* controls the splicing of *feminizer* mRNA resulting in two sex-specific transcripts. The *fem* gene product itself supposedly regulates the splicing of *amdsx* transcripts. In insects, the pre-mRNA of *doublesex* (*dsx*) undergoes alternative splicing giving rise to sex-specific variants in *dsx* protein structure. These then are held responsible for differential regulation of gene expression patterns between the sexes. Until now there are still many open questions concerning the function of *amdsx* splice variants and of other sex determining genes in honey bees. Thus, our goal was to characterize the expression of *amdsx* variants and other sex-determining genes expression (*csd*, *fem*, *dachshund* and *intersex*) during embryonic and larval development. In general, the genes studied have antagonistic expression profiles in sexes and castes of *A. mellifera*. Our results oppose the idea of sex-specific expression of *amdsx* variants. The correlation between *femM* and *amdsxM* for worker, queen and drone (R-worker = 0.62; R-queen = 0.59; R-drone = -0.66) suggest that females of *A. mellifera* can also activate by default the regulatory cascade of sex determination. We could show that the expression profiles of these sex-determining genes are sexually dimorphic, especially during embryonic development, indicating that these genes indeed play a role in sexual differentiation in this insect.

7-7 MALE COMPETITION IN THE ANT GENUS *CARDIOCONDYLA*

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Around the animal kingdom in many groups males compete for access to females, which is also true for such small insects like ants.Whereas in most ant species, mating takes place during a short and highly localized nuptial flight, sexuals of the genus *Cardiocondyla* usually mate inside the nest and show a large diversity of male reproductive tactics . The resulting seraglio situation, which allows individual males to monopolize mating with all female sexuals, has resulted in the evolution of unique, wingless fighter males. In some species of *Cardiocondyla*, such “ergatoid males” have fully replaced the ancestral winged males, while in other species both male types co-occur. Ergatoid males of most species kill all freshly eclosing ergatoid rivals and, in a few species, also adult competitors, using their strong mandibles. However, in particular in species from xeric environments several ergatoid males may peacefully coexist in a nest. We investigate reproductive tactics in the genus *Cardiocondyla*. In my project I performed experiments on *Cardiocondyla venustula*. Wingless males of *C. venustula* use their strong mandibles to kill freshly eclosed rival males and also engage in short fights with other adult males, but in addition show a novel behavior hitherto not reported from social insect males: they spread out in the natal nest and defend “territories” against other males. Ant males therefore show a much larger variety of reproductive tactics than previously assumed.

7-8 INCONSPICUOUS MATURED MALES OF *RETICULITERMES SPERATUS* PARTICIPATE IN REPRODUCTION IN ORPHANED COLONIES

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Differentiation of neotenics is easily induced by orphaning the worker termites of *Reticulitermes speratus* (Kolbe). Although it has previously been reported that the sex ratio of neotenics is female biased, the background of this phenomenon and the gonad developmental process of workers in artificially orphaned colonies are unknown. Although both male and female conspicuous neontenics can emerge as a response to the orphaning of the colony, we observed that the majority of the reproductive males that are formed do not moult into the neotenic morph and are therefore inconspicuous in the population. Participation of the inconspicuous reproductive males in sexual reproduction was confirmed by genetic analysis. Slight pigmentation of the abdominal sternites is the only characteristic of the inconspicuous reproductive males that enables them to be distinguished from normal male workers. The presence of the female ergatoid (one type of female neotenic) did not induce production of inconspicuous reproductive males during the 12 week experiment time.

7-9 ONE DAY STAND: THE REVEALING STORY BEHIND HONEYBEE MALES AND THEIR SPERM

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Because sexual selection operating after mating can both attenuate or amplify sexual selection taking place before mating, focusing on an isolated episode of selection will only provide an incomplete picture of the processes of sexual selection operating throughout the life of an individual. Yet, few studies have tried to integrate pre- with postcopulatory sexual selection. Here we provide a first insight into the interplay of these two selection episodes in the western honeybee (*Apis mellifera*). Honeybee males (drones) gather at specific drone congregation areas (DCAs), were they fly in loops 20m up in the air waiting for a virgin queen to appear. Once this occurs, hundreds of drones chase the queen forming a "mating comet" behind her. Successful males quickly grab and copulate with the queen, dying shortly after and leaving her free to mate again. After copulating with 10-20 males within a short period of time, queens fly back to their hives and never remate again. The collected sperm from all males are then stored in a specialized organ, the spermatheca, and used for egg fertilization throughout the queen's life. The drones and their sperm are thus under sexual selection, the former to copulate with a queen and the later to gain access to the sperm stores and then to fertilize an egg. By comparing a sample of drones captured during their mating flights at a natural DCA, with a control sample of drones that were not found in the DCA, caught inside their maternal colonies, we assessed the relationship between male traits contributing to their mating potential (body size, wing fluctuating asymmetry) with sperm traits likely to influence fertilization success (sperm viability, sperm counts and sperm morphology). Our results will be discussed along with their implications for future research.

7-10 CASTE PRODUCTION IN ORPHANED INCIPIENT COLONIES OF *RETICULITERMES*: ITS RELATION TO GENETIC SYSTEM AND PARTHENOGENESIS

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In the termite genus *Reticulitermes* it has been reported that the caste differentiation of workers *vs* royals (nymphs and alates) is affected by a sex-linked genetic effect. Crossing experiments suggested that the genotypes of a single X-linked locus (*wk*) regulated caste fate of offspring in conjunction with pheromones produced by the reproductives. While *Reticulitermes* incipient colonies with an original king and queen usually do not produce nymphs, the genetic model predicts that the colony will start to produce offspring with nymph-oriented genotypes (*wkAA* and *wkBY*) when the original queen is replaced by an ergatoid reproductive derived from workers.To elucidate the factors regulating nymph production in incipient colonies, we investigated the caste production pattern in experimentally orphaned colonies of *R. speratus*. From incipient colonies of about 100 individuals icluding a royal pair, we removed (1) king, (2) queen, and (3) both king and queen. As control, we also setup control colonies (4) without removal of reproductives. These colonies were maintained at 25ºC for 240 days, and every 30 days the newly differentiated workers and nymphs were sexed and counted. Ergatoid reproductives emerged in the treated colonies (1-3), while no ergatoids differentiated in the controls (4). In the control colonies, offspring differentiated only into male and female workers. Female nymphs were produced in each treatment colony, while only treatment 2 produced male nymphs. Microsatellite genotype analysis revealed that most female nymphs were produced by parthenogenesis, which should have nymph-oriented genotype (*wkAA*). These results indicate that the nymph production starts if the original king or queen disappears. Parthenogenesis is inhibited in an incipient colony with a royal pair. In such a colony, the presence of the genetic caste system and the inhibition of parthenogenesis induce offspring genotype to be worker-oriented.

7-11 THE GENETICS OF THELYTOKOUS PARTHENOGENESIS IN THE ANT *CERAPACHYS BIROI*

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With the increased ease of developing genetic markers for non-model systems it has become clear that many social insects are characterized by highly idiosyncratic modes of reproduction. These include species where workers are genetic hybrids while queens are purebred, as well as species that use obligate or caste-specific parthenogenesis to produce new females. In this presentation, I will focus on the underlying mechanism and genetic consequences of parthenogenesis in the ant *Cerapachys biroi*. This species has been introduced by humans to many tropical and subtropical islands around the world. Queens have never been collected and virgin workers produce new worker-offspring by thelytokous parthenogenesis in the lab. Using a combination of nuclear microsatellite markers and mitochondrial haplotyping, I assess the mode and prevalence of parthenogenesis, the genetic composition and distinctness of colonies in different field populations, and I attempt to reconstruct the number and sequence of worldwide introduction events.

7-12 WHAT MAINTAINS DIFFERENTIATION BETWEEN MALE AND FEMALE GENOMES IN HYBRID WOOD ANTS?

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Usually hybridization leads to problems because of incompatible gene combinations. Occasionally, hybridization can result in novel evolutionary lineages or unusual modes of inheritance. Hybridizing wood ant species from southern Finland show a unique outcome of hybridization, as the males form two highly divergent gene pools, while all the females are hybrids. This results in large-scale differentiation between male and female genomes. Differentiation is maintained throughout generations, but how? One hypothesis is that selection eliminates hybrid males because of recessive incompatibilities. In haploid males recessive incompatibilities are expressed, but in diploid heterozygous females they are masked. An alternative hypothesis is that differentiation between sexes is created already at the prezygotic level, where two chromosome sets, maternal and paternal, segregate as blocks with low recombination. Differentiation between the sexes is maintained if the maternal set is fertilized producing daughters and the paternal set is left unfertilized producing sons. These hypotheses are tested with family data, where the mother queens, their mates and offspring have been genotyped.

7-13 ALTERNATIVE REPRODUCTIVE TACTICS AND SEX ALLOCATION IN THE ANT *HYPOPONERA OPACIOR*

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The tiny neotropical ant *Hypoponera opacior* exhibits alternative reproductive morphs in males and queens associated with distinct sexual behaviours. Our long-term study reports strong seasonality in sexual production with two separated mating seasons in early and late summer. Alate reproductives emerge in June, swarm during the monsoon season and establish new colonies independently. In contrast, wingless worker-like (ergatoid) reproductives appear in late August, mate within their natal or adjacent nests and either do not disperse or establish new nests in the vicinity. These divergent dispersal patterns allowed us to analyse the impact of local factors on investment strategies by comparing sex allocation between and within the two reproductive events. The optimal sex ratio for ergatoid reproductives should be influenced both by competition for matings between brothers (local mate competition) and rivalry among young queens for workers, nest sites or food (local resource competition). The greater relative importance of local resource competition was demonstrated by a male-biased sex ratio for wingless reproductives despite similar relatedness asymmetries during both reproductive seasons. Furthermore, microsatellite analyses revealed that nests with highly related ergatoid males raise a male-biased sex ratio rather than a female-biased ratio as expected under local mate competition. We propose that local resource competition in this viscous population of *H. opacior* is so strong because successful nest foundation by ergatoid queens is highly constrained by the number of workers accompanying queens.

7-14 COMPARATIVE ANALYSIS AND EVOLUTION OF UNUSUAL MATING SYSTEMS IN THE ANT GENUS *CATAGLYPHIS*

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The occurrence of multiple functional queens in social insect colonies (polygyny) and of multiple mating by queens (polyandry) have long been recognized as posing a potential challenge to kin selection theory, because they decrease the relatedness among workers and the brood they rear and, hence, dilute the inclusive fitness benefits from helping. Yet, polygyny and/or polyandry have been reported in several species, including termites, bees, wasps and ants. Understanding the ultimate and proximate factors favoring polygyny and polyandry remains a major issue in evolutionary ecology because the factors that account for their evolution are hard to verify. The ant genus *Cataglyphis* includes approximately a hundred species. All species reportedly form relatively small colonies of about a few hundreds to a thousand of workers. Although *Cataglyphis* ants have been the focus of much behavioral and physiological works, their reproductive strategies still remains surprisingly poorly studied. Here, we report the first comparative analysis of the evolution of social structure and mating system in this genus. Comparison of the breeding system (monogyny or polygyny), queen mating frequency, mode of dispersal, population structure, and asexual reproduction of workers by arrhenotokous and/or thelytokous parthenogenesis in several species, reveals a great diversity in reproductive strategies. First, polyandry is the rule in the whole genus, with queens mating with up to 15 different males in some species. Second, no relationship occurs between colony queen number and queen-mating frequency. Third, in several species where colonies are headed by a single-queen, colony reproduction proceeds by fission - a feature of ant species with large colony size. The evolution of these uncommon reproductive strategies will be discussed in a phylogenetic context.

7-15 MICROGYNES IN THE REPRODUCTIVE STRATEGY OF *ECTATOMMA RUIDUM*

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Microgyny (miniaturized queens) in the reproductive system of an ant species is not widely represented in the ant world: only a few cases in 20 genera, grouped in 4 subfamilies. Moreover, microgynes can have a variety of roles in their "host" species: from alternative short-distance dispersal morphs to inquiline parasites. Most of the time microgynes are associated with polygyny while macrogynes are monogynous morphs of the same species. In the poneromorph group, microgyny is only known in two populations of Ectatomminae: a well studied one of *Ectatomma tuberculatum*, where microgynes are queens of an inquiline species (*Ectatomma parasiticum*), and a less known one of *Ectatomma ruidum*. In this last species, microgynes don't act as parasites according to behavioural observations. We used mitochondrial and newly developed nuclear markers in order to investigate the status as well as the role of microgynes in the *Ectatomma ruidum* population. We confirmed that microgynes and macrogynes are from the same species and we showed that this species is almost exclusively monogynous and monandrous: supernumerary dealated queens of both morphs are actually daughters of the mother queen. Relatedness between nest mate workers ($r = 0.74$) is not significantly different from 0.75, and an apparently polygynous nest can be headed by a microgyne as well as a macrogyne. We didn't find any inbreeding or isolation by distance in this population, indicating that gynes are inseminated by unrelated males and can found a new nest far from their natal nest. Even if we can say that microgynes are not a specific dispersal morph in this species and that their presence must be related to nest density, their role remains enigmatic.

7-16 CASTE DIFFERENTIATION IN FEMALE-FEMALE COLONIES OF THE TERMITE *RETICULITERMES SPERATUS* (RHINOTERMITIDAE)

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In the rhinotermitid termite *Reticulitermes speratus*, facultative parthenogenesis is known to occur occasionally and females cooperate to found a colony with partner females. Genetic factors concerned with caste determination between nymphs and workers were found in this species, and parthenogenetic eggs produced by female reproductives are known to develop into nymphs in the absence of reproductives. Nymphs can molt into secondary nymphoid reproductives, and nymphoids asexually produced by the primary queen are known to be involved in the main reproductive force in natural colonies of this species. Consequently, in female-female colonies, if queens would like to produce nymphoids, conflicts to produce nymphs might occur between two queens. To elucidate reproductive conflicts between pairs of females and caste differentiation of their offspring, incipient female-female colonies were established under laboratory conditions, and the process of colony development was observed at several stages after colony foundation. Individual genotypes of each colony member (queens, eggs, larvae, workers, soldiers, nymphs and nymphoids) were discriminated by microsatellite DNA markers. Although a precise reproductive cycle in pairs of females was observed, ovarian development and vitellogenesis in each female were similar. Nymphs were observed at 6 months or later, and nymphoids were observed in some colonies 14 months after colony foundation. Microsatellite DNA analyses showed that the numbers of eggs, larvae, workers and soldiers produced by each female did not differ significantly in almost all colonies; however, the numbers of nymphs and nymphoids produced by each female were markedly biased in all colonies examined. The results suggest that no conflicts existed between the two females in terms of laying eggs, but some conflicts concerning the production of nymphs and nymphoids existed with colony development.

7-17 Inbreeding avoidance in drone congregations of a stingless bee (*Scaptotrigona mexicana*)

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Drone congregations (DCs) are a widespread phenomenon among stingless bee species (Meliponini). Despite their common occurrence (often outside or near to conspecific colonies) the ultimate and proximate reasons for their formation are still not well understood. An adaptive explanation for the formation of DCs is inbreeding avoidance. Inbreeding is especially costly for many Meliponini species, since they combine the complementary sex-determining system (CSD) of hymenoptera with monandry. Diploids that are homozygous at the CSD locus develop into sterile males and can cause a high colony level fitness loss. To understand what mechanisms - especially the dispersal of drones - avoid inbreeding in mating systems of eusocial bees, the genetic structure of a drone congregation of the stingless bee *Scaptotrigona mexicana* was analyzed over a time window of several weeks. Microsatellite markers initially developed for other bee species were used for the genetic analysis. Despite this cross-species amplification, the PCR reactions were very successful on eight loci resulting in consistent and variable microsatellite fragments. The data of this study showed an almost panmictic structure of the drone congregation. Additionally, it could be shown that the drones did not originate from colonies in the vicinity of the drone congregation. This study could show that there are effective mechanisms in the reproductive strategies of *S. mexicana* to avoid inbreeding and to overcome the considerable genetic load of CSD system and monandry.

7-18 Female-male conflict in the clonal ant *Vollenhovia emeryi*

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Vollenhovia emeryi has an extraordinary parthenogenetic reproductive system. Genotype analysis shows that female sexuals are produced asexually from unfertilized eggs, and workers are produced sexually. Furthermore, males arise from fertilized eggs, with elimination of the maternal genome (Ohkawara *et al.* 2006, Kobayashi *et al.* 2008). In this ant with female and male clonality, females and males inherit only maternal and paternal genome respectively. The uncommon reproductive system posees some important questions about conflict between sexes. It is predicted that queens (females) will prefer an extreme female-biased allocation in sexual production, whereas males will prefer extreme male-biased allocation, leading to intense female-male conflict. In the present study, we examined sexual allocation patterns in long winged queen (LQ) colonies of *V. emeryi*. In a total of 318 colonies, 233 (73.2%) produced only female sexuals, and 24 (7.5%) produced only male sexuals. Hence, queens more nearly achieved their preferred sex ratio in sexual production. However male production is essential for queens to produce workers. How are male sexuals produced in LQ populations? In other word, who undertakes the thankless task of providing males? Toexamine this question, male production pattern was further analyzed. Some colonies (7.5%) exhibiting male biased allocation had infertile queens or were queenless. This suggests that males are produced when the reproductive potential of queens is low, and queens may normally control development of males. Moreover genotype analysis showed that some males in the LQ population are produced from unfertilized eggs like normal ants. This also means that the production of some males is controlled by queens. In addition to this hypothesis, we will introduce other hypotheses about male production.

7-19 Egg production and caste allocation in the clonally reproductive ant *Vollenhovia emeryi*

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Recently, a few species of ants have received attention because of their extraordinary parthenogenetic reproductive strategies. In the little fire ant, *Wasmannia auropunctata*, female sexuals are asexually produced from unfertilized eggs and workers are sexually produced. Furthermore, male sexuals arise from fertilized eggs (Fournier *et al.* 2005). It is suggested that females and males inherit only maternal and paternal genomes, respectively. It has been reported that only one species of myrmicine ant, *Vollenhovia emeryi*, has a similar reproductive strategy (Ohkawara *et al.* 2006). The reproductive strategy of female and male clonality provides a new approach to test certain hypotheses concerning kin selection and social evolution. Concerning this ant species, relatedness values of the mother queens toward their female sexual, worker and male offspring were 1.0, 0.5 and 0, respectively. Considering the uncommon relatedness asymmetries among colony members, the most adaptive sexual allocation of the queen is to invest most of the resources for the sexual production of females. However, male egg production is also essential for the production of workers that developed from fertilized eggs. Under these conditions, how queens should allocate resources to produce each caste of eggs is a unclear. In the present study, in order to examine the caste allocation, we identified castes of eggs laid by queens of *V. emeryi* by using polymorphic genetic marker. Results demonstrated that queens produced mostly female-destined eggs in proportion to that of sexual egg production. However, many queens produced only male-destined eggs in sexual production. In addition, male egg production tended to be independent of production of the eggs of other caste, suggesting that queens cannot completely control the production of male eggs. This could indicate the existence of a conflict between both sexes in sexual production.

7-20 The evolution of asexuality in fungus-growing ants

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The prevalence of sexual reproduction over asexual reproduction among eukaryotes testifies to the evolutionary benefits of genetic recombination. Documented instances of asexuality challenge evolutionary biologists to understand the special circumstances that might confer an advantage to asexual reproductive strategies. One of those rare evolutionary exceptions is the asexual fungus-gardening ant, *Mycocepurus smithii*. Evidence accumulated from different populations in South and Central America demonstrates that queens of *M. smithii* are capable of thelytokous parthenogenesis - the production of diploid (female) offspring from unfertilized eggs (Fernández-Marín *et al.* (2005) *J. Nat. Hist.* 39, 1735-1743; Rabeling *et al.* (2007) *J. Insect Sci.* 7, 402; Rabeling *et al.* (2009) *PLoS One* 4, e6781; Himler *et al.* (2009) *Proc. R. Soc. B* 276, 2611-2616). To understand the evolutionary framework of asexual reproduction in *M. smithii*, I will present results from morphological, physiological, population genetic and phylogenetic studies in order to address the following three questions: First, is *M. smithii* a single asexually reproducing species, or a cryptic species complex? Second, do *M. smithii* populations reproduce asexually throughout the species extensive distribution range, or, in short, is *M. smithii* obligately asexual? And third, could *M. smithii* potentially be a long term asexual species? The results of this study will be discussed in the context of the biodiversity of reproductive systems in eusocial insects.

7-21 PHYLOGEOGRAPHY OF THE PARTHENOGENIC ANT, *PLATYTHYREA PUNCTATA*: HIGHLY SUCCESSFUL COLONIZATION OF THE WEST INDIES

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Sexual reproduction is the dominant reproductive mode of life on earth yet parthenogenic examples can be found in most taxa. Parthenogenesis is often thought to be adaptive in expanding and younger populations, whereas sexual reproduction should be more adaptive in older populations. We describe the distribution of parthenogenesis and the molecular phylogeography of a the parthenogenic ant *Platythyrea punctata*. This species has a very wide distribution, ranging from Central America to most if not all islands in the West Indies. We analyzed intracolonial relatedness using microsatellite markers, observed egg laying in virgin workers and constructed a phylogeny using mitochondrial and nuclear markers. We have found parthenogenesis to be very widespread on the mainland and the islands, both in older and younger populations. It would seem that parthenogenesis evolved prior to the evolution of this species This species appears to have rapidly expanded throughout the Central American mainland and islands in the West Indies. Divergence estimates indicate that this occurred in the mid to late Pleistocene.

7-22 MULTIPLE QUEENS, MULTIPLE PATERNITY AND GENETIC DIVERSITY IN THE ANT GENUS *PLAGIOLEPIS*

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Between- and within-species variations in reproductive strategies have important implications for population and colony structure and, ultimately, for the evolution of social behaviour. To date, the factors that account for the diversification in reproductive tactics are hard to verify, because benefits and costs are hard to quantify. Comparison of reproductive systems between different but related species can help to solve this difficulty. Using DNA microsatellite makers, we performed a comparative analysis of the reproductive strategies in five species belonging to the ant genus *Plagiolepis*: four free-living and one inquiline social parasite. Our data show that all species sampled evolved multiple-queen colonies and a high level of inbreeding. By contrast, large variation between species in queen-mating frequency occurs. Queen-number is not correlated with queen-mating frequency. We analyse the impact of the different reproductive strategies on the colony and population structures, and discuss their evolution in a phylogenetic context.

Oral Presentations

Brood parasitism and inquilinism in social insects

8

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Socially parasitic reproductive strategies in stingless bees

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In recent years, several cases of intraspecific social parasitism have come to light, particularly in honeybees and bumblebees. Here I report results that demonstrate several other novel cases of intraspecific social parasitism in two other groups of highly eusocial insects, the stingless bees and the vespine wasps. In stingless bees, it is demonstrated that parasitizing other hives provides queens with an alternative means to found a new colony. Previously, it was believed that in highly eusocial bees, queens only have two options to establish new colonies: to leave the hive with a swarm of workers or to supersede a failing mother queen. Genetic data, however, show that queens of the native Brazilian stingless bee *Melipona scutellaris* also occasionally employ an entirely different, much more selfish strategy: to act like "cuckoos", invading and taking over unrelated hives nearby. In the same species, the workers themselves are also shown to engage in a form of reproductive parasitism. When the queen happens to die, it is shown that the workers derived from the superseded queen keep on producing their own male offspring for many months after the new queen becomes established, thereby effectively parasitizing the next generation workforce.

Social parasites versus brood parasites: what they do and do not have in common

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As several previous authors have pointed out, there are interesting parallels to be drawn between avian brood parasites, which exploit the parental care of other bird species, and social parasites, which exploit the workforce of insect societies. Here I consider the coevolutionary dynamics of the interactions between these parasites and their hosts. Among brood parasites, the extent of adaptation and counteradaptation is strongly dependent on the harm that the parasite inflicts on host fitness. But what affects how virulent parasites will be in the first place? Theoretical work suggests that the extent of dispersal may be key: when a parasite and its kin repeatedly interact with the same host and its kin, virulence is less likely to be adaptive. I use data from the literature to assess the validity of this idea for both brood parasites and social parasites. I then compare and contrast the nature of the ensuing adaptations and counteradaptations in brood parasites and in social parasites.

Brood parasitism of neighbors by inquilines in *Tamalia* galling aphids

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In the manzanita gall aphid, *Tamalia coweni*, foundress females induce plant galls in which they reproduce, and from which offspring disperse to induce further generations of galls. Gall-inducer foundresses have the option of initiating their own gall, or joining established galls to form communal groups. Such intraspecific, facultative inquilinism sets the stage for potential reproductive conflict among foundresses. A sibling species, *Tamalia inquilina*, acts as an obligate brood parasite, invading gall space and outcompeting gall-inducers for reproductive benefits within galls. Thus gall-inducers, by tolerating co-occupants of galls, may have facilitated the origins of obligate inquilinism in this system. Modes of dispersal from natal galls appear to differ markedly between gall-inducer and inquiline: most or all offspring of gall-inducers are winged and disperse aerially, whereas some inquilines are wingless and disperse on foot, presumably seeking additional galls to invade. I established field trials in which offspring exiting natal galls were collected in sticky traps. My data indicate inquilines dispersing on foot are significantly more likely to be so trapped than are gall-inducers; hence, inter-gall dispersal may be important for the inquiline species. In a second experiment, I marked dispersing juveniles by injecting fluorescent powder into natal galls. Relative rates of entry into nearby galls were then obtained by inspection of gall contents. Preliminary data suggest dispersing inquilines are most likely to invade nearest neighbor galls, yielding low rates of dispersal. These dispersal patterns may have significant consequences for population structure and, ultimately, evolutionary change in inquiline *Tamalia*.

A population genetic survey of a clonal reproductive parasite of *Apis mellifera scutellata* in South Africa

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The honey bee population of South Africa is divided into two subspecies: an arrhenotokous population in the north (*A. m. scutellata*), and a thelytokous population in the south (*A. m. capensis*). A stable hybrid zone separates the two populations. However, on at least three occasions (two historical and one current) the *scutellata* population has become infested by reproductive workers derived from the *Capensis* population. These parasitic workers lay eggs in host *scutellata* colonies parthenogenetically, resulting in yet more parasites. Genetic analyses have shown that the current infestation derived from a single worker that lived more than 10 years ago. The longevity of this infestation is surprising because an asexual lineage is expected to show a decline in vigour over time due to increasing homozygosity and an increase in mutational load. The decline is expected to be particularly acute in honey bees, where homozygosity at the sex locus is lethal. To understand the mechanisms that may contribute to the longevity of this lineage we surveyed 51 colonies from throughout the zone of infestation. We genotyped putative parasites at two sets of tightly linked loci (Shaibi *et al.* 2008 *Mol. Ecol. 8*, 1034), one set linked to the sex locus (*Sex*), and one linked to a region thought to be involved in the regulation of theltoky (*The*). We confirm that there is indeed a single clonal lineage of parasites. The lineage shows minor variations arising from recombination events, but no mutations were observed. Within the clonal lineage the *The* loci show remarkably high levels of heterozygosity. This heterozygosity may be maintained by selection against homozygotes, or by a reduction in recombination frequency within the lineage. The relative merits of these alternative hypotheses will be discussed. Surprisingly, the *Sex* loci are invariably homozygous. Yet the individuals are unequivocally diploid females, and are heterozygous at the sex locus itself.

EXPERIMENTALLY INDUCED SOCIAL PARASITE WORKERS ARE NOT SUBJECTED TO QUEEN CONTROL IN THE BUMBLEBEE *BOMBUS TERRESTRIS*

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Despite their high level of cohesion, insect societies are a privileged target for selfish individuals who divert the efforts and the social organization of a colony to their own ends. Indeed, workers often exhibit selfish reproductive strategies and lay eggs, either in their own or in foreign colonies. Nevertheless, very little is known about the mechanisms responsible for this worker social parasitism. We investigated them in the bumblebee *Bombus terrestris*, a monogynous and monandrous species where intercolonial worker social parasitism has been shown. We introduced groups of five reproductive workers either in their mother or in a foreign colony, at a time where no workers normally reproduce. Using behavioural observations and ovarian measurements, we found that while workers introduced in their own colony revert to sterility, they maintain a high level of ovary activation when introduced in a foreign colony and also are significantly more often in direct contact with the queen. Those workers may thus act as parasites, acting both on their physiological and behavioural advantage on other workers. This provides a good support for worker social parasitism in *B. terrestris* and shed some light on the control of worker reproduction.

HOW SPECIALIST AND GENERALIST CUCKOO BUMBLEBEES SNEAK INTO POWER OF REPRODUCTION

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Bumblebees belonging to the subgenus *Psithyrus* are obligate brood parasites of nest-building bumblebees of the genus *Bombus*. There are cuckoo bumblebees such as *B. vestails* that are specialised in usurping the nests of only a single host species, *B. terrestris*, while generalists like *B. bohemicus* usually parasitizes nests of *B. lucorum*, *B. terrestris* or *B. cryptarum*. After nest usurpation and killing of the host queen the parasitic female has to control worker reproduction in order to accomplish and maintain reproductive dominance. Our aim was to examine in a comparative approach, if parasitic bumblebees monopolize reproduction by physical or chemical means, and if the parasite females are able to produce a primer pheromone or mimic the fertility signal of the host queen in order to prevent worker reproduction. Bioassays with callow workers of the host *B. terrestris* were conducted using various gland extracts, cuticle surface extracts and mixtures of synthetic compounds of breeding cuckoo females as well as living parasites. The results clearly prove that *B. vestalis* and *B. bohemicus* females are able to suppress host worker ovarian development, when host workers were under direct influence of the *Psithyrus* female. Furthermore, we could demonstrate that cuckoo females in order to uphold the reproductive skew in a similar way as the host queen does in unparasitized colonies, mimic a fertility signal, a mixture of wax-type esters that we also identified in egg-laying host queens and workers.We thank the German Science Foundation (AY 12/2-2) for financial support.

Poster Presentations

Brood parasitism and inquilinism in social insects

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8-1 SOCIAL PARASITISM AND EGG RECOGNITION IN *FORMICA* ANTS

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Nests of social insects are an attractive resource in terms of nutrition and shelter and therefore targeted by social parasites. Social parasitism regularly leads to the demise of the host colony, and the pressing question is whether prospective hosts can evade parasitism by discriminating against eggs laid by parasite queens. Colonies of the ants *Formica fusca* and *F. lemani* are subject to temporary parasitism by *Formica rufa* group species. Young mated queens of the parasite species enter colonies of the host and harness the work force to raise parasite eggs. We investigated whether host workers are able to discriminate between nest mate and parasite eggs. We found that workers of both host species discriminate between nest mate and parasite eggs in favour of their own eggs. Moreover, workers of *F. fusca* are more fastidious than *F. lemani*, rejecting some of the nestmate eggs. Nevertheless, most host colonies reared some parasite eggs, paving the way for parasitism. In the absence of con-colonial eggs more parasite eggs were reared, suggesting that the presence of con-colonial eggs facilitates recognition.

8-2 VIOLENT FIGHTS AND FACIAL PATTERNS IN THE HOST COLONY TAKEOVER BY THE CUCKOO WASP *POLISTES SULCIFER*

Alessandro Cini*[1], Irene Ortolani[1], Claudia Bruschini[1], Lisa Poggi[1], Rita Cervo[1]

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The conspicuous variability in facial patterns (shape and size of black spots on the yellow clypeus) of some *Polistes* wasps have been recently investigated opening new scenarios in wasp communication field. The visual channel is now recognized to play a role together with the well known chemical channel. In particular, in *Polistes dominulus*, facial pattern seems to affect the aggressive response of opponents during contests. *P. dominulus* is the host of the obligate social parasite *Polistes sulcifer*, which violently attacks and usurps pre-emergence colonies. The parasite has a particular facial pattern, usually with the lower part of the clypeus black and connected to the black mandibles. In this study we evaluate whether the parasite facial pattern is involved in the usurpation process visually amplifying the mandibles width, giving information about the parasite dangerousness. Laboratory lure presentation experiments with manipulated and unmanipulated facial patterns demonstrated that the black lower part of the parasite clypeus reduces the aggressiveness of the hosts. To assess the relative importance of this visual trait together with other parameters (e.g. body size) in determining parasite success we performed laboratory usurpation trials. Our results showed that parasite facial pattern does not influence usurpation outcome, which is indeed strongly affected by the relative size of opponents. We hypothesize that this visual trait could play a role in the following stages of colony integration.

8-3 IS THERE SPECIALISATION IN POLICING IN THE HONEYBEE *APIS MELLIFERA*?

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Worker policing, in which worker laid eggs are selectively removed by other workers, is a widespread phenomenon in the eusocial Hymenoptera that helps to maintain the reproductive monopoly of the queen. Worker policing was first discovered in the honey bee, *Apis mellifera*, but since then has also been found in ants, wasps and Asian honeybees. Despite worker policing being a much studied phenomenon, as yet relatively little detailed behavioural studies have been done on it. For example, in the honeybee, the most studied organism in this respect, no data are available on whether or not particular bees or patrilines specialise in policing and what age group of bees carry out policing. Here, we provide the first study that looks into these issues. By following cohorts of individually marked bees and studying their behaviour in policing assays inside an observation hive, we show that policing was carried out by bees ranging in age between 10 and 36 days, and with their average age being 21 days. In addition, there was large variation in the amount of policing carried out by different bees, and some of the policing bees could be observed during several of the consecutive policing trials. These results strongly suggest that some bees specialise in policing in honeybee colonies. Currently, a genetic analysis is underway to determine whether bees from different patrilines also police to different extents, which will allow us to determine the genetic heritability of policing behavior.

8-4 Are caterpillars of the social parasite *Maculinea alcon* capable of changing their host's chemical profile?

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Insect societies have achieved very sophisticated ways to cooperate with nestmates and to aggressively reject non-nestmates. The necessary cues to discriminate between nestmates and non-nestmates in social insects are mainly based on chemicals, the cuticular hydrocarbons. These cues however are not static within a colony but rather an ever changing entity derived from genetic and environmental components. Some social parasites, such as the butterfly *Maculinea*, having evolved to break the chemical code to enter ants' nests and survive on the food and the shelter provided, could take advantage of the rather plastic discrimination system. We hypothesised that caterpillars of *M. alcon* could consolidate their integration into their host colony, and potentially facilitate additional parasitism by kin, by producing sufficient amounts of extra hydrocarbons to directly alter the colony's odour through their trophallactic interactions with their hosts. Results of a controlled lab experiment however did not confirm this hypothesis, instead we could document changes in the cuticular hydrocarbon profile of ant colonies, living under stable controlled conditions, over the course of a single month.

8-5 Effects of host social structure and host predisposition to parasitic eggs on social parasitism in *Formica fusca*

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Social parasitism is brood parasitism that is unique to social insects. In temporary social parasitism a parasite queen enters a host colony, kills the host queen and uses host workers to raise her own offspring. Rearing parasite offspring is harmful to the inclusive fitness of the host workers, and they should thus discriminate against parasite brood. We are interested how parasite queens areable to circumvent the egg discrimination behaviour of the host. *Formica fusca*, the common black ant, is a host for several species of temporary social parasites. In previous studies it has been shown that it has excellent ability in chemical recognition of eggs, but also that this recognition can be manipulated. Predisposition to alien conspecific eggs before their own queen started laying induces acceptance of alien eggs by workers. This might also be the loophole temporary parasites exploit in order to get theireggs reared by the host. We have studied how previous experience of parasite eggs affects acceptance of parasite eggs by *Formica fusca* workers in queenless colonies, and whether the effects are similar for two different potential parasite species *F. aquilonia* and *F. truncorum*.

8-6 Worker reproductive parasitism in social bees

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Insect workers typically refrain from personal reproduction in favour of rearing the queen's offspring, thus increasing their inclusive fitness. In some situations, a foreign worker may enter a colony, activate her ovaries and lay eggs, which are reared at the expense of the host colony. This is known as worker reproductive parasitism (WRP). If a colony loses its queen and cannot rear another, its only reproductive option is for its workers to lay eggs and rear a final cohort of drones before the colony dies. Hence, workers must curtail policing (removal of worker-laid eggs), rendering the colony vulnerable to WRP. WRP may also involve "cheating", or workers laying eggs despite the presence of their queen. In the thelytokous Cape honeybee *Apis mellifera capensis*, workers activate their ovaries during reproductive swarming events in an attempt to become the mother of the next queen, and a substantial proportion of replacement queens in *A. m. capensis* are indeed worker produced. I study the circumstances in which workers will exploit opportunities for cheating, as well as sources of and defences against WRP in *A. mellifera* and the Asian hive bee *A. cerana*. As in the Cape honeybee, we expect workers of arrhenotokous (sub)species to activate their ovaries during reproductive swarming as this provides them with the opportunity to produce drones that will mate with a new queen. By sampling drone brood of *A. mellifera* colonies in the lead-up to reproductive swarming and genotyping them to determine their maternity, any contribution by the workers to the production of drones can be determined. In *A. cerana*, 1-6% of workers have active ovaries under queenright conditions, yet no male offspring can be attributed to workers. Do queenright *A. cerana* workers with active ovaries actually lay their eggs? This addresses the bigger question of whether cheating in *A. cerana* is prevented by coercion by peers (policing) or self-restraint by individuals.

8-7 Induced anti-social parasite defense in host ant colonies

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Slavemaker ants, obligate social parasites, regularly conduct slave raids. Raided host colonies suffer severe fitness costs due to the regular killing of the queen and the loss of workers and brood during the raiding events. Host species developed general defense mechanisms such as parasite recognition as well as aggressive behavior directed towards slavemakers. Here we demonstrate that in addition, hosts exhibit context-specific defenses against slavemaker raids. We investigated the induced behavior of free-living host colonies induced by a five minute within-nest encounter with a dead slavemaker worker. We found that host ants discriminated between slavemaker ants and conspecifics and that the encounter with the slavemaker, but not with another non-nestmate conspecific or a different ant species, induced a longer lasting strong aggressive response directed towards non-nestmate conspecifics. This response may be adaptive if an encounter with a slavemaker reliably indicates an attack on the host colony in the near future and if aggression raises the chances of surviving such destructive raiding event. This induced elevated aggression was maintained over three days, indicating the ability of host ants to remember past encounters with slavemakers for at least three days. After 17 days the response had disappeared by then the benefits of constant high aggression level might be counter-balanced by potential costs associated with it. This is the first example of an induced anti-social parasite defense in social insects.

8-8 GIANT *DINOPONERA* AND TINY *PHEIDOLE*: A KIND OF INQUILINISM?

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In ants there are reports of complex symbiotic interactions such as social parasitism, parabiosis, xenobiosis, cleistobiosis and inquilinism. We investigated the interactions between colonies of *Pheidole* and their hosts *Dinoponera quadriceps* and *Dinoponera mutica*, discussing possible set of evolutionary implications for both genders. We found colonies of *Pheidole* in nine of the seventeen *D. quadriceps* nests excavated and five of six *Dinoponera mutica* nests excavated. In the literature, there is a report of the occurrence of *Pheidole dinophila* colonies living exclusively within the *Dinoponera australis* nests, and *Pheidole rudigenis* which can also be found within and outside the *D. lucida* nests. There is also a previous observation of colonies of an unidentified species of *Pheidole* living exclusively within the nest of another Ponerinae - *Diacamma sp1*. Our observations suggest that this interaction is possibly symbiotic, because both species involved have mutual benefits in the interaction and this is probably only required for the species of *Pheidole* involved, once these ants were not found living outside the nests *Dinoponera*. Within the artificial nests of *Dinoponera*, we observed a peaceful coexistence between the tenant and the giant ants. Workers of *Pheidole* visited and manipulated the garbage of *Dinoponera* nests, but we did not observe any worker preying on eggs or larvae of *Dinoponera*. We also never observed any other aggressive interaction between the two species. *Dinoponera* workers were observed carrying workers and soldiers of *Pheidole* to the interior of the nests. Therefore shelter and food access are benefits for the colony of *Pheidole*, while *Dinoponera* colonies have their nest cleaned. Further investigations will be carried out about this interaction and the kinship of the *Pheidole* inquiline group that relates to *Dinoponera*.

8-9 ZOOMING IN ON THE MIMICRY STRATEGIES OF THE SOCIALLY PARASITIC BUTTERFLY *MACULINEA ALCON*

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Caterpillars of the endangered butterfly *Maculinea alcon* are obligate social parasites of *Myrmica* ants. For social integration into ant nests, the caterpillars mimic the chemical signals of the ants. When a population exploits a single *Myrmica* species it clearly mimics the signals of that host, but when it exploits several host species, the situation is more complex. Most studies of chemical mimicry have combined several caterpillars to extract sufficient quantities of surface chemicals for accurate gas chromatography. It is therefore unclear whether (1) selection acts such that every caterpillar's profile is a compromise that mimics the chemical profiles of all local host species or (2) that selection acts on sub-populations to mimic only a single species, and the "compromise profile" is an artefact of combining caterpillars for the extract. We collected *M. alcon* caterpillars from Danish and Swedish populations where they use exclusively *Myrmica rubra* or *Myrmica ruginodis* ants, or both species simultaneously. We analysed the chemical profiles of single caterpillars before they had been adopted by their respective host. Initial results show that overall chemical profiles reflect geographic origin rather than host ant use, reflecting genetic differentiation between populations, but that there are some interesting patterns for particular compounds overlaying this background.

8-10 PHYLOGENETIC PERSPECTIVE OF ALTERNATIVE MODES OF SOCIAL PARASITISM IN NORTH AMERICAN *LASIUS* (*ACANTHOMYOPS*) ANTS

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The evolution of social parasitism in North American *Lasius* ants is studied through a phylogenetic analysis of character evolution in the temporary social parasitic species of the subgenus *Acanthomyops*. Phylogenetic reconstruction is based on DNA and morphology, with other subgenera of *Lasius* used as outgroups. With the sampling used here, the social parasites of *Acanthomyops* are found to be monophyletic, and there are two distinct lineages with respect to the method of invasion. One lineage uses glandular secretions that originate deeper in the genus *Lasius* as a way to confuse hosts. Another lineage relies upon armor and physical force to invade a host colony. Our results are consistent with earlier proposals that certain species are hybrids. We reject Emery's Rule in its strict form, that social parasites attack their close relatives.

Oral Presentations

Insect-microbe symbioses as evolutionary innovation

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The role of endosymbionts in ant nutritional ecology and evolution

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Symbioses and their entailing coevolutionary processes have been identified as a major driving force in ecological function and evolution by opening niches otherwise unavailable to the organisms engaged in the interaction. The bacterial endosymbiont *Blochmannia* is harbored in specialized cells intercalated into the midgut cells of *Camponotus* and related genera. *Blochmannia* plays a role in nutritional upgrading for their hosts by providing essential amino acids and may be regarded as a key innovation facilitating the success of these ants by enabling them to sustain on nitrogen-poor diets such as plant-exudates or honeydew. Recent studies have shown that other genera of arboreal ants utilizing largely plant-derived food sources possess a gut microflora with a similar function, comprised mostly of an ant-specific clade of *Rhizobiales*. I will give an overview of the role of endosymbionts and gut microflora in ant nutrition. and will compare the mechanisms of interaction with the host between the two. In addition I will discuss the role of ontogeny of the individual host for the association with the different bacteria as well as the impact of bacteria on the colony level.

Passalid beetles: Partitioned microbial gut communities

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The importance of microbial associates of many organisms has been known for more than a century but more recently has attracted renewed interest with the development of new technology and increased funding exemplified by the Human Microbiome Project. In many cases, including humans, the number of microbial cells greatly exceeds the number of cells comprising the organism. In associations with microbes the host organisms benefit from acquisition of large numbers of functional genes already present in the microbial symbionts. The enzymes are particularly important for degradation of intractable plant polysaccharides (e.g., pectin, cellulose, and hemicellulose). The termite hindgut is acknowledged as a model system for studies of the degradation of plant polysaccharides, but other wood-ingesting insects, including beetles (Cerambycidae, Lucanidae, Passalidae, Scarabaeidae, Tenebrionidae) also have microbes with similar functions. The adult subsocial beetle, *Odontotaenius disjunctus* (Passalidae), has a partitioned gut with compartments that vary in pH, oxygen availability, and microbial community. The gallery walls are coated with a mixture of frass and macerated wood. This material, rich in nutrients and microbes, is fed to larvae and newly molted adults. Initially, we were interested in the yeast (e.g., *Schefferomyces stipitis*) present in the hindgut of *O. disjunctus*. This yeast is notable for the holdfasts by which it is attached to the relatively unelaborated hindgut lining and by its ability to ferment xylose. In addition to *S. stipitis* recent studies have demonstrated that other xylose-fermenting yeasts are associated with other passalid species. A far more diverse community of organisms (parabasalids and prokaryotes) is present in the anterior part of the hindgut, a region of dramatic topographic elaboration. Based on their taxonomic identity, sulfate reducers, methanogens, cellulose decomposers, and nitrogen-fixing prokaryotes inhabit this gut region.

ECOLOGICAL GENETICS OF A MUTUALISM: DO LEAFCUTTER ANTS CULTIVATE FUNGI ADAPTED TO LOCAL ECOLOGICAL CONDITIONS?

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Variation in adaptive traits along an ecological cline can provide evidence for adaptive evolution. The mutualism between leafcutter ants and their cultivated fungi provides test cases for symbiont-mediated clinal variation, where adaptive traits of the symbiont interact with adaptive traits of the host to confer adaptation synergistically. To assess symbiont-mediated adaptation across a steep south-north temperature gradient and an east-west precipitation gradient at the northern end of the leafcutter ant distribution, we collected the leafcutter ant *Atta texana* throughout its range in Texas and Louisiana, isolated cultivars for population genetic analyses, and tested for cold-hardiness and desiccation resistance. Behavioral adaptations of the ants to regulate garden temperature interact synergistically with physiological adaptations of the fungi to extend the leafcutter range northward and permit ant-fungiculture under the harsh winter conditions in northern Texas. MaxEnt ecological niche modeling corroborates the importance of the ecological parameters identified by the analyses of clinal variation. Symbiont-mediated adaptations therefore generates ecological zonation in the leafcutter ant-fungus mutualism of *A. texana*, influencing range expansions under past and future climate changes. The patterns of ecological zonation in the leafcutter ant-fungus mutualism parallel patterns in farming practices and distribution of crop phenotypes along clines in human agriculture.

FUNGUS FARMING AND THE EVOLUTION OF SOCIALITY IN AMBROSIA BEETLES

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Fungiculture in insects is known from attine ants, macrotermitine termites and ambrosia beetles. The first two groups are eusocial and exhibit some of the most highly developed social organizations known among all organisms. In contrast, the social organization of ambrosia beetles is largely unknown. Adult female offspring in xyleborine ambrosia beetles delay dispersal from their natal nest, during which period they often engage in various brood care and fungus maintenance tasks. Dispersing females transmit spores of mutualistic ambrosia fungi (Ascomycetes) in special organs from the natal gallery to their new nest. These transmitted fungi usually dominate the microbial complex within the galleries. Our experiments show that adult females induce growth of the mutualistic fungus that is characterized by massive fruiting structures (sporodochia). These fruiting bodies seem to be an essential component of beetle nutrition. Our data suggest that an important function of delayed dispersal of adult females from their natal nest might be improved growth conditions of their ambrosia fungus creating potential for indirect fitness gains of philopatric females by an increase in sibling production. Sociality in ambrosia beetles probably evolves in close association with the beetle-fungus symbiosis.

EVALUATING METHODS TO ASSESS MICROBE DIVERSITY IN ANTS: TRADE-OFFS BETWEEN TRADITIONAL APPROACHES AND 454 PYROSEQUENCING

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Host-microbe associations can range from mutualistic to parasitic, providing some hosts with vast fitness benefits and others with great costs. Regardless of the outcome of these interactions, microbes shape the ecology and evolution of every living organism, incur strong selective pressures on their hosts, and are recognized as a major force driving host evolution and biological diversification. However, we are just beginning to understand the diversity and function of microbes in ants. In our recent research, we try to grasp the diversity of microbes associated with ants using several techniques. We targeted both bacteria and fungi using cultivation and cloning approaches. Additionally, we employed next generation sequencing techniques as approximately 99% of microbes are not cultivable and diversity is often underestimated in cloning approaches. Our results indicate an extreme diversity of both fungal and bacterial associates of ants. At the same time, we highlight methodological problems associated with the different techniques employed and evaluate their applicability. This study provides new insights into diversity assessment methods of ant-associated microbes - a group of organisms that may play a key role in the extraordinary abundance and evolutionary success of ants.

TEAMING UP IN DEFENSE: SYMBIONTS PROVIDE ANTIBIOTIC COMBINATION PROPHYLAXIS TO WASP LARVAE

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Mutualistic microorganisms are well-known to play a key role in providing nutrients for successful growth and reproduction in many insects. Several recent studies indicate that they can be equally important for the protection of the host and its nutritional resources against pathogen attack. Digger wasps of the genera *Philanthus* and *Trachypus* (Hymenoptera, Crabronidae) cultivate symbiotic *Streptomyces* bacteria in specialized antennal gland reservoirs and apply them to their brood cells, where they are later taken up by the larvae and incorporated into the cocoon silk. On the cocoon, the symbionts provide protection against pathogenic fungi during the long phase of hibernation. Here, we report on the identification of streptochlorin and eight different piericidin derivatives as the antimicrobial substances that defend the larval cocoon against potential pathogens. Using imaging mass-spectrometry (LDI imaging), we were able to visualize the most abundant antibiotic substances directly on beewolf cocoons. The abundance of antibiotics is much higher on the outer surface than on the inside of the cocoon, which probably serves both to enhance the protective activity against invading pathogens and to reduce potentially harmful side-effects on the beewolf larva itself. In agar-diffusion bioassays, the single substances exhibited differential bioactivity against various soil microorganisms as well as specific entomopathogenic fungi and bacteria. However, the complete cocktail of nine antibiotics ensured an efficient protection against all microbial strains tested and thereby provides the first case of an antimicrobial defense strategy in nature that parallels the combination prophylaxis used in human medicine. Defensive mutualisms with actinobacteria might constitute a general and widespread theme in the ecology and evolution of arthropods, and the study of the secondary metabolites involved promises to uncover novel drug candidates for human medicine.

Poster Presentations

Insect-microbe symbioses as evolutionary innovation

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9-1 UNRAVELING THE FUNCTIONAL SIGNIFICANCE OF MULTIPLE *WOLBACHIA* INFECTIONS IN WORKERS OF *ACROMYRMEX* LEAFCUTTER ANTS

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Wolbachia alpha-proteo bacteria are found as intracellular symbionts in many insects, where they often affect host fitness as reproductive parasites by causing cytoplasmic incompatibility, parthenogenesis, male-killing and/or feminization. In other associations the host has become dependent on these bacteria, because they are nutritional mutualists or necessary for successful reproduction. Many ants also harbour *Wolbachia* strains, but the consequences of these infections are typically not well understood. We used quantitative PCR and fluorescent *in situ* hybridization to study the dynamics of *Wolbachia* infections across different life-stages (larvae, pupae, adult workers) in *Acromyrmex* leaf-cutter ants where multiple *Wolbachia* strains have been identified previously. Preliminary data from *Acromyrmex octospinosus* laboratory colonies revealed infection rates of 100%, significant changes in the proportional representation of strains between larval and adult stages, and indications of different locations of strains in adult tissues. We are now seeking to confirm this in samples from field colonies and hope to come closer to understanding the consistent presence of *Wolbachia* in sterile ant workers that cannot pass these symbionts on to future generations.

9-2 SUBSTRATE PREPARATION BEHAVIORS FOR CULTIVATION OF SYMBIOTIC FUNGUS IN ATTINE ANTS

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Despite their importance for the evolution of the symbiosis between Attine ants and their fungal cultivar, substrate preparation behaviors have been the focus of few studies. This study aimed to comparatively examining these behaviors in *Acromyrmex disciger*, *Apterostigma pilosum*, *Mycetarotes parallelus*, *Myrmicocrypta sp.*, *Trachymyrmex fuscus* and *Trachymyrmex sp.* Nov. to describe the patterns of their evolution. Behavioral observations were carried out with a set of micro cameras and the behavioral frequencies were analyzed by principal components. Our findings revealed that the process can be divided into three parts: physical treatment, chemical treatment, and incorporation. Two behavioral patterns were revealed. The first is exhibited by basal species (*Myrmicocrypta sp*, *A. pilosum* and *M. parallelus*) and is characterized by the absence or low frequency of chemical treatment behaviors, while the second pattern is exhibited by derived species (*Trachymyrmex sp.* Nov., *T. fuscus* and *A. disciger*) and is characterized by great fragmentation of the substrate and deposit of fecal fluid. This suggests that the evolution of the process is marked by an increase in the importance of the chemical treatment, leading to the adaptations observed in leaf-cutting ants.

9-3 Assessment of yeasts associated with waste material of *Atta sexdens rubropilosa* (Hym. Formicidae) laboratory colonies

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Leafcutter ants harbor a plethora of microorganisms in their fungus gardens in despite the cultivated fungus they use for food. Recently, several yeast species were found to be associated in the fungus gardens but their potential roles in the symbiosis are poorly understood. The aim of this study was to profile the yeast community associated with waste material of two *Atta sexdens rubropilosa* nests reared in the laboratory. Fragments (1 g) of recently deposited waste material (new) and the same substrate after exposure of abiotic factors (old) were suspended in a solution of 0.2% of peptone and 0.05% Tween 80. After serial dilution, 150•L aliquots were inoculated in 2% malt agar supplemented with 50 mg/L of penicillin-G and streptomycin sulfate. Plates were incubated at 25º C in darkness for 7 days. A total of 38 strains were recovered. Sequencing of the D1/D2 domains (26S rDNA) coupled with microsatellite primed-PCR of 31 strains resolved 18 ascomycetes and 13 basidiomycetes. In general, the prevalent species in this study was *Trichosporon jirovecii* (25.8% of total isolates) followed by *Candida chiropterorum* (19%), *Stephanoascus ciferrii* (19%) and *Debaryomyces nepalensis* (9.7%). The strains *Candida orthopsilosis*, *Hyphopichia burtonii* and *Pseudozyma* were also recovered but in minor proportions (6.45%). We did not observe differences in the yeast communities between the new and old types of waste. However, the observed yeast species composition is different from previous published reports. Thus, our results suggest that yeast community from the waste material may resist harsh environmental conditions after exposure to abiotic factors.

9-4 Laccase gene expression as a possible key adaptation for herbivorous niche expansion in the attine fungus-growing ants

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Fungus garden enzyme activity is crucial for sustaining societies of attine ants. The evolutionary diversification of this clade has likely been influenced by enzymatic specialization in connection to changes in foraging niche (De Fine Licht *et al.* 2010 *Evolution*), particularly when the ancestral leaf-cutting ants shifted from a diet of mostly fresh but shed plant material to actively cutting leaves. However, the way in which leaf-cutting ants managed to overcome the chemical defences of leaves has remained poorly understood. Here we document that laccases may have played an important role in allowing the leaf-cutting ants to become generalist functional herbivores. Laccases are polyphenol oxidase enzymes (PPOs) that are best known for their ability to degrade lignin in saprophytic and wood-pathogenic fungi. We found that laccase activity was primarily expressed in newly constructed garden sections where secondary leaf compounds are most likely to hinder decomposition. A combination of genomic and transcriptional analyses showed that there are at least eight copies of putative laccase coding genes in a draft genome of the fungal symbiont *Leucocoprinus gongylophorus* and that these are differentially expressed. We hypothesize that fungus garden laccases originally had a lignin-degrading function in the fungi that the attine ants domesticated, consistent with the gardens of lower attine ants processing only dry plant substrate. Our results would then suggest that some of these laccases were co-opted for the derived function of neutralizing phenolic secondary defences in leaves. Selection for increased gene expression of fungal laccase genes in the ancestral leaf-cutting ant symbiont could thus have gradually increased the ant's capacity to process a higher proportion of fresh leaves in their forage. We are currently investigating the molecular evolution and expression profiles of these multiple laccase genes.

9-5 ECTOSYMBIONTS AND IMMUNOCOMPETENCE IN THE LEAF-CUTTING ANT *ACROMYRMEX SUBTERRANEUS SUBTERRANEUS* FOREL, 1893

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Associations with symbiotic organisms can serve as a strategy of social insects to deal with pathogens. Antibiotics produced by attine ectosymbionts (Actinobacteria) suppress the growth of *Escovopsis spp.*, the specialized parasite of attine fungus garden. These bacteria are clearly visible in *Acromyrmex* workers living inside fungus garden, conferring them a whitish appearance. Foragers do not have this whitish coat, suggesting that there is a decrease in bacteria number on cuticle when workers assume this function. Our objective was to evaluate if the presence/absence of symbiotic bacteria covering the whole ant cuticle is related to immunocompetence differences (ability to produce an immune response). The parameter used to measure immunocompetence was the encapsulation rate of a standard antigen, a nylon microfilament. We used three three years-old *Acromyrmex subterraneus subterraneus* colonies, containing six liters of fungus. The treatment was performed in three groups of workers (~ 2.4 mm head capsule): foragers (FOR), gardener ants with bacteria covering the whole body (COM) and without bacteria covering the whole body (SEM). W also eliminated the bacteria by antibiotic treatment and verified worker immunocompetence. COM ants showed lower rate of encapsulation than FOR and SEM ($p < 0.001$). Lower encapsulation rate did not seem to be a cost imposed by bacteria since their elimination did not change encapsulation rate. Instead, we propose that bacteria confer protection to young workers until maturation of their immune system. Although it was shown that bacteria have a specific action against *Escovopsis spp.,* new studies shed light in their action against generalist entomopathogenic fungi. Grants: CNPq and FAPEMIG

9-6 LOW DIVERSITY AND HIGH SPECIFICITY OF BACTERIAL COMMUNITIES IN THE BUMBLE BEE GUT

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Social insects are associated with a diverse array of bacteria, and their significance for their hosts range from pathogens to obligate symbionts. While the availability of molecular tools has enabled a deeper look into these interactions, a considerable part of past research has been concentrated on relatively few social insect species and has often focused only on endosymbiotic bacteria. There are some indications for an important role of gut bacteria in the honey bee (*Apis mellifera*), especially for pathogen resistance and food conservation, but almost nothing is known about the diversity, distribution and role of gut bacteria in wild bee species. In an ongoing research project we are therefore studying the gut bacterial communities in bumble bees (Hymenoptera: Apidae, Bombini) from different species and geographical locations, spanning a good part of the bumble bee phylogenetic tree. We are using a combined approach of 16S terminal restriction fragment length polymorphism analysis and cloning and sequencing of the same gene to characterize the bacterial members of the bumble bee gut flora. Focussing mainly on the central European species, we are presenting first insights into the diversity and distribution of these bacteria and compare them to those found in the honey bee. We are also in the process of testing the influence of these bacteria on the resistance of bumble bees against colonization of an intestinal trypanosomatid parasite (*Crithidia bombi*) and will present an outline of this experiment.

9-7 YEASTS ISOLATED FROM FIELD NESTS OF *ACROMYRMEX HEYERI* (HYMENOPTERA: FORMICIDAE) FROM ARGENTINA.

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This is the first report describing the isolation and identification of yeasts from fungus gardens and waste material of field nests of *Acromyrmex heyeri* from Santa Fe province, Argentina. This ant species usually defoliate monocot plants which are used as substrate for their symbiotic fungus. A total of 44 yeasts strains were isolated during the summer and winter sampling periods. Yeast strains with ascomycetous affinities such as *Pichia* and *Yarrowia* were rarely found; on the other hand, basidiomycetous species in the genera *Cryptococcus* and *Rhodotorula* were prevalent. Overall, the waste material and the fungus gardens were both quite similar in species composition. In addition, our data suggest that the yeast community in such substrates may be shaped by the seasons. These findings corroborate previous observations on yeast diversity in fungus gardens of different leafcutter species.

9-8 A POPULATION-LEVEL STUDY OF ANT-FUNGAL CULTIVAR COEVOLUTION IN THE *CYPHOMYRMEX WHEELERI* GROUP

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The transition from hunter-gatherer to fungus-farmer occurred approximately 50 million years ago in the ancestor of fungus-growing ants (Formicidae: Attini). To date, over 230 fungus-growing ant species obligately depend on the cultivation of fungus for food and, in return, the ants nourish, protect, and disperse their fungal cultivars. Propagation of cultivars is vegetative (as asexual clones) within nests, and also between parent and offspring nests; the foundress queen carries a fungal pellet from the natal nest and uses it to start her own garden. The long-standing assumption that fungi are only transmitted vertically (from parent to offspring) has been shown to be incorrect. Rather, cultivars are occasionally transmitted horizontally between different (sometimes very distantly related) ant colonies, and some cultivars appear to be closely related to free-living fungal populations. Understanding ant-cultivar coevolution at species- and population-levels ultimately informs our interpretation of the evolution of attine agriculture. We have conducted phylogenetic analyses of ants and their cultivars in the *Cyphomyrmex wheeleri* species group, which appears to be the sister group to the "higher" Attini. This work reveals extensive fungal cultivar exchange between members of this species group. The implications of these results are that, except for a few cases, each ant species appears to be fixed on a single fungus. The implications are further complicated by our discovery, based on mtCOI haplotypes, that *C. longiscapus* and *C. muelleri* may consist, respectively, of as many as six and three cryptic species. Reconstructing the shared evolutionary histories of the farming ants and their fungal cultivars elucidate the sequence of evolutionary events that produced attine agriculture.

9-9 STINGLESS BEES AND FUNGI: ARE THEY A SPECIALIZED FOOD FOR *SCAPTOTRIGONA* (APIDAE, MELIPONINI) LARVAE?

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The Meliponini are eusocial bees characterized by liquid massive feeding offered to the larvabefore queen oviposition, in individual brood cells, organized in combs. They live in largecolonies, normally also inhabited by other species of arthropods and microorganisms. The aim of this work was to study the relationship between the larvae of the stingless bee *Scaptotrigona aff. depilis* and a fungus that can grow on the surface of their larval food, developing only at the moment of egg hatching. We studied the frequency of the fungi presence inside cells during larval development. We also made observations on the larvae behavior to see how the fungus grew and if it was controlled. The fungus was present in all the colonies, inside all the brood cells containing larvae one to three days old. It appeared inside the brood cells, at the border of larval food, and was eaten by the larva, until the end of its development, when the prepupa occupied all cell. Although the larva has its own individual food produced by workers (pollen, honey and gland secretions), the constant presence of the fungus inside the individual brood cell and the way the larva eats it suggest a close relationship between them. This kind of fungus was found until now only in species of *Scaptotrigona* genus. Further molecular studies in 3 *Scaptotrigona* species will show if they belong to the same fungi species. The presence of this fungus in all brood cells suggests a close relationship, until now unknown in social bees.

9-10 EXPLORING POTENTIALLY NOVEL SYMBIOSES BETWEEN FUNGUS-GROWING TERMITES AND LIGNO-CELLULOSE DEGRADING BACTERIA

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Fungus-growing termites (Macrotermitinae) are major decomposers in tropical areas of the Old World, where they form some of the most complex colony and mound structures of any insect group. Although other symbiotic relationships have played an essential role in termite evolution (including intestinal microbes), only the Macrotermitinae have evolved a mutualistic association with fungi of the genus *Termitomyces* (Tricholomataceae, Basidiomycotina). These fungi aid in the degradation of plant material, and are housed in a special structure in the nest (the fungus comb), which is maintained by the termites through the continuous addition of predigested plant substrate. The termite workers play a crucial role in this substrate preparation, because all forage material passes through the termite gut before incorporation in the fungus comb. Here we explore whether bacteria with ligno-cellulolytic properties are present in guts (potentially aiding predigestion) and in the fungus comb (where the predigested substrate is utilized by the mutualistic fungus). Using targeted microbial isolations, we show that bacteria with ligno-cellulytic properties can readily be obtained from fungus-growing termite colonies. We characterize the abilities of a subset of these microbes to degrade lignin and cellulose, and present a draft genome of a candidate lignin-degrader in the genus *Sphingomonas*. Our findings suggest that the fungus-growing termite system may involve previously undescribed symbiotic associations with ligno-cellulose degraders, potentially aiding in substrate degradation. Current and future work will aim to obtain a better understanding of these associations, using both microbial isolations across multiple colonies from each of the three major fungus-growing termite genera (*Macrotermes natalensis*, *Microtermes sp.*, and *Odontotermes sp.*) from South Africa, in addition to community metagenomics and whole-genome sequence analyses of bacteria with ligno-cellulytic potential.

9-11 A SURVEY OF *ESCOVOPSIS SP.* ASSOCIATED WITH BRAZILIAN FUNGUS-GROWING ANTS (HYMENOPTERA: FORMICIDAE)

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Fungus gardens of attine ants are threatened by specialized microfungal parasites in the genus *Escovopsis*. The prevalence and distribution of this virulent parasite is known mostly for attine species in North and Central America. Here, we surveyed *Escovopsis spp.* associated with higher and lower attine ants from seven different sites in Brazil, spanning all major Brazilian ecoregions. Culture-dependent techniques revealed that 11 out of 65 (17%) nests were positive for *Escovopsis sp.* after at least two isolation attempts for each nest. This figure is consistent with previous reports on the prevalence of *Escovopsis spp.* associated with fungus-growing ants from South Brazil, but comparatively lower than the prevalence reported for attine ants from Central and North America. Two strains identified as *Escovopsis aspergilloides* based on their morphology were isolated from *Sericomyrmex luederwaldti* and an unidentified species of *Trachymyrmex*. To our knowledge, this is the first report of this fungal species from South America, as the only previous report was from gardens of *Trachymyrmex ruthae* on the Caribbean island of Trinidad. In addition, most *Escovopsis* strains were isolated in nests kept in sealed plastic containers for less than ten days; however, nests maintained for up to 42 days also contained *Escovopsis sp.*, corroborating published observations of long-term, sustained infections by this normally virulent parasite.

9-12 DID FUNGUS GARDENS OF LEAFCUTTER ANTS EVOLVE PECTINASES ANALOGOUS TO THOSE IN PHYTOPATHOGENS TO ATTACK LIVE PLANT CELLS?

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Leafcutter ants live in symbiosis with a basidiomycete fungus that they grow on a substrate of fresh plant material and use as food for themselves and their brood. These fungal symbionts originate from saprotrophic ancestors, i.e. from clades that normally do not attack live plant tissues. As the fungal symbionts of lower attine ants continue to process dead organic material, it seems reasonable to assume that domesticated attine fungi did not originally have physiological adaptations to overcome the physical and physiological defenses mounted by live plant tissue. In contrast, plant pathogenic fungi are able to actively enter live plant tissue by breaking down the plant cell walls with an array of concentrated degradation enzymes, of which pectinases are the most prominent. The fungal symbionts of the higher attine ants are characterized by the possession of unique swollen hyphal tips (gongylidia) on which the ants feed. Previous work has shown that some fungal enzymes pass unharmed through the ant intestines to be transferred to the actively growing parts of fungus gardens where the ants deposit the fecal droplets on a substrate of fresh plant tissue. To investigate whether the leafcutter ant symbiont *Leucoagaricus gongylophorus* has evolved adaptations to handle living plant tissue, we used proteomics methods to determine the identity of the fecal droplet proteins. Several of these proteins appeared to be pectin degrading enzymes that continue to be highly active in the fecal droplets of the ants. We further used qPCR to establish that these proteins are more highly expressed in gongylidia than in mycelium, suggesting that these pectinases have been actively selected to help degrade fresh plant tissue after passing through the ant guts. This may imply that the evolutionary derived fungal symbiont of *Atta* and *Acromyrmex* leafcutter ants has evolved an aggressively invasive mechanism that is analogous to what plant pathogenic fungi use to enter live plant tissue.

9-13 THE LEAF CUTTER ANT *ATTA TEXANA* (ATTINI, FORMICIDAE) CHOOSES TO CONSTRUCT CHIMAERIC GARDENS BY FUNGAL INTERCROPPING

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Interspecies or intraspecies cooperation can be stabilized evolutionarily if choosing partners favor beneficial partners and discriminate against non-beneficial partners. We quantified such partner choice (symbiont choice) in the leafcutter ant *Atta texana* (Attini, Formicidae). We offered the ants genotypically distinct fungal cultivars from *Atta texana* and *Acromyrmex versicolor* in a cafeteria-style preference assay. Symbiont choice was measured as the ants' tendency to choose one or more cultivar(s) from several pure (axenic) cultivar fragments and convert a given fungal fragment into a garden. Microsatellite DNA-fingerprinting enabled us to identify the cultivars chosen by the ants for their gardens. In 91% of the choice tests, *A. texana* workers combined multiple cultivars into a single intercropped, chimaeric garden, and the cultivars coexisted in such chimaeric gardens for as long as four months. Coexistence of distinct fungal genotypes in chimaeric gardens appears to contradict a recent model of cultivar competition postulating that each cultivar secretes incompatibility compounds harming other cultivars, which presumably would preclude the intercropped polyculture observed in our experiments. Symbiont choice by ants and any competition between coexisting cultivar strains in chimaeric gardens do not appear to operate fast enough in our laboratory assay to convert chimaeric gardens into the monocultures observed for *Atta texana* under natural conditions.

9-14 NEW PERSPECTIVE ON THE RELATIONSHIP BETWEEN TERMITES AND SYMBIONTS: REPRODUCTIVES LOSE SYMBIONTS AFTER COLONY MATURATION

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Termites are well-known social insects, and lower termites have symbiotic microbes associated with wood digestion and nitrogen provision in their hindguts. Because this symbiosis is usually recognized and has been investigated in the sterile workers, understanding of the relationships between symbionts and other castes is still lacking. Here, to elucidate the dynamics of symbionts in reproductives with colony development of *Reticulitermes speratus*, the hindgut sizes and quantities of major symbiotic protozoan species (*Trichonympha agilis* and *Pyrsonympha grandis*), based on the *alpha-tubulin* gene expression, of primary and secondary reproductives were examined together with the observations of ovarian and testicular development. Incipient colonies were established under laboratory conditions, and reproductives were sampled 30, 50, 100 and 400 days after colony foundation. Alates before colony foundation had highly reduced hindguts, and *alpha-tubulin* expression levels of both symbionts were low; however, the hindguts of a queen and king developed 30 days after colony foundation, and *alpha-tubulin* expression levels of both symbionts were significantly higher than in alates. The hindguts of the queen and king, with well-developed ovaries and testes, greatly reduced again 400 days after colony foundation, and *alpha-tubulin* expression levels were significantly lower than in previous stages. Moreover, greatly reduced hindguts and quite low-level *alpha-tubulin* expression were observed in physogastric female nymphoids (secondary reproductives) obtained from a field colony. Our results showed that reproductives lost their symbionts instead of developing reproductive organs after colony maturation. This is probably a new perspective on the relationships between termites and their gut symbionts, and gives important insight into our understanding of the reproductive division of labor in eusocial termites.

9-15 SUCCESSION OF FILAMENTOUS FUNGI IN THE WASTE MATERIAL OF *ACROMYRMEX BALZANI* (HYMENOPTERA: FORMICIDAE)

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The waste material produced by leaf-cutting ant colonies harbor various microorganisms potentially harmful to the development of the colony. The present study analyzed and compared the community of filamentous fungi found in the waste material of *Acromyrmex balzani* both when newly deposited (new) and after exposure for a period of time to abiotic conditions in the external environment (old). The new and old substrate samples were collected from the waste of four *Acromyrmex balzani* nests and suspended in a solution of 0.2% of peptone and 0.05% Tween 80. After serial dilution, 150µl aliquots were inoculated in 2% malt agar supplemented with 50 mgl^{-1} of penicillin-G and streptomycin sulfate. The dishes were incubated at 25 ºC in darkness for 7 days. After incubation the filamentous fungi load was quantified and representative isolates were selected. Isolates were identified by morphological characteristics (macro and micromorphology). From the 30 isolates recovered, 15 genera have already been identified. Isolates in the genera *Aspergillus* (23.5%), *Cladosporium* (20.6%) and *Penicillium* (14.7%) were predominant in the new waste but occurred in lower proportions in the old waste. On the other hand, the prevalent genera in old waste were *Trichoderma* (5.9%) and *Mucor* (8.8%). *Absidia sp.*, *Acremonium sp.*, *Rhyzopus sp.* and *Verticillium sp.* appeared only when waste had aged and faced harsh conditions, while new waste presented one species from *Scopulariopsis sp*. The results of this study suggest the existence of a succession of fungi throughout the waste's aging process so that some fungi present in newly deposited waste cannot be found once the waste ages, whereas others are only found when the waste is at an advanced stage of decomposition.

Oral Presentations

The social evolution of fusion and exclusion

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COLONY FUSION AND THE EVOLUTION OF ALLORECOGNITION SPECIFICITY IN MARINE INVERTEBRATES

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The path to the evolution of stable, multicellular life cycles must have been filled with ongoing cycles of conflict, cooperation, cheating, and conflict resolution. Three more-or-less ubiquitous features of multicellular life cycles can be understood in terms of conflict resolution: meiosis, a unicellular bottleneck, and self/nonself recognition (allorecognition). Allorecognition appears to play an exceptionally important role in all organisms whose life cycles involve aggregation (e.g., cellular slime molds) or intergenotypic fusion (e.g., colonial marine invertebrates). Yet, as Ross Crozier noted over two decades ago, in these contexts, simple population genetic models cannot easily account for the evolution of the genetic polymorphism necessary for precise allorecognition. This talk explores the role of allorecognition systems in the evolution and maintenance of multicellular life cycles, focusing on the costs and benefits of intergenotypic fusion, and the seemingly paradoxical evolution of allorecognition specificity.

FUSION, SEGREGATION, AND GENES FOR KIN RECOGNITION IN A SOCIAL AMOEBA

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Social amoebae, *Dictyostelium discoideum*, fuse with others by the thousands when they starve. If those others are non-clonemates, competition can occur, forcing some genotypes to become sterile stalk cells. But small groups pay the cost of reduced motility. What should the adapted amoeba do? An interesting pair of cell adhesion genes, tgrC1 and tgrB1, share a common promotor, are highly variable, as expected under balancing selection, and appear to be involved in kin discrimination. They are expressed at the same time, early in aggregation, and, when knocked out, cause the aggregate to behave as if no clonemates are available. The aggregate gathers, then disperses, repeatedly, presumably under the control of different signals. This system might be an ideal means of discriminating, and clones that are genetically more distant at these loci are less likely to engage in social fusion. And yet in the laboratory genetically different clones sometimes fuse into common fruiting bodies. In contrast, wild fruiting bodies from dung, a rich food source for social amoebae that prey on bacteria, are over 85% clonal. We have a great deal to learn about the intricacies of kin recognition from this system and are particularly excited about future research using clones of identical genetic background except the tiger genes.

FATAL MEETINGS IN THE WOOD? INTRASPECIFIC COLONY ENCOUNTERS IN WOOD-DWELLING TERMITES

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Insect societies are characterised by reproductive division of labor which is evolutionary stabile because related individuals cooperate. Hence fusion of colonies are expected to be rare as they reduce the indirect fitness gains of helping workers. Wood-dwelling termites live in a single piece of wood which serves at the same time as food source and shelter. If two colonies are founded independently in the same tree, they will inevitably meet when feasting up the wood. In the drywood termite *Cryptotermes secundus* more than one third of all field colonies face this fate of intraspecific encounters. The results of such encounters can be avoidance (rare) or aggressive (reproductives kill each other) or peaceful (no aggression among individuals) fusions. We investigated several factors influencing the outcome of encounters. In contrast to expectation relatedness was not a decisive factor. But larger colonies were more likely to be successful winners of aggressive encounters than their smaller counterparts. Extended investigations also elucidated the longterm consequences of social interactions between related and non-related nestmates for the occurence of conflicts.

WHEN ARE 'GREENBEARD' RECOGNITION SYSTEMS INTRAGENOMIC OUTLAWS?

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A ‘greenbeard’ gene, or set of linked genes, encodes both a mechanism for assorting with other carriers (or non-carriers) and a helping (or harming) trait that benefits other carriers in particular. Greenbeards have the unique potential to mediate cooperative coalitions among carriers who share genetic relatedness only at the greenbeard locus, and it is for this reason that greenbeards might appear to cause intragenomic conflict. We use population genetics arguments to clarify when greenbeards can be classified as intragenomic outlaws, considering cases of helping and harming both among non-kin and within established kin groups. We find that the outlaw status depends critically on the scope for, and the cost of, cheating within the complex, i.e., whether ‘falsebeards’, which assort with greenbeards but only reap the benefits, can arise. The analyses help to clarify the roles of inclusive fitness and assortment in greenbeard-like interactions, which have been implicated at many levels of biological organization.

EXPERIMENTAL EVIDENCE FOR CROZIER'S PARADOX

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Cooperative behaviors, behaviors that benefit other individuals, are widespread. However, to understand cooperation we have to explain how cheating, i.e. profiting without contributing, is kept at low frequency. Kin selection is the predominant solution for this problem. Kin selection requires that cooperation is preferentially directed towards related individuals, and one way to achieve this is via genetic kin recognition. However, Crozier argued that in the short term positive frequency dependent selection will eliminate the genetic polymorphism required for such recognition, since common genotypes will experience more cooperation and thereby increase in frequency. Here we study somatic fusion as a model for cooperation and kin recognition. Sharing somatic tissue via fusion seems to be an extreme form of cooperation. The potential for such fusion is widespread, but the fitness consequences of fusion are generally unknown. In fungi, successful somatic fusion is usually restricted to clonally related individuals regulated by highly polymorphic recognition loci. We study somatic fusion between mycelia of the fungal species *Neurospora crassa*. First we show that in cultures started from a high density of spores, there is a highly significant positive correlation between total fitness and the degree of successful fusion. This result demonstrates that fusion between genetically identical mycelia is net beneficial (i.e. B-C>0) and thus cooperative. We then show experimental evidence for Crozier's theoretical prediction that, in the short term, positive frequency dependent selection acts against polymorphism of recognition alleles. With these findings we discuss which counteracting evolutionary forces maintain the extensive recognition polymorphism observed in nature.

IMPERIALISM AND THE RISE AND FALL OF APHID EMPIRES

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Most social aphids are found within plant galls, inside of which clonally-derived family groups feed. Specialized larval castes forego reproduction and perform various cooperative tasks, including group defense. When unrelated aphids move between clones, conditions are ripe for conflict, because galls and cooperative defense are shared resources that are vulnerable to exploitation. A key unknown is whether conflict is costly in aphid social groups. I show that diversity within groups is negatively correlated with performance in the North American social aphid, *Pemphigus obesinymphae*. A substantial fraction of productivity is invested into invading the clones of both conspecific and heterospecific aphids. I describe the consequences of clonal fusion, and suggest that when unrelated individuals move between groups, social aphids may experience conditions consistent with a tragedy of the commons. Nevertheless, this form of imperialism by aphids may form the ultimate basis for social evolution in this poorly-understood group. These results emphasize the strongly convergent properties associated with conflict across the spectrum of animal and microbial sociality.

Poster Presentations

The social evolution of fusion and exclusion

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10-1 Pleometrosis is not the answer

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The prevailing hypothesis for the origin of multiple unrelated primary reproductives in mature colonies of the Neotropical termite *Nasutitermes corniger* has been pleometrotic colony foundation, the establishment of new colonies by a group of founding alates. While this has been shown to be the mechanism by which polygamous colonies are formed in another derived termite, *Macrotermes michaelseni*, it has not been verified in *N. corniger*. Through field observations and laboratory experiments, we have discovered that pleometrotic colony foundation is extremely rare in *N. corniger*. Further, incipient colonies with more than a monogamous pair of reproductives invariably have more kings than queens, the opposite of the sex ratio in mature polygamous colonies, indicating that mature polygamous colonies in this species do not result from pleometrosis. Incipient colonies will, however, accept additional reproductives, and our preliminary experiments suggest that young colonies readily fuse while retaining queens and kings from both colonies. Experimental fusion of mutually-tolerant mature colonies has previously been reported in the literature. We propose that colonies of *N. corniger* "meet and merge" during development, and that supernumerary kings are later eliminated leading to queen-biased sex ratios in mature polygamous colonies.

10-2 The evolution of kin recognition: production, detection, and action components

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The evolutionary stability of genetic kin recognition systems is not well understood. Although highly variable cue loci make kin recognition possible, using those loci for altruism selects against cue variability, a problem known as Crozier's paradox. However, models of kin recognition have not fully taken into account the three components generally recognized. They include the production component (cues), and the action components (behavior), but do not explicitly model the perception component. Here we explicitly include the perception and evaluation of cues, particularly the possibility that alleles at a perception locus can accept or reject specific cue alleles. Such allele-specific perception provides the kind of feedback required to prevewnt common cue alleles from going to fixation. Such common cue alleles provide poor information about kinship and therefore are selected to be rejected or ignored. The evolutionary dynamics are complex, but the model provides a partial solution to Crozier’s paradox. It can sometimes explain how multiple cues can be maintained in populations, though it does not appear to explain how vary rare cue alleles are initially favored.

10-3 COLONY STRUCTURE OF *KALOTERMES FLAVICOLLIS* (ISOPTERA, KALOTERMITIDAE): PRELIMINARY RESULTS FROM TWO MOLECULAR MARKERS

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180 *Kalotermes flavicollis* individuals from nine colonies sampled in Feniglia (Tuscany, Italy) were molecularly analyzed using a 303 bp fragment of the mitochondrial control region and the Inter-SINE fingerprinting methodology. The mitochondrial analysis highlights the presence of two highly divergent lineages. The 7 and 8 haplotypes scored for lineage A and B, respectively, show an intra-lineage variability ranging from 0.1% to 0.15% and a mean inter-lineage diversity of 8.8%: this obviously leads to a clear-cut separation in variability frequencies distribution. While lineage A was recognized in a previous analysis as a *K. flavicollis sensu strictu* lineage, the taxonomic status of the B lineage is actually under discussion. Within each colony the number of haplotypes ranges from 1 to 9: in particular, haplotypes of both A and B lineage occur in six colonies (KF1-6). On the other hand, one colony (KF9) shows three haplotypes of the B lineage, while the remaining two colonies (KF7 and KF8) share the same haplotype from lineage A. The PCA analysis performed on 15 I-SINE loci indicates that individuals from colonies KF7 and KF8 are clearly separated from individuals of colony KF9, while samples from mixed colonies superimpose to individuals from colonies of the A lineage.On the whole, mixed colonies can be the result of colony fusion processes or of the hosting of winged individuals swarming from other colonies. In any case, interbreeding between these highly differentiated lineages appears to occur and colonies are not closed and isolated structures as generally proposed for the genus *Kalotermes*. The haplotype pattern together with PCA data further suggests a preferential direction in crosses towards lineage A. Future investigations, performed on a wider dataset, will clarify these aspects.

Oral Presentations

Kin structure variation, gene flow and social adaptation

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Exploring the causes and consequences of social structure variation in ants

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Social insects vary greatly in the number of breeders per colony. The ancestral social structure in which eusociality evolved consisted of one single-mated queen and her offspring, so that workers raised highly related brood. Secondarily, polygyny – the presence of multiple queens reproducing in the same colony – became common in some taxonomic groups, particularly in ants. This seems paradoxical at first: why would workers or resident queens accept supernumerary queens if this decreases their inclusive fitness? Socially polymorphic species provide a good opportunity to explore the causes and consequences of changes in social structure. We studied some life-history, genetic and behavioural correlates of social structure variation in *Formica selysi*, a pioneer ant species that colonizes riverbanks. Our study population contains single-queen and multiple-queen colonies. The shift in social structure correlated with changes in multiple life-history traits, such as colony size, colony life-span, sex allocation, body size and disease resistance, but did not alter nestmate discrimination. There was no sign of genetic differentiation between social forms at neutral markers. Moreover, we found evidence that queens originating from each social form may either found new colonies independently or be accepted by workers in established colonies, and that mating can occur between individuals originating from alternative social forms. Taken together, these findings indicate that gene flow between social forms may be maintained via multiple routes. Overall, our data suggest that the alternative social forms are not stabilized by pronounced differences in behaviour or genetic incompatibilities that would restrict gene flow, but might be preserved by the spatial and temporal heterogeneity of the environment.

Is gene flow merely a spanner in the works of adaptation?

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Gene flow has a number of potentially important effects on the evolution of social behaviour. Two theoretically neat packages, namely the development of adaptations by natural selection and the measurement of genetic similarity in terms of relatedness, are both likely to be complicated by gene flow. The extent to which adaptations will be refined by geographical location will also be affected by gene flow. A natural first guess is that gene flow merely destroys adaptation, reducing the fineness of the fit of organism to environment. But there may also be ways in which gene flow can enhance and deepen adaptations. Bringing together population genetics and studies of adaptation is a major challenge, as it will tend to expose the inadequacies in our understanding of both.

TEMPORAL SHIFTS IN QUEEN NUMBER IN A SOCIALLY POLYMORPHIC ANT POPULATION

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In social insects, the maintenance of colonies with multiple queens requires additional evolutionary explanations, compared to single queen colonies where all workers are siblings. Positive selection favouring multiple queens is needed to counteract the cost of sharing reproduction and cooperating with more distant relatives or non-kin. For example, multiple queen colonies may be favoured in risky or saturated environments, because young queens are able to join pre-existing colonies and new nests often form by budding off from established nests. We investigate a *Formica selysi* population that contains both single-queen (monogyne) and multiple-queen (polygyne) colonies. Ultimately, we seek to determine what factors favour each social structure and how these two forms can co-occur in the same environment. In this study, we explore whether the social polymorphism is stable through time. We address this question with a 10-year data set, a time-scale that is approximately equivalent to the average life-span of a monogyne colony in this population. We compare the genetic structure of colony members collected between 2000 and 2009 at 11 polymorphic microsatellite loci in order to determine changes in social structure and in relatedness among colony members. In our population, monogyne colonies appear to be significantly more likely to become polygyne than the reciprocal shift. These monogyne colonies gain queens either through queen adoption or colony turnover. Given the apparent lack of genetic differentiation between social forms that has been shown in previous studies and the directional shift in queen number that we document here, we consider whether the hypotheses that have been proposed to explain polygyny in other species are applicable to *F. selysi*. We investigate whether monogyne and polygyne colonies tend to occur in different microhabitats from one another, and we explore the potential role of habitat instability in maintaining this polymorphism.

POPULATION STRUCTURE OF *APIS CERANA* IN THAILAND: AN INTERPRETATION TO ASSESS *VARROA* PARASITISM PATTERN

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The Asian cavity-nesting honeybee, *Apis cerana* Fabricius, is recognized as the original host of the infamous *Varroa* mite, a major brood parasite of the economically important western honeybee, *A. mellifera*. During the past decade, population genetics and biogeographic studies have revealed substantial genetic and morphological variations and mitochondrial DNA (mtDNA) population structure of *A. cerana* in Thailand. In our earlier study using mtDNA data, we found that *Varroa* populations in Thailand have specific links with local *A. cerana* populations, suggesting local co-evolution between the host and the parasite. However, by examining 18 microsatellites polymorphic loci (n = 45) and 3 AFLPs profiles (n = 184) of Thai *A. cerana*, the current results showed that the nuclear DNA differentiation of *A. cerana* populations are not entirely congruent with the mtDNA population differentiation, which may be explained through male-biased gene flow of the drones. The local populations of *Varroa* in Thailand are also not specific to the nuclear DNA population differentiation of *A. cerana*. These findings suggested that the pattern of parasitism of *Varroa* may be explained through local adaptation rather than overall genetic incompatibility between the host and parasite. Nevertheless, we cannot rule out that host specificity and the observed *Varroa* distribution in Thailand are caused by the geographic variations in one or a few genes of *A. cerana*. To test the hypotheses, cross-infection experiments of *Varroa* on different populations of *A. cerana* are essential.

GEOGRAPHIC VARIATION IN CONFLICT, COOPERATION, AND COOPERATIVE BREEDING AMONG UNRELATED ANT QUEENS

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Species exhibiting geographic variation in social traits provide important opportunities to test a basic tenet of sociobiology, that ecological factors often drive the evolution of social cooperation. Our comprehensive survey across the range of the desert ant, *Messor pergandei*, shows that their queens exhibit distinctly different social behaviors during colony foundation. In part of the species' range, queens establish new colonies alone (haplometrosis). Staged encounters among newly mated queens from one area show that they are extremely aggressive towards other queens when they encounter them, while in another, they are not. In the remainder of the species' range, queens establish a new colony in groups (pleometrosis). Queens are unrelated, yet they cooperate in rearing the first brood. Experiments show that queens from one part of the pleometrotic region then become aggressive, and fighting occurs until one queen remains. Genetic analysis of mature field colonies shows that they have a single queen, exhibiting secondary monogyny. In the remainder of this region queens do not fight after producing the initial brood, and queens coexist in laboratory colonies long-term. Analysis of mature field colonies reveals multiple reproductive queens, consistent with primary polygyny. *Messor pergandei* is a model system to test evolutionary theory proposed to explain the evolution of social behaviors and cooperative breeding in the absence of kin selection. It displays the entire range of breeding systems across its range, from haplometrosis to secondary monogyny to primary polygyny. Queens also vary in the underlying social behaviors by which those breeding systems are mediated. Moreover, the regional distributions of different social behaviors suggests that the study of this species can result in important tests of the roles that local adaptation, gene flow, and past history can play in explaining current social phenotypes.

VARIATION IN SOCIAL STRUCTURE WITHIN AND BETWEEN POPULATIONS OF THE HARVESTER ANT *POGONOMYRMEX CALIFORNICUS*

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Social insect colonies exhibit striking diversity in social organization including variation in queen number. The number of queens per colony varies both inter- and intraspecifically which in turn has major impacts on the social dynamics of a colony and the inclusive fitness of its members. To understand the evolutionary transition from single to multi-queen colonies we examined a species which exhibits intraspecific variation both in the mode of colony founding and for the queen number of mature colonies. The California harvester ant *Pogonomyrmex californicus* exhibits both variation in queen number at colony founding (metrosis) and in adult colonies (gyny). Throughout most of its range, *P. californicus* colonies are haplometrotic (one queen) but some populations exhibit pleometrotic (multiple queen) foundings which occur between unrelated individuals. Intrinsic differences in queen behavior lead to the two types of populations observed. Also, even though populations exhibit strong tendencies on average toward haplo- or pleometrosis, within population variation also exists among queens for behaviors relevant to metrosis and gyny. We examine these behavioral differences. We also investigate the phylogenetic relationships between focal populations and the amount of gene flow between them with both mitochondrial sequences and microsatellites. We present results which document the geographic extent of pleometrotic populations and provide genetic evidence that pleometrosis leads to primary polygyny (polygyny developing from pleometrotic foundress associations) a phenomenon which has received little attention and is poorly understood. Our results are important in understanding the evolution of polygyny in *P. californicus* and for understanding how such strong interpopulation variation is maintained for adjacent populations in the face of gene flow.

Poster Presentations

Kin structure variation, gene flow and social adaptation

11

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11-1 Split sex ratios due to queen replacement and the evolution of eusociality

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Eusociality constitutes one of the most extreme forms of altruism in biology. In these systems worker castes specialize in brood care and develop lifetime obligate sterility. The question as to the conditions that lead to such behaviour being favoured in a population remains to be fully answered. Here we develop Hamilton's suggestion that a haplodiploid genetic system favours brood care, applying it to populations with split sex ratios owing to queen replacement. We show that queen replacement leads to queenright (original queen present) colonies specializing in the production of females, and queenless (old queen dead, and replaced by a reproductive worker) colonies specialize in the production of males. These split sex ratios favour the expression of eusocial behaviour by workers from queenright colonies, lending some qualitative support to the haplodiploidy hypothesis in the context of populations with queen replacement.

11-2 Study of hybrid zones in the *Trigona carbonaria* complex (Hymenoptera, Apidae, Meliponini) through molecular tools

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In Australia, the endemic *Trigona carbonaria* group of stingless bees comprises at least 4 similar species (*T. carbonaria*, *T. hockingsi*, *T. davenporti* and *T. mellipes*) whose identification is difficult due to morphological variations along latitudinal clines and the occurrence of sympatric species. Nest architecture is the primary means used for distinguishing the species. The present work aimed to genetically analyse populations of *T. carbonaria* and *T. hockingsi* populations along Queensland state in order to understand the genetic structure of the contact zone between the two species. A total of 282 samples have been collected within the region of the two putative contact zones in South and North Queensland. Sequencing of two mitochondrial genes (16S and COI) resulted in highly well assigned molecular species identifications. Based on this marker, *Trigona carbonaria* has been shown to be monophyletic while *T. hockingsi* is paraphyletic. Interestingly, *Trigona hockingsi* from North Queensland possessed different haplotypes from bees from the Southern region. Also, it was possible to distinguish other species using the molecular data such as *Trigona sapiens*, *Trigona clypearis* and *Austroplebeia*, later confirmed by morphological traits. We also genotyped nine polymorphic microsatellite loci for all samples using primers designed from the *T. carbonaria* genome. Genetic distance of Nei and F_{ST} values were low between geographic sub-groups of each species. However, Fisher's exact test results pointed to genotypic differentiation for most pairs of sub-groups, as would be expected between groups from different species. We could not identify introgressions of species specific mitochondrial haplotypes into specific genotypes, discriminated by exclusive alleles from most loci. In spite of morphological traits not being sufficiently clear for species discrimination, no evidence of hybridization has been found in the present work. Financial support: CNPq; Endeavour Awards

11-3 Reproductive conflicts in the ponerine ant, *Pachycondyla verenae*

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Although social groups are characterized by cooperation, they are also often the scene of conflict. When colony members are not clones their reproductive interests differ and selfish behaviour and conflicts can occur. The ponerine ant *Pachycondyla verenae* is facultatively polygynous, moreover, some mature nests present a high level of polygyny for a species of this genus (n = 10 nests, range of queens = 2-18), possibly favoured by ecological constraints. Here we will present results from behavioural observations and experiments, genetic analyses, and GC-MS analyses of cuticular hydrocarbons. Through these studies we can analyse the behavioural control of conflicts, and its consequences for the kinship structure of the colony. High levels of polygyny may result from colony foundations established by unrelated queens, who stay together throughout the lifetime of the colony, or through readoption of daughter queens. We will present data on how the colony behaves when ecological constraints are removed, as well as colony manipulations such as orphaning. Through CHC analyses we will determine the existence of a "fertility signal" in dominant individuals within the colony. We used 9 microsatellite loci designed specifically for this species to analyse polyandry among queens as well as reproductive skew among workers. The results are compared with the previously studied and closely related *P. inversa* and *P. villosa*, which exhibit some biological differences, and lower levels of polygyny.

11-4 Opportunity for direct reproduction: do workers prepare for queenless conditions?

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In many eusocial Hymenoptera, queenless conditions provide the only opportunity for workers to successfully achieve direct reproduction. Therefore, given the importance of the event, workers may have been selected to "prepare" for queenless conditions. In the following study we tested this hypothesis using the facultatively polygynous ant *Leptothorax acervorum*. First, we investigated whether workers which go on to reproduce under queenless conditions behave differently to other workers under queenright conditions. Our methods involved filming the behaviour of individually marked workers under queenright and then queenless conditions. We then used ovary dissections and film footage to identify which workers had reproduced in the queenless stage. Finally, we compared the behaviour of these workers to non-reproductive workers using data collected from the queenright films. A difference in behaviour between future reproductive and non-reproductive workers would imply that some workers "prepare" for the future opportunity to reproduce, whilst others exhibit "self-restraint". Second, we investigated workers' response as a function of the social and genetic structure of their colonies by testing whether workers within monogynous colonies are more prepared for queenless conditions than those within polygynous colonies, given that monogynous colonies are more likely to become queenless. We removed the queens (and their eggs) from monogynous and polygynous colonies and took daily egg counts for a month. We then compared the latency and frequency of egg-laying between previously monogynous and polygynous colonies. In this poster we present the findings of our study.

11-5 Worker policing of queen reproduction

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In species of eusocial Hymenoptera with multiple queen colonies, there is potential conflict over reproductive partitioning (skew) among coexisting queens. Theoretical treatments of skew often assume that control rests with those individuals competing directly over reproduction (the queens) and overlooks the influence of a third party (the workers). Given that skew among queens impacts on the indirect fitness of workers, and workers are influential in other reproductive conflicts - such as sex ratio - workers are predicted to influence skew. We investigated the role of workers in controlling skew in functionally monogynous colonies of the ant *Leptothorax acervorum*. Detailed behavioural observations on un-manipulated nests revealed that worker aggression predicted which queen became the reproductive. Furthermore, experimental removal of the original queen showed that worker aggression again predicted which queen became the new reproductive. We found no support for "queen control" as the frequency of aggressive interactions among queens was low and did not increase after queen removal. Our work shows that workers can indeed influence queen reproduction in a species with distinct morphological castes, and importantly allows worker interests to prevail as supported by further genetic analysis.

11-6 Genetic structure of a drone congregation area of *Tetragonisca angustula* (Apidae, Meliponini) in São Paulo city, Brazil

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The stingless bee *Tetragonisca angustula* is commonly known as Jataí. Although this species is considered dependent of forest, recently it has been observed a high number of nests in urbanized areas. The substrates for nesting provided by human buildings and the deforestation can be the causes. The drone congregation areas (DCA) analysis is a form to clarify aspects concerning reproductive biology and also an indirect way to know the density of nests in a specific area. The goal of this study was to analyze a DCA of *T. angustula* and infer the number of colonies contributing to the DCA in an urbanized area. The males were collected in front of the nest entrance which was built in a brick wall cavity, in the city of São Paulo, next to the University campus. Ninety six males were screened for six microsatellite loci. The number of alleles per locus was determined by FOFpop v2.0 and ranged from 5 to 16 (mean 10.5). All loci were polymorphic taking account the 95% criteria. At least 8 colonies have contributed to the DCA. It is very intriguing the recent migration we are observing for some Meliponini species to urbanized areas. The loss of natural nest substrates (tree trunks) and the presence of gardens and green areas in the cities besides the wall cavities for nesting are attracting some species to this urban environment.

11-7 GENETIC AND PHENOTYPIC DIVERGENCE IN *APIS MELLIFERA* POPULATIONS FROM HIGH AND LOW ALTITUDE REGIONS OF THE KENYA MOUNTAIN

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Understanding the factors that lead to the genetic differentiation and phenotypic divergence of natural populations is of general interest in evolutionary biology. Evolutionary forces such as natural selection and random genetic drift as well as demographic factors may influence patterns of genetic variation and phenotypic divergence. However, the relative importance of these factors in producing the spatial distribution of genetic and morphological variation in nature remains an unresolved question. We used two subspecies of *Apis mellifera* distributed in mountain regions of Kenya in high (*A. mellifera monticola*) and low (*A. mellifera scutellata*) altitude to answer this question.We sequenced highly variable regions of mitochondrial DNA and genotyped six polymorphic microsatellite loci from three high and three low altitude populations. These genetic data were complemented by morphometrical analysis on several hundred individuals. Our preliminary results indicate a differentiation between high and low altitude populations of *A. mellifera* subspecies. The spatial distribution of genetic diversity and phenotypic divergence is discussed in respect to possible ecotypic differentiation and adaptive population divergence. This project is funded by the HHU (SFF-grant to M.H.)

11-8 KILLING THE WRONG KIND OF EGGS: THE COST OF WORKER POLICING

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Kinship, coercion and constraint are the three key factors in the resolution of conflicts in insect societies. Policing, the destruction of worker-laid (w-l) male eggs, is an important form of coercion in social insects. The highly effective worker policing in honey bees kills most (98-99%) w-l eggs. Workers are on average closer related to the queen's sons than to sons of other workers and so increase their inclusive fitness by preferentially rearing the queen's sons. In most previous studies of worker policing in the honey bee, eggs were transferred using forceps which can cause damage. This may have resulted in the proportion of queen-laid (qu-l), in error, and w-l eggs that are policed being overestimated. Here we used unmanipulated male and female qu-l eggs in order to estimate the cost of policing in honey bees. Our three main aims were to quantify: 1. the proportion of qu-l male and female eggs killed and their decrease depending on subspecies, sex and day; 2. the replenishment of accidentally killed male and female qu-l eggs by qu-l eggs; 3. whether replenishment depends on the age of an egg when removed.Our results show that only a small proportion of qu-l female eggs (4.6%) and male eggs (10.2%) was killed. Previous studies show that only 1-2% of w-l eggs are mistakenly not destroyed. This is much better than randomly killing qu-l or w-l eggs (2x50%) lacking any information. Besides, most eggs were replaced, especially when removed at a young age (~90%, $p < 0.001$). Interestingly, male eggs were replaced quicker than female eggs ($p < 0.001$). The proportion of w-l eggs was higher in *A. m. mellifera* than in *A. m. carnica* ($p = 0.028$). However, there was no correlation between the proportion of w-l eggs and the proportion of qu-l eggs that were policed ($p = 0.59$). W-l eggs that already survived 0-24 hours were as likely to be policed as 1 day old qu-l eggs ($p = 0.2$) but more likely than 2 day old qu-l eggs ($p = 0.029$). For statistical analyses in R GLMMs were applied.

11-9 Gene flow across the solitary-social transition in the socially polymorphic sweat bee *Halictus rubicundus*

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Eusociality has evolved just a few times in the bees. Interestingly, some sweat bee (Halictidae) species straddle this major evolutionary transition; they are socially polymorphic, exhibiting both solitary and social behaviour. Previous studies of socially polymorphic sweat bees have suggested a genetic basis to the social transition. We have attempted to address whether there is a genetic underpinning of this transition in the socially polymorphic sweat bee *Halictus rubicundus*, which exhibits solitary behaviour in the cooler north of its European range and sociality in the warmer south, by inferring levels of gene flow among populations exhibiting solitary or social behaviour. Our population genetic data reveal subtle genetic differentiation between populations in the UK and Ireland, though the level of differentiation between social and solitary populations is no greater than expected by geography alone (isolation by distance). The only major barrier to gene flow seems to be water (the Irish Sea). These data suggest little or no reproductive isolation between solitary and social populations of *H. rubicundus*. It is more difficult to infer whether there is, or is not, a genetic underpinning to the social transition with our indirect, population genetic data. They suggest that either there is no genetic underpinning (social phenotype is a plastic response to environment) or that there is a simple genetic architecture to the switch in social phenotype.

11-10 Colony structure in three species of the ant genus *Myrmecia*

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Ants of the genus *Myrmecia* have retained many biological traits that are considered to be basal in the family Formicidae. Studying these ants may provide valuable insight into the early stages of formicid social evolution. Using microsatellites, we investigated colony structure in three *Myrmecia* species, viz. *M. brevinoda*, *M. pilosula*, and a newly discovered cryptic species of the *M. pilosula* complex, *Myrmecia sp*. We observed, as a first ever for the genus *Myrmecia*, the co-occurrence of polydomy, polygyny and polyandry, in all three species. Mating frequencies of queens varied strongly within species, but the maximum frequencies were high in all of them, ranging from 9 to 12. Queens within colonies were lowly related in *M. brevinoda* and *M. pilosula*, but unrelated in *Myrmecia sp*. In *M. pilosula* and *M. sp.*, the colony founding mode could be budding, as indicated by Mantel tests. Our data for *M. brevinoda* are in support of the "genetic-variability" hypothesis that polygyny and polyandry should be negatively correlated due to their costs and the benefits of the resulting genetic variability.

11-11 As an ant flies: sex-biased dispersal and inbreeding in the ant *Formica exsecta*

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Dispersal behavior is a key determinant of population structure, and therefore also dictates the extent to which animals are susceptible to loss of genetic diversity owing to inbreeding and drift. As human induced habitat changes render populations more prone to inbreeding, it is essential to understand how dispersal and mating behavior affect gene flow and population structure in the wild. Yet, data on dispersal and its effectiveness in reducing inbreeding are scarce, particularly in social insects that may also be particularly vulnerable to inbreeding owing to their low effective population sizes. We investigated the relationship between mating behavior, dispersal and inbreeding in the narrow-headed ant *Formica exsecta*, using a long-term data set on field colonies and queens caught after the mating flight. We used microsatellite markers to measure dispersal distances of queens and males by assigning them to their colonies of origin within the study area. The observed flight distances were surprisingly short, with queens establishing new colonies as close as 60 meters from their natal colony. Males fly further, but not more than 150 meters on average. Neighborhood sizes estimated from population genetic data were highly compatible with the true estimates of flight distances. Dispersal distances were not associated with inbreeding level of the future colony, which implies that dispersal does not play a significant role in inbreeding avoidance. Furthermore, multiple mates of queens were often related, which implies only a few colonies contribute to mating swarms at any one time. However, actual sib-mating was rare, so inbreeding in the population is caused by pairing of individuals from related, yet not the same colonies. Our results show that mechanisms curbing sib-mating, such as mating flights and sex-biased dispersal, are not effective in preventing inbreeding in this population.

11-12 Temporal dynamics of the male effective population size in bumblebees (Hymenoptera: Apidae)

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Bumblebees are of major ecological and economic importance. As in all social Hymenoptera, sociality and haplodiploidy leads to a generally reduced effective population size (N_e) associated to reduced population fitness. Using microsatellites on a large field sample of drones and workers of *Bombus terrestris* and *B. lapidarius* we tested two hypothetical scenarios of drone dispersal potentially increasing effective population size: 1) genetically distinct drone-cohorts sequentially pass through an area or 2) Drones belong to one large temporarily unstructured population with extended mating flight range. We used two different colony assignment approaches, deriving natal queen genotypes either from weekly separate drone sub-samples or from the overall sample, referring to the two Hypotheses. The majority of the drones in our sample-area originate from colonies farther away from the sampling location than the workers foraging range. Our results indicate a clear genetic differentiation between local, worker contributing, and foreign, only drone contributing colonies leading to an increase of the populations gene-pool. However, analysis of colony assignment variance and temporal distribution of drone-contributing colonies suggested Hypothesis 2 to be the more parsimonious one. Though queen dispersal remains to be studied, we argue that N_e is bumblebees may be increased through extended male mating flight ranges, estimated with 10.54 km^2 and 15.98 km^2, *B. terrestris* and *B. lapidarius*, respectively, almost doubling local colonies foraging range in both species. Accordingly, population genetic characterizations indicate strong, genetically highly diverse populations with no signs of inbreeding.

Oral Presentations

Integrating molecular and morphological approaches to elucidate social insects phylogenies

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THE IMPORTANCE OF MORPHOLOGY IN THE AGE OF MOLECULAR SOCIAL INSECT PHYLOGENETICS

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Molecular data have eclipsed morphology as the primary source of phylogenetic characters for social insect taxa. Molecular data are also increasingly impacting other areas of systematics such as species discovery and identification. In general, the study of morphology is becoming increasingly marginalized in the genomics era. I discuss case studies from my own work on ants and bees that illustrate the continued importance of morphological data for phylogenetics. These examples include: incorporating fossils into phylogeny; inferring divergence dates; assessing the impact of relict taxa; establishing preliminary phylogenetic hypotheses; corroborating novel molecular clades; creating diagnostic morphological keys based on molecular phylogeny; and studying the evolution of adaptive characters. This is far from an exhaustive list of how morphology can impact molecular phylogenetics. As we embrace the vast potential offered by genetic and genomic data for phylogenetics, we also have much to gain in continuing to forge new advances in morphological systems. The more that we can develop a positive interplay between morphology and molecules, the better this enhanced perspective on social insect evolution can be realized.

MORPHOLOGY AND MOLECULES, THE FIRST COMPREHENSIVE, TOTAL EVIDENCE, PHYLOGENETIC ANALYSIS OF THE HYMENOPTERA

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The first comprehensive analysis of higher-level phylogeny of the order Hymenoptera is presented. The analysis includes representatives of all extant superfamilies, scored for 392 morphological characters, and sequence data for four loci (18S, 28S, COI and Ef1α). Including three outgroup exemplars, 100 terminals were analyzed. Relationships within Apocrita are resolved. Well supported relationships include: Orussidae is sister to Apocrita; Evanioidea is monophyletic; Aculeata is sister to Evanioidea; Proctotrupomorpha is monophyletic; Ichneumonoidea is the sister-group of Proctotrupomorpha; Platygastroidea is sister to Cynipoidea, and together they are sister to the remaining Proctotrupomorpha; Proctotrupoidea s.s. is monophyletic; Mymarommatoidea is the sister-group of Chalcidoidea; Mymarommatoidea + Chalcidoidea + Diaprioidea is monophyletic. Weakly supported relationships include: Stephanoidea is sister to the remaining Apocrita; Ceraphronoidea is sister to Megalyroidea, which together form the sister-group of (Trigonaloidea (Aculeata + Evanioidea)); Diaprioidea is monophyletic; Xiphydriidae is sister to Orussidae + Apocrita. Symphytan relationships are well resolved as follows: (Xyeloidea (Pamphilioidea (Tenthredidoidea (Cephoidea (Siricoidea (Xiphydrioidea (Orussoidea + Apocrita))))))).

MOLECULES, GUTS AND SYMBIONTS REVEAL THE ORIGINS AND EVOLUTIONARY DIVERSIFICATION OF TERMITES

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Our understanding of termite phylogenetics has been revolutionised by molecular methods, not only when considering the termites themselves but also for the gut microbial symbionts that they harbour. In addition, recent studies of the comparative anatomy of the worker gut have provided numerous phylogenetically-informative characters. In this talk I will discuss the main findings from analyses of these three data sources, and how they illuminate aspects of termite evolution and ecology. In particular I will discuss what the phylogenetic evidence now tells us about the origins of eusociality in termites.

COLONIZATION OF MADAGASCAR BY FUNGUS-GROWING TERMITES RAISES ADAPTIVE RADIATION HYPOTHESIS

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The mutualism between fungus-growing termites (Macrotermitinae) and their mutualistic fungi (*Termitomyces*) began in Africa. The fungus-growing termites have secondarily colonized Madagascar and only three of the eleven genera of fungus-growing termites were previously reported in Madagascar, based on their morphology: *Odontotermes*, *Ancistrotermes*, and *Microtermes*. Successful long-distance colonization may have been severely constrained by the obligate interaction of the termites with fungal symbionts, and the need to acquire these symbionts secondarily from the environment for most species (horizontal symbiont transmission). Consistent with this hypothesis, we show that all extant species of fungus-growing termites of Madagascar are the result of a single colonization event, ca.13 mya, of termites belonging to the genus *Microtermes*. This genus is one of the only two groups with vertical symbiont transmission, and the only one of the putative Malagasy genera. Vertical symbiont transmission may therefore have facilitated long-distance dispersal since both partners disperse together. In contrast to their termite hosts, the fungal symbionts have colonized Madagascar multiple times, suggesting that the presence of fungus-growing termites may have facilitated secondary colonizations of the symbiont. These findings indicate that the absence of the right symbionts in a new environment can be a limiting factor for the success of long-distance dispersal of symbioses that rely on horizontal symbiont acquisition. Furthermore, the observed incongruence between morphological and phylogenetic data suggests that *Microtermes* found there free niche space that has allowed it to diversify on the island into *Ancistrotermes*-like and *Odontotermes*-like morphotypes. This adaptive radiation of *Microtermes* on Madagascar needs to be further tested using detailed morphological data.

12. *Integrating molecular and morphological approaches to elucidate social insects phylogenies*. Oral

NEW FINDINGS IN ANT PHYLOGENETICS: IMPLICATIONS FOR MORPHOLOGICAL EVOLUTION

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Recent advances in our understanding of ant phylogeny have come about primarily through the application of molecular phylogenetic methods. The employment of DNA sequence data from multiple nuclear genes has resulted in robust, well-supported trees for several major clades of ants. These studies indicate that some features of ant morphology are reliable indicators of phylogenetic relationship while others are subject to greater homoplasy than previously suspected. The challenges of integrating molecular and morphological data in ant systematics are discussed.

ON THE DIVERSIFICATION OF CASTE: INSIGHTS FROM A NEW PHYLOGENY FOR THE ANT GENUS *CEPHALOTES*

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Morphological castes are a central theme in the evolution of colony organization in insect societies. Nevertheless, how caste structure changes and diversifies within social insect lineages remains poorly understood. Phylogenetic comparative methods provide valuable tools for advancing understanding of caste evolution. However, for any potentially informative lineage, such methods require a robust phylogeny and detailed comparative data on caste structure. Both are usually lacking. Here, we report on new phylogenetic analyses of the charismatic ant genus *Cephalotes*, and on related comparative studies of caste evolution in the group. *Cephalotes* displays one of the most striking patterns of soldier evolution and diversification of any extant social insect lineage. Our molecular phylogenetic analysis, which uses multiple nuclear and mitochondrial genes, provides a valuable test of the pioneering morphological phylogeny from a decade ago. We also explore combined molecular and morphological approaches for inferring the *Cephalotes* phylogeny. We find support for changes to the previous morphological phylogeny that are particularly important for interpreting soldier evolution in the genus. Building on these insights, we use new morphometric data, from a phylogenetically broad sample of *Cephalotes* species, to evaluate classic and untested ideas on caste evolution and diversification. Specifically, we address whether caste diversification in *Cephalotes* conforms to a hypothesized series of 'major steps' from ancestral monomorphism through to discrete castes. Contrary to this proposed series of steps, we identify complex patterns of gains and losses of specialized soldier traits. In particular, we show that the degree of discretization between castes, and the size range of soldiers, is highly labile. We propose adaptive hypotheses for why these aspects of *Cephalotes* caste structure are so changeable, and outline ongoing work to test these ideas.

PHYLOGENY OF THE TRIBE SOLENOPSIDINI AND CRYPTIC SOCIAL PARASITIC SPECIES, AN ECLECTIC APPROACH TO SPECIES DELINEATION

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The Solenopsidini tribe (Hymenoptera: Formicidae: Myrmicinae), first proposed in 1893, has challenged taxonomists for over a century. Not only has the monophyly of the tribe been called into question but there are also unsatisfactory generic delineations. Our work begins with an overview of the tribe then delves deeper into the three largest genera in the tribe, *Solenopsis*, *Monomorium*, and more specifically, *Megalomyrmex*. The genus *Megalomyrmex* comprises 31 described species. Eight *Megalomyrmex* species, classified in the *silvestrii* species group, are associated with fungus-growing ant or attine hosts while the other *Megalomyrmex* species are free-living predators. Within the *silvestrii* species group, there exists a gradation of parasitic behavior that includes lestobiotic parasitism (e.g., *M. mondabora*), agro-predatory association (e.g., *M. wettereri*), and xenobiotic parasitism (e.g., *M. wettereri*, *M. symmetochus* and *M. silvestrii*). All *silvestrii*-group species appear to be obligate associates of attine ants except for *M. silvestrii*, which is a facultative parasite that has been found both free-living and associated with fungus-growing ant hosts. Using our phylogenetic results from three genes (mtCOI, CAD, EF-1 alpha F2) and over 200 taxa we test the monophyly of the tribe Solenopsidini and the included genera. Our results recover two distinct *Monomorium* lineages and reject monophyly for the tribe. Also, using additional data from two genes (protein-coding EF-1 alpha F1 and wingless) for *Megalomyrmex* species, we test Darwin's predation hypothesis (i.e., predatory behavior is ancestral to social parasitism) and the monophyly of the four morphology based *Megalomyrmex* species groups proposed by Brandão. Further, we delimit cryptic social parasitic species using an eclectic approach to species delimitation, comparing phylogenetic, ecological, morphological, and venom alkaloid data.

SYSTEMATICS AND EVOLUTION OF THE MALAGASY *CREMATOGASTER* (*DECACREMA*), WITH REFERENCE TO BIOGEOGRAPHY AND NESTING BEHAVIOR

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In Madagascar ants of the subgenus *Crematogaster* (*Decacrema*) are a prominent component of the canopy fauna of many forest ecosystems. Malagasy *Decacrema* currently include four nominal species and two subspecies. Recent extensive inventories of the Malagasy ant fauna now allow for comprehensive taxonomic revision of this species-group. In this study, species within *Decacrema* in Madagascar are delimited using a combination of morphological and molecular characters, informed by species distributions. Both morphology and phylogenetic analyses of 2665 bp sequence data from the nuclear markers arginine kinase (ArgK), long wavelength rhodopsin (LWRh) and rudimentary (CAD) strongly suggest two new species and one elevation from subspecies to species level, while confirming the validity of one of the described species. Boundaries of the three remaining described species remain insufficiently resolved with respect to each other, but both the nuclear data and morphology point towards a case of synonymy for one of these species. *Decacrema* biogeography and evolution in Madagascar are discussed in the light of phylogenetic and taxonomic results, and relationships to *Decacrema* from other biogeographic regions are inferred within an outgroup framework. Further evaluated on the basis of the phylogeny are the evolution of nesting behavior and its implications for the dominance of these ants in the forest canopy.

12. *Integrating molecular and morphological approaches to elucidate social insects phylogenies.* Oral

UNCOVERING TERMITE SPECIES RICHNESS

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Termites (Isoptera) play an important role in tropical ecosystems, where they can constitute up to 10% of all animal biomass and as much as 95% of the soil insect biomass. So far, about 2600 termite species in over 280 genera have been described. Their species richness has been extensively studied in tropical rainforests where termite diversity is highest and where soil-feeding termites dominate. By contrast, only little is known about termite diversity in African savannas where fungus-growing termites (Termitidae, Macrotermitinae) prevail. Studying the latter is largely hampered by the difficulty of morphological species identification. Macrotermitinae lack complex guts that can serve as a reliable means of species identification in soil feeders. Thus, identification based on soldier traits is often only possible to the genus level. Here we present the results of a combined morphological and molecular approach to identify termites and determine their species richness in an African savanna ecosystem, the Pendjari National Park (Benin). We performed a large-scale survey covering six years to obtain a representative collection. After morphological identification samples were sequenced for three gene fragments (cytochrome oxidase I, cytochrome oxidase II and 28SrDNA; total length about 1900bp) to infer putative species from phylogenetic trees. Our results show that morphological traits proved to be inconsistent and unreliable means of species identification for several termite taxa. Especially among fungus growers many cryptic species were revealed. These results show that often a combined approach is necessary to identify termites reliably: first, specimens need to be morphologically identified to the genus level and then, samples from difficult genera should be sequenced.

Poster Presentations

Integrating molecular and morphological approaches to elucidate social insects phylogenies

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12-1 Phylogeography of the Weaver ant *Oecophylla smaragdina* supporting southern Indian refugia hypothesis

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Weaver ants *Oecophylla smaragdina* are spread widely over a large area in the tropical forests from India to Australia. In the previous study on the phylogeography of weaver ants, a deep genetic separation was found between a Bangladeshi population and an eastern Indian population, even though the geographic distance between the two populations is relatively narrow. The genetic distance between the Bangladeshi population and other South-East Asian populations was small. Therefore, the Bangladeshi population was considered to have come from the same refugia as those South-East Asian populations, but this group does not include the Indian population which has probably dispersed from another refugia. To validate this hypothesis, we collected ant samples from 48 colonies of 22 localities in India and Sri-Lanka and analyzed mitochondrial DNA Cytb and COI regions. In addition, the DNA data of ant samples collected from 13 localities in South-East Asia were cited from the present previous study on the molecular phylogeny of weaver ants. In the phylogenetic analysis, excepting one Indian population, a clear separation was confirmed between the Indian and other populations. Although Colombo (Sri-Lanka) population clustered with the majority of Indian populations, the genetic differentiation between the two groups occurred in early stage. In the median-joining Network, most dominant haplotype No.4 from Southern India was placed positioned at the center, and other Southern Indian haplotypes were connected to the haplotype No.4. On the other hand, haplotypes from Eastern or Northern India were not connected directly to the haplotype No.4. These results suggest that the Indian populations underwent strong bottleneck effects and dispersed relatively recently. Overall, the present study suggests that there was a South Indian refugia in ancient time.

12-2 Origin, diversification and molecular systematics of the leaf-cutting ant *Atta sexdens*

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The leaf-cutting ant *Atta sexdens*, one of the major agricultural pests in the Neotropics, is a complex of three currently recognized subspecies. We collected individuals from 100 nests in Brazil, Peru and Venezuela, spanning much of the geographic range of the species complex. We sequenced mitochondrial loci encompassing Cytochrome Oxidase I, an Intergenic Spacer, the Leucine tRNA and Cytochrome Oxidase II. Phylogenetic reconstruction using parsimony and Baysian methods showed that the *Atta sexdens* complex is composed of a more basal North Lineage restricted to the Amazon rainforest and two derived lineages: the Central Lineage, widespread across Northeast, Central, Southeast and South Brazil, and the South Lineage, restricted to the extreme southern tip of Brazil. Lineages were supported by high bootstrap and posterior probability values, were in demographic equilibrium according to the mismatch distribution, were reproductively isolated and highly genetically divergent, as suggested by F_{ST} values. A Mantel test indicated that genetic divergence was poorly correlated with geographic distance suggesting forces other than isolation by distance have promoted diversification. One of these forces may be related to ancient specialization in exploiting rainforest vegetation by the North Lineage. Further acquired abilities may have allowed the ants to exploit other environments, escaping the Amazon Region to colonize more open and arid habitats such as the Cerrado. The environmental plasticity of this derived group of ants is evident in the extant individuals which are pests for a great variety of native and introduced crops. A later vicariant event seems to have occurred to separate the Central from the South Lineage. Taken together, our results suggest that the current taxon known as *Atta sexdens* may comprise three distinct species.

12-3 SYSTEMATICS AND EVOLUTION OF MESOAMERICAN *STENAMMA*: A NEW PERSPECTIVE ON A TEMPERATE ANT GENUS

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Since its description in 1839 the genus *Stenamma* Westwood has been perceived as a group of cryptic, leaf-litter ants with strong affinity to the northern temperate zone. Forty-five extant species currently belong to the genus, and of these, thirty-nine occur in the Holarctic region. This pattern, however, does not reflect the true distribution of the genus, and is likely the result of both poor sampling and taxonomic neglect. Recent collecting efforts, in conjunction with greater use of the leaf-litter sifting technique, have revealed that the Mesoamerican region contains an assemblage of *Stenamma* species that rivals the Holarctic region in diversity of species, morphology, and behavior. Combining molecular phyogenetics with traditional morphological-based taxonomy, I show that Mesoamerican *Stenamma* form a clade comprising nine species groups and over thirty species. With these results, I discuss morphological evolution within the genus and present a new paradigm on the origin and diversification of *Stenamma*.

12-4 INVESTIGATING THE PHYLOGENETIC SIGNIFICANCE OF MELANISM IN *BOMBUS MUSCORUM L.*

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The DNA barcode, a c640 bp region of the cytochrome c oxidase (CO1) mitochondrial gene has fast become an invaluable tool for the ecologist and taxonomist alike. Although primarily used for species level identification, CO1 barcoding is shedding light on groups of taxa with ambiguous biogeographic and phylogenetic characteristics. One such group is the moss carder bee *Bombus muscorum*, a bumblebee distributed across Europe and northern Asia for which a number of blond and melanic varieties exist. The melanic forms are primarily found on islands and have been recorded on islands off the coasts of England, Ireland, Scotland and Norway (including the Faroes), as well as sporadically across Fennoscandia and European Russia. Taxonomically, melanic forms have been variously recognised as distinct species, subspecies or varieties. In an effort to resolve these issues we sequenced the CO1 barcode region for 60 specimens obtained from a number of Irish and UK mainland and island populations. Of the 600 nucleotides, only a single polymorphism was observed, with all specimens having either a cytosine (C) or a thymine (T) at position 150. British specimens were of the C haplotype, whereas the Irish specimens were of the T or C haplotype. Considering that previously generated sequence data for a cryptic species aggregate of *Bombus* revealed intra and interspecific estimates of variation of 0.36% and 2.8% respectively, our results (0.16% intraspecific variation) indicate that British and Irish *B. muscorum* (whether melanic or blond) are a single species. Our results also indicate that melanism in *B. muscorum* has no underlying phylogenetic significance (e.g. remnants of a Lusitanian distribution or edge of geographic range effect) and the presence of melanic forms on islands is due to convergence. However the environmental factors associated with island life and selective pressures that contribute to this convergent (and presumably adaptive) trait remain, as yet undetermined.

12-5 TAXONOMY AND PHYLOGENY OF THE ANT SUBFAMILY HETEROPONERINAE

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Heteroponerinae Bolton (2003) comprises three ant genera: *Acanthoponera* Mayr, *Aulacopone* Arnol'di, and *Heteroponera* Mayr. *Acanthoponera* is an exclusively Neotropical genus with four described species. *Heteroponera* shows a disjunct distribution, in South America and Australasia and includes twelve Neotropical and four Australasian nominal species. The genus *Aulacopone* is known by a single species represented by a couple of dealate gynes from Azerbaijan (southwestern Asia) which position within the subfamily is unclear. Heteroponerinae ants are predominantly predators and occasionally feed on plant exudates. Nests can be found in the ground or in the vegetation, and may contain a few dozen individuals. The recent increase in sampling effort of leaf litter ants in tropical wet forests has revealed new and interesting records of Heteroponerinae, including new species and extending considerably the ranges of the previously known ones. The present study represents the first species-level taxonomic revision of the subfamily as a whole and the first attempt to analyze the internal phylogenetic relationships of Heteroponerinae based on morphological characters. The monophyly of the subfamily and its genera is confirmed. The position of the monotypic genus *Aulacopone* remains unclear within Heteroponerinae. Seven species are recognized in the exclusively arboreal genus *Acanthoponera*, of which three are described as new. Seven species-complexes (three Indo-Australian and four Neotropical) including 19 species (six Indo-Australian and 13 Neotropical) are recognized for *Heteroponera*, five of them described as new. *Heteroponera flava* Kempf is synonymized with *H. panamensis* (Forel); *H. georgesi* Perrault is synonymized with *H. microps* Borgmeier; and *H. robusta* Kempf is synonymized with *H. dolo* (Roger). Field observations allied to data recorded in museum specimen labels provide important additional information on the natural history of the group.

12-6 MOLECULAR PHYLOGENY OF FIRE ANTS: IMPLICATIONS FOR THE EVOLUTION OF SOCIAL ORGANIZATION AND WORKER POLYMORPHISM

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Fire ants (*Solenopsis geminata* species-group) are one of the best studied groups of ants, no doubt due to the Red Imported Fire Ant, *Solenopsis invicta*, which has become an invasive pest in the southern USA. Considering that this species has become a model species for the study of ecology, behavior, life history, and social organization, it is surprising that the evolutionary history of this species and its close relatives is still not well understood. To address this shortcoming, I present a multi-locus nuclear gene phylogeny of the fire ants with nearly complete taxon sampling (only two of 21 nominal species are lacking). I discuss the challenging taxonomy and systematics of this group and well as the implications of the phylogenetic hypothesis for the evolution of polygyny and worker size polymorphism in fire ants.

12-7 THE BASAL GROUP OF THE GENUS *CREMATOGASTER* FROM MORPHOLOGICAL EVIDENCE (HYMENOPTERA: FORMICIDAE)

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The ant genus *Crematogaster* is one of the most hyperdiverse genera including more than 900 available species-level names. The genus is traditionally divided into 16 subgenera. Up to the present, no attempt of the phylogeny of subgenera within *Crematogaster* has been made. The present study suggests that the genus is divided into the two subgenus-groups from the morphological characters of the clypeus and petiole. One is the *Orthocrema* subgenus-group including *Apterocrema*, *Eucrema*, *Neocrema*, *Orthocrema* and *Rhachiocrema*. The other is the *Crematogaster* subgenus-group including *Atopogyne*, *Colobocrema*, *Crematogaster*, *Decacrema*, *Mesocrema*, *Nematocrema*, *Oxygyne*, *Paracrema*, *Physocrema*, *Sphaerocrema* and *Xiphocrema*. The *Crematogaster* subgenus-group shares the distinct synapomorphic characters: anterolateral margin of clypeus produced anteriorly; petiole without node-like process posteriorly. Among the *Orthocrema* subgenus-group, the subgenus *Rhachiocrema*, known only from Indo-Australian region, is supposed to be a monophyletic taxon and located in a basal position of the phylogeny. This idea is supported by the morphological evidences of the petiole with long peduncle and postpetiole with convex sternite.
In addition, the present study focuses on the Asian *Orthocrema* species because the subgenus is most diverse among the *Orthocrema* subgenus-group but the monophyly is highly dubious. Three species groups, *C. (O.) binghamii*, *biroi* and *quadriruga* species groups are recognized. The identity and justification of the grouping are discussed.

12-8 BIOGEOGRAPHY AND DIVERSIFICATION OF THE PACIFIC ANT GENUS *LORDOMYRMA* EMERY

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Aim: This study addresses the origins of terrestrial biodiversity of the Fijian islands using the ant genus *Lordomyrma* (Hymenoptera: Formicidae: Myrmicinae) as a model system. We derive the evolution of the genus and determine its closest extra-Fijian relatives from geological data, molecular phylogenetic reconstruction and divergence estimates. *Location:* Ant taxa were sampled in the Southwest Pacific, Melanesia, Southeast Asia, Australia and mainland China. *Methods:* Phylogeny and divergence estimates of the ant genus *Lordomyrma* based on four nuclear genes (28S, ArgK, LW Rh, CAD) plus data on Indo-Pacific geological history are used to address current hypotheses regarding the origins of the Fijian biota. *Results:* The genus *Lordomyrma* probably originated in mainland Asia, with subsequent colonization of Australia and the Pacific. The Fijian *Lordomyrma* clade is monophyletic, and originated c. 8.8 Ma, when it diverged from a sister group in Papua New Guinea. *Main conclusions:* The colonization of Fiji by *Lordomyrma* is probably a result of long-distance dispersal from New Guinea, possibly aided by island hopping across the Vitiaz Arc. The timeline of diversification in *Lordomyrma* is broadly congruent with the Miocene fragmentation of the Vitiaz Arc and the Pliocene emergence of Vanua Levu. The biotic shuttle hypothesis, which posits `Eua Island as the source of Fijian endemics, is rejected based on the sister relationship of Fiji and New Guinea lineages, as well as on the Miocene submergence of the terrane below sea level. The diversity of Fijian *Lordomyrma* results from the radiation of a single lineage, which diverged from a New Guinea sister group. The genus appears to have originated in Asia rather than in Australia.

12-9 PATTERNS OF DIVERSIFICATION IN THE NEOTROPICAL ANT GENUS *CEPHALOTES*

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Cephalotes is a diverse genus of ants distributed throughout the Neotropics. Known as the turtle ants, the 131 fossil and extant members of this group comprise the third largest ant genus in the Neotropical region and the sixteenth largest worldwide. Despite this great diversity, only one phylogenetic study of *Cephalotes* has been conducted, and the evolution of this genus has yet to be examined using molecular data. In this study we reconstruct a species tree from multiple gene trees, encompassing about half of the described species within *Cephalotes*. Our molecular data support the monophyly of most previously recognized clades, containing morphologically similar species. However, the higher-level topology of our molecular tree differs substantially from previous work. Using fossils from Dominican amber and a relaxed molecular clock approach, we obtained a timescale for the *Cephalotes* phylogeny. We used our time-calibrated tree to explore diversification dynamics within the genus. Our results indicate a rapid burst of diversification in *Cephalotes*, shortly after the origin of the genus in the Paleocene.

12-10 TAXONOMIC REVISION OF THE AFRICAN DRIVER ANTS

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African driver ants hunt in massive swarm raids on the forest floor and in the vegetation. Their colonies are the largest single-family insect societies in the world. They play significant roles in afrotropical forest ecosystems as extremely polyphagous predators, prey for several mammal species, and hosts to a plethora of commensalists and parasites. Studies on these fascinating ants have long been severely hampered by considerable taxonomic confusion. For example, under the name *Dorylus nigricans*, a species originally described in 1802 on the basis of two male specimens collected in Sierra Leone, more than 15 subspecies and varieties have been recognized at one time or another. We used morphological data to distinguish the workers and males of different species and mitochondrial and nuclear DNA sequence data to establish male-worker associations. An identification key to the workers of all recognized species is provided.

12-11 PHYLOGENETIC ANALYSES OF EARLY EVOLUTIONARY TRANSITIONS IN FUNGUS-FARMING ANTS

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Understanding the evolutionary position of key taxa within the fungus-farming ants (Myrmicinae: Attini) is imperative for reconstructing the evolutionary history of the different attine agricultural systems. For example, the study of Schultz and Brady indicated that the phylogenetic position of the recently erected genus *Mycetagroicus* is transitional from lower to higher agriculture, leading to targeted field work that identified the cultivar of *Mycetagroicus cerradensis*. In a second example, Lattke (1999) described the bizarre species *Apterostigma megacephala* from Perú and Colombia. Recently, several specimens of this species have been collected in the state of Pará, Brazil. The morphology of this species suggests that it may occupy a position intermediate between the *auriculatum* Leucocoprineae-cultivating and *pilosum* Pterulaceae-cultivating species groups of *Apterostigma*, and that it therefore may provide information about the only shift away from Leucoprinaeae cultivation in the entire tribe Attini. In a third example, a primitive attine species that may represent a new genus may provide information about the early evolution of agriculture in ants. The phylogenetic position and the evolutionary implications will be discussed for each of the latter two examples (*A. megacephala* and the 'new genus').

12-12 AUSTRALIAN *MONOMORIUM* ANTS: MORPHOLOGICAL PLASTICITY OR UNFATHOMABLE DIVERSITY?

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Monomorium Mayr (Hymenoptera: Formicidae) is a highly abundant, widespread and diverse genus of ants. Several Australian 'species' show considerable morphological and chemical variation, while evidence from ecological and preliminary molecular research suggests significant cryptic diversity. However, rigorous morphological criteria that support separate species have proved difficult. In this study we are using sequence data from multiple loci to generate a phylogeny for the Australian species in order to test the current species group relationships. In addition, we generate hypotheses for species boundaries in the widespread and morphologically diverse seed harvesting *M. 'rothsteini'* complex using a combination of molecular and morphological data. Preliminary results indicate that the Australian *Monomorium* species groups, as currently understood, are not reflected in the molecular phylogeny and that several groups may be para- or polyphyletic. Our results also provide preliminary evidence for a large number of species in the *M. rothsteini* complex and in other morphologically diverse species.

12-13 THE EARLY EVOLUTION OF THE SOCIALLY PARASITIC ANT GENUS *MYRMOXENUS*: RECONSTRUCTION OF THE PHYLOGENETIC TREE

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'Social parasitism' is the exploitation of social animal societies by other animals. Ants have become model organisms for studying this fascinating phenomenon. As shown in previous studies, the small myrmicine tribe Formicoxenini is a particularly hot spot in the evolution of social parasitism, with six phylogenetically independent origins of slave-making alone (Beibl *et al*. 2005). The formicoxenine genus *Myrmoxenus* exhibits a wide variety of socially parasitic scenarios, with transitions from active slave-raiding to workerless inquilinism and different sexual behavior. A previous study aiming at the elucidation of the phylogeny of this genus based on mitochondrial DNA (Beibl *et al.* unpublished) remained incomplete because of quantitative (too short sequences) and qualitative (lack of some species) shortcomings and the origin and relationships within this genus could not be clarified. The present study has three objectives: (1) To accumulate more data by using additional mitochondrial and nuclear genes. (2) To create a phylogenetic tree only with each gene to test for its robustness. And (3), to combine information from all studied genes to reconstruct a comprehensive phylogenetic tree.

12-14 TAXONOMY AND DISTRIBUTION OF THE GENUS *APIS*

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Two new taxa have recently been added to the genus *Apis*. *Apis indica*, the yellow Plains bee of south India and Sri Lanka is a separate species from *A. cerana*. The Giant Philippines Honey Bee, *A. breviligula*, is a separate species from *A. dorsata* (Lo *et al*. 2010). The taxonomy, distribution and evolution of Asian honey bees will be reviewed.

12-15 MALE-BASED REVISION OF MALAGASY ANTS

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Male ants are valuable as a new source of morphological data to reconstruct the hypotheses on the morphological evolution of ants. Conflicts found between molecular and morphological data require revising the existing ant taxonomic system, which is mainly based on workers' characters. Specialized characters of males have different functions from workers, and could lead to new diagnostic characters and new landmarks for comparative studies, as well as pointing out hidden homoplasies. However, most of the morphological information about males is still scattered in many sources as a part of species descriptions, and a large part of these are not standardized and useless for inter-generic comparison. Systematizing this morphological data is necessary. This study reconsiders the usefulness of male ant morphological characters as diagnoses of genus and subfamily ranks. At this time, subfamilies Ponerinae, Proceratiinae, and Dolichoderinae have been examined. New diagnostic characters were proposed for the mandible, labrum, maxillary palp, mesopleuron, fore and hind wings, abdominal terga, pygostyle, and genitalia. A lack of the pygostyles, a reduction in number of the tibial spurs, and a change in shape of the labrum were recognized as genus rank characters in each subfamily.

Oral Presentations

Patterns and processes of aging and lifespan: how special are social insects?

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SOCIAL AND UNIQUELY AGED? MECHANISMS OF HONEY BEE AGING PLASTICITY

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Honey bee life-history exemplifies that aging can become so strongly influenced by social relationships that senescence correlates only weakly with chronological age. Molecular mechanisms that influence honey bee longevity include gene networks with functions in socio-behavioral plasticity. This plasticity plays an important role in the bee's social allocation of intergenerational care and food-resources to adaptive schemes of growth, reproduction, and survival at the level of the colony. Opportunities and limitations on colony resource-allocation emerge from the biology of worker honey bees (female helpers), in which genes that affect individual lifespan have pleiotropic effects on nutrient sensing and sensory physiology, behavioral biases for care-giving and foraging, and on preferences for collecting food-resources rich in protein (pollen) or carbohydrate (nectar). In terms of proximate mechanisms, regulatory connections between worker biology of aging, socio-behavioral plasticity, nutrient sensing, and biases of individual resource-acquisition and -transfers are supported by functional genomic research on insulin-like/TOR (target of rapamycin) pathways and vitellogenin/juvenile hormone signaling, as well as studies of metabolic resilience and brain function. In terms of ultimate explanations, the flexibility of honey bee senescence may be best accounted for by theories of aging that consider how natural selection for survival can be conditional on patterns of intergenerational care-giving and food-sharing.

SUCCESSFUL AGING AND SUSTAINED GOOD HEALTH IN THE EUSOCIAL MAMMAL, THE NAKED MOLE-RAT

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Naked mole-rats (*Heterocephalus glaber*), like wasps, ants and honey bees are eusocial, living cooperatively in large colonies with only a single breeding female. These mouse-sized mammals are the longest-living rodents known exhibiting a maximum lifespan of 30 years and therefore living 5 times longer than expected on the basis of body size. Their extended longevity and lower extrinsic mortality, like that of the eusocial insects, is correlated with group living in protected habitats. Reproductively active queen bees, ants, termites and mole-rats live longer than non-breeding individuals even though they partition a large proportion of available energy into reproduction rather than somatic maintenance. Unlike the eusocial insects, non-breeding naked mole-rats retain their ability to breed throughout their long-lives, yet non-breeders are sexually monomorphic and show pronounced differences in bone and neural traits from those of breeders. Very old animals (>28 years), like humans, show signs of age-associated pathologies including sarcopenia, osteoarthritis and cataracts as well as the accumulation of lipofuscin pigments and, like younger animals, show no signs of tumorigenesis. Indeed, for at least 80% of their long-lives naked mole-rats maintain normal activity, body composition, reproductive and physiological functions and show no obvious age-related gradual increases in mortality rate. As such their long lifespan is attributed to sustained good health and pronounced cancer resistance. Clearly physiological and biochemical processes in this species have evolved to dramatically extend both their good health- and lifespan, our quest is to determine the mechanisms they employ to retard the aging process.

SENESCENCE IN A NATURAL POPULATION OF THE WESTERN HARVESTER ANT, *POGONOMYRMEX OCCIDENTALIS*

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Information on the existence and significance of senescence of colonies of social insects in natural populations is largely absent due to the rarity of long-term studies, and the relative longevity of many species. In this talk we discuss senescence of colonies (that is, senescence of the queens) of the western harvester ant, *Pogonomyrmex occidentalis*, from our long-term study population. At the level of the population, we present evidence concerning mortality and reproductive senescence. At the level of individual colonies we examine the performance of colonies in the years preceding their deaths. From the initial cohort of 1000 colonies we examine the differences between those that have died and those that remain alive to determine the hallmarks of colonies that are going to die, and how far in advance of death we can detect differences.

KIN-SELECTED CONFLICT AND THE EVOLUTION OF LIFESPAN AND AGEING IN *BOMBUS TERRESTRIS*

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Recent extensions to the evolutionary theory of ageing have incorporated processes of social evolution likely to affect ageing. One hypothesis stemming from this is that social systems with intergenerational resource inheritance are predicted to exhibit kin-selected, parent-offspring conflict over the timing of inheritance. Preliminary evidence exists for such kin-selected conflict in the bumble bee *Bombus terrestris*. Workers have been reported to lay eggs in their natal colony at high frequency only following the death of their queen. Worker harassment of the queen, often followed by worker reproduction, has also been widely observed. Fitness optima, in regards to the timing of resource handover, differ between queens and workers in colonies of *B. terrestris* as the two parties are differentially related to worker offspring. Workers are predicted to harass and even kill their queen when they stand to obtain more inclusive fitness from worker reproduction than from reproduction by the queen. Those workers that initiate harassment are also predicted to go on to reproduce after the queen's death. Also, when the queen's fecundity drops to the point where she gains more inclusive fitness from allowing offspring to reproduce rather than continue to reproduce itself, the queen is predicted to rapidly age and die. Here, we present preliminary results from an ongoing research programme designed to test these predictions in *B. terrestris*. Specifically, we have tested whether the level of worker reproduction is simply a function of the duration of the queenless phase in the colony cycle, or whether it covaries with queen quality. Results from this experiment will help us construct a realistic inclusive fitness model for the effects of worker interests on queen lifespan and hence understand social influences on ageing more completely.

ANTIOXIDANT PRODUCTION AND LIFE SPAN IN ANTS

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Division of labor in social insect colonies is manifested in the morphology and physiology of caste members. In addition, natural selection has refined the intrinsic life spans of castes - most notably in ants, where queens can live more than 20 years, workers only year or two, and males die shortly after mating. Apart from few known examples, the female castes have genetically identical background and also the haploid males share all genes with females, suggesting the variation in life spans may arise from differential gene expression. One hypothesized group of genes at the bottom of life span determination is those producing antioxidants. Antioxidants, according to Free radical theory of ageing, hinder senescence by neutralizing oxidative agents produced by the cell metabolism. Further, despite the males do not invest in somatic maintenance, they are expected to invest in sperm viability and protect it from agents that cause their rapid somatic deterioration. Here we investigated whether antioxidant production correlates positively with caste life span and whether males produce more antioxidants in their germ line compared to somatic tissue. We compared expression of four antioxidant genes (sod1, sod2, sod3, and gst) in virgin and established queens, workers, and males of the ant *Formica exsecta*, using quantitative real-time PCR. In addition, to analyze tissue specific expression, we dissected somatic and germ line tissues from a subsample of males and females. Antioxidant transcripts were quantified both when individuals were at rest and after exposing them to oxidative stress, applying pure oxygen gas or paraquat, to study if variation in their production is conditional to stress. Finally, we assayed the amount of antioxidant proteins catalase and glutathione in all castes. Our results demonstrate significant differences in expression of many antioxidants between castes and tissues, and shed light to whether they can account for life span determination.

SUBCASTE AND AGE-RELATED PATTERNS OF BEHAVIORAL DEVELOPMENT AND NEURODEGENERATION IN THE ANT *PHEIDOLE DENTATA*

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Social insects provide powerful systems to test evolutionary theories of aging and examine neurophysiological underpinnings of behavioral development and senescence. Selection for lifespan and neural processes underscoring task performance and senescence can vary widely among worker subcastes, providing opportunities to examine functional neuroplasticity. As workers age, brains undergo synaptic restructuring and neuropil growth, and brain and behavior are likely to decline with advancing age. We investigated neurodegeneration in minor and major workers of the ant *Pheidole dentata* using immunohistochemistry and confocal microscopy to visualize and quantify neural apoptosis in different brain regions. We found that the number of apoptotic cells in minor worker brains increased with advancing age. Minor and major workers were assayed for their ability to nurse, follow chemical trails and provide defense to examine how senescence affects task performance. Subcaste-specific associations between behavioral development, neural plasticity, and ageing provide a nexus for understanding how selection shapes brain evolution according to caste and task specialization in ants.

Poster Presentations

Patterns and processes of aging and lifespan: how special are social insects?

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13-1 Early olfactory experience modifies neural activity in the antennal lobe of a social insect at the adult stage

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The antennal lobe (AL), the first olfactory centre of the insect brain, is organized in glomeruli. These neuropiles are well-defined globular structures that encode odor information in spatiotemporal patterns of activity. Whether and how such patterns are modified in the long term after an early olfactory experience (i.e. in the first days of adulthood) remains unknown. We used *in vivo* calcium imaging technique to measure the odor-evoked responses in the AL of 17-day-old honeybees which either experienced an odor associated with sucrose solution at the age of 5 -8 days or were left untreated. We found that precocious olfactory experiences associated to reward (i.e. 5 -8 days after emergence) enhanced the activity and the number of activated glomeruli in the long-term, i.e. at the age of 17 days. Furthermore precocious learning seems to modify the spatial response of the patterns. Such effects were not limited to the experienced odor, but generalized to other perceptually similar odors. Behavioral experiments under the proboscis extension responses (PER) paradigm revealed a similar trend in which the increased responses to the experienced odor extended to perceptually similar odors in treated bees. Then we show that early olfactory conditioning during young adulthood affects neural activity in the honeybee AL on a long-term scale (9-12 days) in two different ways: i) increasing the general activity of the AL and ii) modifying the spatiotemporal response of the patterns. Those changes might underlie a long-term olfactory memory for the early-experienced odor.

13-2 Effect of slave ratio on the survival probability of the slave-maker amazon ant *Polyergus rufescens* Latr.

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Many different types of relationships exist in ants. Besides competition, specific mutualistic and social parasitic relationships are quite frequent in these eusocial insects. Amongst these dulosis is a most intriguing form of social parasitism. A slave-maker ant species' colony usually attacks so-called slave ant colonies and steals their pupae. The emerging young slave workers accept the host ants as conspecifics. The exact role of the slaves differ in each slave-maker species, but primarily they act as caretakers of the host ant's brood, while they can also assist nest building or foraging activities, even participate in raids sometimes. It is reasonable to assume that a certain optimal proportion of slaves should be present in a dulotic colony in order to function properly. Less is known about the effect of slaves on the lifespan of adult slave-makers, although usually slaves by feeding adult slave-makers could have some effect on the survival probability of adult slave-makers. Could there be an optimal ratio of slaves from the point of view of adult slave-maker lifespan? *Polyergus rufescens* is a common obligatory slave-maker ant species in Europe. We analyzed the effect of slave ant's ratio on the survival probability of *P. rufescens* in the absence of brood. Monospecific (controls) and heterospecific groups of same size were set up in laboratory conditions. Five different heterospecific groups were established with increasing proportion of slaves: 20%, 40%, 50%, 60% and 80%. The results show that slave-makers lifespan significantly increases with the increasing proportion of slaves until 50-60%. Contrary to expectations group composition seems not to affect the survival probability of slaves. Nevertheless increasing feeding demand from the part of slave-makers would suggest otherwise.

13-3 DIFFERENCES IN MALE ACCESSORY GLAND PROTEINS OF THE ANT SPECIES *CARDIOCONDYLA OBSCURIOR*

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In the ant species *Cardiocondyla obscurior*, two different male morphs occur. Previous studies have shown that queens that mated with a winged male live longer than queens that mated with an ergatoid, wingless male. Under normal environmental conditions, colonies regularly produce wingless males, which mate with queens that stay and reproduce in the nest or found new colonies by budding. The winged morph is produced only when conditions deteriorate. Then, young queens are presumably forced to disperse to found new colonies without the help of workers. Winged males might assist them ultimately by prolonging their lives, so that they are able to establish new societies solitarily, away from the mother nest. Proximately, accessory gland proteins, which are transferred during copulation, are likely to play an important role in the life-prolonging effect for queens mated with winged males. In solitary insects like *Drosophila melanogaster*, seminal fluid proteins are well known for their impact on the lifespan of the females. However, little is known about the role of accessory gland proteins in ants. We therefore investigated the differences of the protein content of the accessory glands of the two occurring male morphs in *Cardiocondyla obscurior* by 2D- gel electrophoresis.

13-4 MORIBUND ANTS MAY CEASE TO AVOID ILLUMINATED ZONES

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A growing number of studies devoted to factors involved in the mediation of behaviour of social insects reports behavioural modifications induced in response to shortened life expectancy. These modifications include increased probability of switching to extranidal activities and of participation in risky tasks. Moribund, wounded and infected individuals are also known to leave their nests to die in isolation. We will report a short review of these recent findings including our own data demonstrating that workers of a nocturnal ant species *Campontus melanocnemis* show increased photopositivity during the last few days preceding their death. Minor workers of *C. melanocnemis* were reared in controlled illumination conditions from the stage of an egg. Their illumination preferences were then tested by housing small groups of individually marked ants in H-shaped dark-light choice nests consisting of two test tubes equipped with water reservoirs and connected by a narrow passage. One of the tubes was kept in darkness and the second one was exposed either to 12:12 LD or to constant white light. The ants which survived until the end of the experiment (during 40 or 80 days) were as a rule photonegative, but moribund workers showed increased photopositivity during the last few days preceding their death. Their photopositivity was not related simply to their inability to leave the illuminated zone, as it was observed also in the subset of moribund workers which remained active during the last two days of their life. We also compared the survivorship of ants kept in constant darkness and at 12:12 LD. Exposure to illumination did not lead to increased ant mortality, which implies that increased photopositivity of moribund *C. melanocnemis* was the effect of physiological changes preceding their death, and not the cause of their mortality. Our findings provide thus an important cue concerning proximate causal factors underlying behavioural modifications observed in moribund ants.

13-5 Demography of social insects; understanding the evolution of aging phenotypes

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While the extraordinary lifespan of queens in eusocial societies has been well studied, the question of why workers' lives are generally shorter has been neglected. This is linked to one basic issue in sociobiology: How are the resources in of a colony allocated into colony growth and reproduction? Here I present a theoretical optimization model that uses the hierarchical trade off within insect colonies to explain how different aging phenotypes could have evolved. The model points to the significance of two factors. First, any investment that would generate a longer intrinsic lifespan for workers is lost if the individual dies from external causes while foraging. As a consequence risky environments shall favour the evolution of workers with a reduced lifespan. Second, shorter lived workers require less investment leaving more for the rest of the colony. Benefits of a reduced investment into workers should be increasing with colony size. A comparative study using lifespan and group size data across eusocial taxa reveals that the difference in life span between queen and worker life span is more pronounced with increasing group size.

13-6 Maintaining the brain's functional integrity in long-lived honey bees - possible candidates

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Worker honey bees (*Apis mellifera*) display a flexible but extreme bimodal distribution of longevity patterns. Nest bees during winter (diutinus) can outlive their sisters in summer by several months. Yet, in comparison to hibernating animals, little is known about how brain function might be affected by the honey bee's winter stage. We asked, whether and how functional integrity of the honey bee brain can be retained during winter. Behavioral integrity, as measured as learning ability and sensory responsiveness, was assessed in bees that were sampled throughout Norwegian winter. Contrasting these bees from outside locations with bees from an artificial flight room, we assessed possible effects of behavioral state on learning ability. Potential determinants of functional senescence were examined by screening for proteins involved in longevity regulation. In long-lived winter bees no decline of learning ability and sensory responsiveness was detected. In contrast, when the winter stage was experimentally disrupted, declined learning function was observed 15 days after foraging was initiated in the flight room. We next show that manipulating the social environment by reducing brood load can counteract the decline of the measured brain functions, even after outwintering. As an intrinsic route for maintained brain function despite old chronological age, our data suggests an unexpected localization within the brain for proteins involved in sib care and aging regulation.

13-7 Gene expression of mated, sham-mated and virgin *Cardiocondyla obscurior* queens

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Mating has a positive effect on the life span of *Cardiocondyla obscurior* queens: mated queens and queens mated with sterilized males ("sham-mated") have similar life spans, despite sham-mated queens laying significantly less eggs than mated queens (Schrempf et al. 2005, Curr Biol 15: 267-270). This contradicts hypothesized reproduction-correlated age-enhancing effects (i.e. Vitellogenin). We investigated genome-wide gene expression patterns in response to mating at two time-points using the *Solenopsis* microarray. Our study dissects the effect of mating and reproduction and points to conservative mechanisms involved with somatic maintenance.

13-8 CAN THE HONEY BEE GUIDE INSIGHTS INTO AGE-ASSOCIATED FUNCTIONAL DECLINE?

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The cognitive functional decline that accompanies senescence and the mechanisms that underlie neuronal aging are of great biomedical interest. Aging research has shown that lifespan extension is possible in many organisms, but enhanced longevity coupled with declined brain function is an undesirable combination that warrants further investigation. Research aimed at enhancing health span will require the use of systems such as the honey bee, a neurobiological model and a species that, like humans, lives in social groups. Our study aims to increase understanding of how neuronal metabolic biology can affect learning and memory in aged animals. To achieve insight, we examined the gustatory responsiveness and associative learning performance of honey bees under hyperoxia, an experimental treatment known to shorten lifespan and increase cellular patterns of dysfunction characteristic of aged organisms. As in previous studies conducted on solitary organisms like *Drosophila* and *C. elegans*, hyperoxia reduced lifespan in honey bees. Further, to test whether observed patterns of oxygen-induced frailty like high mortality and learning impairment can be attenuated, we fed bees a therapeutic agent, Resveratrol, which is known to extend lifespan in yeast, roundworms, fruit flies, and mice. Consistent with previous findings on other organisms, we found that Resveratrol lengthens lifespan in the honey bee. However, under conditions of increased oxidative stress, the lifespan extension response of Resveratrol is abolished. Additionally, Resveratrol alters gustatory responsiveness, a metric of sensory sensitivity that usually remains constant throughout life, but it does not affect olfactory learning performance in honey bees. Further work will be aimed at examining a potential Sirtuin-dependence for honey bee lifespan and gustatory responsiveness.

13-9 REMAINING LIFESPAN DETERMINES DIVISION OF LABOUR IN SOCIAL INSECTS

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There is a strong relationship between longevity and division of labour in social insects. On one hand division of labour affects average longevity of workers. On the other hand expected longevities of workers determine optimal strategy of division of labour. According to mathematical models unhealthy workers, with shorter expected longevity, should start foraging at earlier age than their healthy nestmates. Moreover; at the onset of foraging remaining lifespan of healthy and unhealthy workers should be the same. The above optimal strategy can be achieved by simple regulatory mechanism which is based on variable response threshold. Workers with lower response threshold start foraging at lower level of a yet unknown stimulus. Presence of foragers reduces level of the stimulus in such a way that proportion of foraging workers remains similar. The response threshold of a worker is not fixed but decreases with time and it is strongly correlated with the remaining lifespan of the worker. Results of simulation model based on the above assumptions showed that the simple mechanism presented here can explain temporal polyethism, earlier foraging of unhealthy workers and reversal of foragers to tasks performed earlier in life in absence of nestmates with longer remaining lifespan. It can be predicted that the unknown stimulus determining onset of foraging should be strongly correlated with remaining lifespan of workers.

13-10 ALTRUISTIC COLONY DEFENSE BY POST-REPRODUCTIVE FEMALES IN A SOCIAL APHID

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Recent studies have suggested that extended post-reproductive lifespan will evolve when indirect fitness benefits from a post-reproductive altruistic behavior are greater than the direct fitness benefits from continuing reproduction. Post-reproductive altruism is expected to occur in aphids partly because there is no kin-selected conflict over reproduction within the clones. Here we show the evidence of a post-reproductive altruism in the social aphid *Quadrartus yoshinomiyai*, which forms completely closed galls on the primary host plant. In the gall, a gall founder parthenogenetically produces viviparous offspring, which develop into wingless adults. At maturity, the gall forms an exit hole. Winged adults, which are born from the wingless adults, escape from the open gall via the exit hole and migrate to the secondary host plant. Wingless adults of *Q. yoshinomiyai* discharged adhesive waxy droplets from their abdomen when disturbed. In the field, we found that some wingless adults stuck to natural predators with the droplets, whereby the predators were impeded. Wingless adults clustered around the exit holes of the galls, and removal of them had a negative effect on repelling the predators. The defending adults contained no mature embryo in their abdomen. Field sampling and laboratory rearing showed that wingless adults ceased reproduction before galls open and survived extended periods within the open galls. The abdomen of wingless adults in open galls was filled with the waxy droplets. These results suggest that the wingless adults accumulate waxy substance for colony defense in preparation for increasing predation pressure after galls open, and contributes to their indirect fitness benefits. Our results suggest that the presence of an age-related trait for altruistic behavior promotes the evolution of post-reproductive altruism in this social insect via kin selection under natural selection acting through predation pressure.

13-11 PRODUCTION OF FREE OXYGEN RADICALS AND AGEING IN THE ANT *FORMICA EXSECTA*

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According to the Free radical theory of ageing, senescence of tissues and individuals is caused by oxidative damage from by-products of energy metabolism. Mitochondria are the most important natural source of reactive oxygen species (ROS), that cause damage to cell components and DNA. The link between ROS and ageing has been established in several model organisms and humans. These detrimental effects of ROS are controlled by production of antioxidant proteins. However, during high metabolic activity, ROS can overwhelm these defense mechanisms and create oxidative stress. Social insects such as ants provide an excellent study system for the mechanisms of ageing. The variation in life span between genetically similar individuals is remarkable: ant queens can live up to decades, but males die shortly after mating. In addition, unlike the males themselves, their germ line tissue must stay viable throughout the lifespan of queens. Consequently queens are predicted to invest more in somatic maintenance than males, and male germ line tissue should receive higher protection from oxygen radicals than their somatic tissues. We compared the production of ROS between males and females, and between somatic and germ line tissues in the narrow-headed ant *Formica exsecta*. Winged queens and males were put under oxidative stress by exposure to pure oxygen and forced to fly in flying mills. After the experimental stress we dissected the individuals and quantified the amount of ROS produced in somatic and germ line tissues. The results show that oxidative stress increases the production of free radicals in males, but not in queens. The male germ line tissue is not better protected from free oxygen radicals than somatic tissue.

Oral Presentations

Evolution of morphological novelty in social insects

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On the origins of novelty in development and evolution

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The origin of complex, novel traits is among the most intriguing, and enduring, questions in evolutionary biology. What are the genetic, developmental, and ecological mechanisms, and the interactions between them, that permit novelty to rise from within the confines of ancestral homology and variation? Here we briefly review existing definitions of novelty and how they help us to capture important components of what constitutes novelty in evolution, yet fail to provide good starting points for exploring the early stages of innovation. We then discuss how developmental-genetic perspectives may allow us to move beyond current roadblocks in our understanding of the origins of novel, complex traits. Specifically, we explore the roles of exaptation, developmental plasticity, and relaxed selection in providing developmental and genetic mechanisms by which novel traits may be initiated and elaborated upon within natural populations. We highlight results from recent studies across diverse insects, from horned beetles to fireflies and social insects. We conclude that in our quest to understand the nature of innovation, the nature of development deserves to take center stage.

Developmental mosaics, social buffering, and the evolution of novel castes in ants

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Ant castes are highly diversified but their evolution is poorly known. In addition to the ancestral polyphenism (winged queens and wingless workers), other castes occur in many species (ergatoid -permanently wingless-queens and soldiers). The scattered distribution of ergatoid queens across ants as well as their morphological heterogeneity indicate repeated convergent evolution. Such novel phenotypes may readily evolve in polyphenic social taxa due to both developmental and social reasons.Adult phenotypes consist of modules, i.e. subunits such as organs or body segments, that are more or less connected and imbricated. A module includes both the adult phenotypic character and the underlying developmental process. Queen and worker phenotypes in ants can be seen as assemblages of caste-independent modules (e.g. legs, antennae) and caste-specific modules (e.g. wings, ovaries, spermatheca).We suggest that ants can generate novel phenotypes by replacing some modules in one caste by modules normally expressed in the other caste, i.e. shuffling caste-specific modules. The resulting mosaics have higher chances of viability because their modules have already been tested by natural selection. This contrasts with monomorphic insects where novelty can only be produced through a slow and costly process of trial-and-error. We review morphological and developmental evidence that both ergatoid queens and soldiers are mosaics of winged queens and workers, although size regulation is also involved. Developmental accidents in solitary insects are often selected against after emergence because they cannot function. We suggest that, in contrast, insect colonies shield erratic queen-worker mosaics (intercastes) from immediate selection by providing a safe environment and cooperative nestmates. If intercastes can mate and reproduce, they can evolve into ergatoid queens by genetic assimilation. This is adaptive for reasons of colonial economy. Colonies thus act as incubators of novelty.

FLAPPING WINGS AND STRONG HEADS: NOVEL THORAX ARCHITECTURES IN QUEEN AND WORKER ANTS.

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The evolution of castes in social insects allows queen and worker phenotypes to be highly adapted for different functions within a colony. While the thorax of winged queens is under selection for flight, the thorax of workers is usually considered just a simplified wingless version of that of the queens. However, in comparison with other Hymenoptera, both social and solitary, worker ants show striking differences in the relative size of their thoracic segments that are hard to account for by failure of wing-muscle development alone. In flying Hymenoptera most of the thoracic volume is formed by the second thoracic segment (mesothorax) bearing the large wing muscles; conversely, in all worker ants the first segment (prothorax) is the largest. Furthermore, queen ants of various clades (e.g., Amblyoponinae, Ectatomminae, Myrmeciinae and Ponerinae) develop large prothoraces similar to workers' while retaining functional wing-muscles. We assessed the degree of queen-worker dimorphism and the relative size of thoracic segments in representatives from all major ant clades. We show that the relative size of the prothorax in winged queens is correlated with the type of colony founding behaviour: queens that forage outside the nest (semi-claustral foundation) have a large prothorax similar to workers', while queens that rear the first generation of workers without ever leaving the nest (claustral foundation) have a reduced prothorax. A comparative analysis of thoracic musculature revealed that external prothorax size is a direct reflection of the size and position of the muscles involved in head articulation. We suggest that workers requiring to hunt and carry prey need structural modifications in the prothorax to provide extra strength for the head and mandibles, a modification also acquired by semi-claustral queens. We conclude that the thoracic morphology of each caste in ants should be understood as a novel architecture shaped by selection.

SIZE AND SHAPE IN THE EVOLUTION OF ANT WORKER MORPHOLOGY

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Morphological evolution in the ant worker caste has been traditionally thought as being strongly influenced by selection for colony ergonomic efficiency. Although many studies have focused on the evolution of social characteristics in ants, little is known about the evolution of worker morphology at a macroevolutionary scale. In this study, we carry out a comprehensive analysis of worker morphological variation to investigate the tempo and mode of their evolution, focusing specifically on changes in size and shape. Datasets included both a large sample of species from many different ant genera (N=115 spp) and a set of New World *Pheidole* species (N=96 spp), with separate analyses for major and minor workers, for a total of 1650 measurements. The rate of size evolution was at least five times faster than the rate of shape evolution, with more than 80% of the variance in worker morphology being explained simply by changes in size. Modeling morphological evolution using maximum likelihood methods indicated that there was statistically significant phylogenetic signal in both size and shape and in all datasets. However, even though shape evolution showed a statistically significant deceleration over evolutionary time in the ant genera dataset, the opposite was found in *Pheidole*, with an accelerating rate of morphological evolution. This difference might have resulted from the separation of minor and major morphologies, which could have “freed up” previous limitations in the evolution of the worker castes due to conflicting biomechanical constraints. The results of this study underscore the potential of phylogenetic comparative methods on large datasets in uncovering important aspects of the morphological evolution in worker ants.

WORKER SIZE POLYMORPHISM IN BUMBLE BEES (*BOMBUS IMPATIENS*): EVOLUTION AND MECHANISM

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Bumble bee workers show a high degree of variation in body size within colonies. Worker sizes are approximately normally distributed, but the largest workers can have 10 times the body mass of the smallest. We show that this size polymorphism does not result from food limitation at the colony level, but is a consequence of the spatial distribution of nurse workers. Nurses spend most of their time in the colony center, where they feed larvae at higher frequency. These larvae turn into larger adults than those in the periphery. This is, to my knowledge, the first time an organizational mechanism for producing size variation among workers has been proposed in social insects.The function of size polymorphism has not been clearly established. Smaller workers perform different tasks than larger ones, although specialization of individuals is weak and they often switch tasks. Also, large workers appear to be able to perform small-worker-tasks with equal efficiency. In addition, larger workers tend to have longer potential lifespans than small workers. On the other hand, larger workers may be more costly to produce and maintain, may be more susceptible to starvation, and they may be more likely to reproduce selfishly. These factors have to be taken into account to understand the evolution of worker polymorphism in insect colonies.

THE EVOLUTION OF ERGATOID QUEENS IN THE ANT GENUS *CATAGLYPHIS*: THE ROLE OF NATURAL SELECTION AND CASTE FATE CONFLICTS

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Colony fission or Dependent colony founding (DCF) is a form of parental investment by which workers of a social insect colony accompany young queens during the foundation of a new nest. In ants, DCF is thought to have evolved from Independent colony founding by which queens fly away from their natal nest to found new colonies alone. In the genus *Cataglyphis*, this transition has induced a major loss of queen morphological specialization. Hence, the participation of apterous workers in the propagules of *C. floricola* increases foundation success but constrains dispersal distance with respect *C. emmae*, the closest known ancestor of *C. floricola* which disperse by ICF. This transition has selected for brachypterous queens (short wings) that are incapable of flying. We discovered that in addition to brachypters, *C. floricola* has a second completely wingless queen morph that is very similar to workers. These ergatoid queens are produced in excess before colony fission, but many of them are eliminated by workers upon emergence. We suggest that, contrarily to brachypters, ergatoids derive from selfish totipotent larvae that escape worker control by developing into small queens instead of workers at the cost of the mother colony.

Poster Presentations

Evolution of morphological novelty in social insects

14

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14-1 Regulation of the vitellogenin synthesis by juvenile hormone in *Ectatomma tuberculatum* (Formicidae: Ectatomminae)

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Vitellogenins are proteins precursor of vitellins, the main source of yolk to the insect embryo. They are synthesized in the fat body, released into the haemolymph and transferred to oocytes via receptor-mediated endocytosis. In social insects, the haemolymph vitellogenin levels vary between castes and had influence in caste determination, brood care and foraging tasks, longevity and immune response. The vitellogenin synthesis is regulated at transcriptional level mainly by juvenile hormone. However, this mechanism is poorly described in ants. Workers of the ant *Ectatomma tuberculatum* show active ovaries and produces unfertilized eggs used to brood feeding. In this ant, the ovary development and vitellogenin synthesis are important mechanisms to control the division of labor in workers, and this study evaluated whether vitellogenin synthesis may be regulated by juvenile hormone. Thirty day old workers received topically six µg of juvenile hormone III diluted in acetone. The control group received only acetone. Total RNA was extracted 24 hours after treatments, following quantification of vitellogenin synthesis by quantitative real time PCR using vitellogenin primer from *Solenopsis invicta* (GenBank CB252008). The vitellogenin mRNA level in workers 24 h after exposure to juvenile hormone was 90% lower than in control group ($p < 0.05$), showing that juvenile hormone inhibits vitellogenin synthesis in *E. tuberculatum* workers, likely found in bees. Our findings suggest that the same mechanism of vitellogenin regulation already occur in both bees and ants.

14-2 Beyond the muscle mass: the role of mandibular morphology for different cutting forces in grass- and leaf-cutting ants

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Selection pressure is expected to have increased the mandibular forces in herbivore insects that forage on hard grasses compared to consumers of tender dicots leaves. Therefore, morphological traits involved in mandibular force generation should correlate with feeding habits. We have comparatively studied the mandibular force exerted by *Acromyrmex* and *Atta* leaf- and grass-cutting ants, as well as its correlation with mandibular morphology and head size. Results show that *Atta* leaf- and grass-cutter ants possess similar head sizes, and generate comparable mandibular forces. By the contrary, *Acromyrmex* grass-cutters posses larger heads and exert more bite force than *Acromyrmex* leaf-cutters. Such differential force production goes beyond differences in head mass: for same-sized heads, *Acromyrmex* grass-cutters exert up to two fold more force than *Acromyrmex* leaf-cutters. Intergeneric comparisons show that for the same head mass, *Acromyrmex* grass cutters exert more bite force than any grass- or leaf-cutter *Atta*. This can be explained by the shorter and broader mandibles of *Acromyrmex* grass-cutters, which provide a mechanical advantage over *Acromyrmex* leaf-cutting ants, as well as over *Atta* leaf- and grass-cutters. The mandibular mechanical advantage, expressed as the ratio between mandibular basal width/mandibular length, represents the efficiency with which the force generated by the head muscles is transmitted to the mandibular tip. Hence, the combination of both factors, head mass and mandible morphology, explains better the bite force generated by the investigated *Atta* and *Acromyrmex* species than head mass alone. *Acromyrmex* grass-cutters exhibit therefore morphological traits adapted to the production of higher cutting forces compared to leaf-cutters, while in *Atta*, grass-cutters neither produce higher forces than leaf-cutters, nor possess differentiated morphological traits compared to leaf-cutters.

14-3 NUTRITIONALLY-DRIVEN DIFFERENTIAL EXPRESSION OF *UBX* GENE IS ASSOCIATED LEG DIPHENISM IN *APIS MELLIFERA* CASTES

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Diphenism in the honeybee *Apis mellifera* results from differential feeding of female larvae. Beyond physiological and behavioral differences the morphological is pre-eminent. The appendages such as the hind legs in workers, which are highly specialized pollinators, deserve special attention. The hind tibia of workers has an expanded bristle-free region used for carrying pollen and propolis, the corbicula, while in queens this structure is absent. A comprehensive analysis of gene expression during prepupal leg development indicated that 171 genes are differentially expressed, 68 of which are over-expressed in workers versus 103 in queens. Members of cuticle gene family are differentially expressed between castes, unsurprising in that the cuticle of the corbicula is visibly different in queens and workers. Genes involved in sensorial and nervous system development are also differentially expressed according to caste, possibly reflecting the distinct repertoire of behavior of the castes. *ubiquitin-specific protease7* gene, a Bithorax Complex transcription inhibitor, is expressed 18 fold more in queens. It possibly inhibits *ultrabithorax* expression that is down-regulated relative to workers. Additionally, *ultrabithorax* is expressed at higher levels in the prepupae and early pupae of workers than in queens. Furthermore, immunohistochemistry using FP6.87 antibody against *ultrabithorax* localized it in the tibia and basitarsus of workers and only in the basitarsus of queens settling a time/caste specific localization of this protein during late hind leg development. Differential nutrition reveals the phenotypic plasticity in the honeybees activating specific genes that coordinate the differential development of specific traits in both castes. (Funds: FAPESP)

14-4 MORPHOLOGICAL ORGANIZATION OF THE DORSAL PROTUBERANCE OF *LINEPITHEMA HUMILE* (MAYR) ANT'S LARVAE (HYMENOPTERA: FORMICIDAE)

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The Argentine ant *Linepithema humile*, is an important invasive species due to the levels of infestation that it can reach. However there is little information about its presence, histological organization and function of the dorsal protuberance which is found exclusively in their larvae. This study described it in *L. humile* through Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM), bringing information about this structure to the context of the social organization of *L. humile*. Semithin sections revealed that the epidermis of these larvae have cuticles covering the whole body, formed by a sequence of overlapping lamellas where the inner ones were thicker and presented lower electrondensity while the outer ones were thinner and highly electron dense. Pores or pore-like channels were not observed either in the apex or in any other region of the dorsal protuberance. A thick and acellular region composed by granular material was found under the cuticular layer. Out of this region, the flattened cells formed an epithelial layer. For the dorsal protuberance region, these cells becomes prismatic, and show significant thickening. These epidermic cells presented cytoplasmatic prolongations, as well as a great amount of lamellar rough endoplasmic reticulum. Under this epithelium, a concentration of fat body cells was observed, more numerous in the dorsal protuberance region. This study has indicated that the dorsal protuberance present in the first segment of *L. humile* larvae has no secretory function, since no pores were found. Scanning Electron Microscopy revealed that epidermic secretory cells located at the dorsal protuberance region changed from flattened to prismatic shape by the need of producing a thicker cuticle in this region than that from the rest of the larva. This fact allowed to conclude that in *L. humile* larvae the dorsal protuberance would have the function to make it easier for the worker ants to carry them within the colony.

14-5 Tergal glands in nasute mandibulate soldiers of Neotropical termites (Isoptera, Termitidae, Syntermitinae)

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Although exocrine glands play an essential role in the societies of social insects, their occurrence and function is still poorly known in termites, especially in Neotropical termites. In Isoptera, tergal glands have only been described for alate reproductives and young neotenics and have been considered sexual glands, as they play a role in courtship behavior. In the present study, we describe the occurrence and structure of tergal glands in soldiers of the Neotropical termites *Cornitermes cumulans*, *Procornitermes araujoi*, and *Syntermes* spp. Histological sections of the abdomen of these termites revealed secretory cells arranged similarly to an epithelium, under all tergites. In sagittal histological sections, cell clusters consist of 4-8 gland cells per tergum. The ultrastructure analysis revealed that the tergal glands of soldiers consist of only bicellular units formed by class 3 cells and canal cells. Secretory cells of class 3 exhibit well-developed smooth endoplasmic reticulum, abundant Golgi apparatus, elongated mitochondria, several electron-lucent vesicles, and electron-dense granules, which have paracrystalline structures in *S. nanus*. Scanning electron microscopy revealed the presence of several irregularly arranged pores on the surface of tergite, among which trichoid and campaniform sensilla are distributed. The role of tergal glands of termite soldiers might be linked to the production of allomones, similar to that observed in cockroaches, in which they are involved in defense.

14-6 The hows and whys of size variation in worker bumble bees (*Bombus impatiens*)

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Division of labour as a key feature of social insect societies is considered a major reason for their ecological success. Labour may be divided by body size, as in bumble bees (*Bombus* spp.), where there can be a 10-fold difference in mass between workers of the same colony. Larger workers tend to forage and smaller workers concentrate on intranidal tasks. However, much remains unknown about this important life history feature. For example, is worker body size resource-limited? Does the size distribution change as the colony ages? Lastly, what are the costs of making and maintaining differently-sized workers. We found that colonies maintained a normal size distribution throughout the cycle and with increasing population, despite unlimited resources. We quantified larval feeding in *Bombus impatiens* discovered a significant decrease in feeding rate and pupae size as one moved outward from the centre. Lastly, although there is evidence that the larger workers, who tend to forage, are in fact better at it, there is little evidence that smaller workers are superior nurses. What is the adaptive value of small workers? Here we report on the differential robustness to starvation of small and large worker bumble bees. The presence of small workers, and size variation in general, might impart robustness, not necessarily efficiency, to the system.

14-7 MANDIBULAR GLAND FROM WORKERS OF *MELIPONA QUADRIFASCIATA LEP.* AS A POSSIBLE SOURCE FOR CUTICULAR HYDROCARBONS

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Communication between individuals in social insect colonies employs a large variety of chemical signals that include the hydrocarbons. A quantitative and qualitative variation of these compounds in cuticular surface occurs between workers within the colony, being an important cue that allows individual and the behavioral roles characterization in the society. Bee's mandibular glands have been pointed out as sources of pheromones used in several kinds of communication; however their role in *Melipona* species is not completly clarified. The objective of this study was to characterize the gland cellular morphology and secretion composition variations and compare these parameters with changes in variation of the cuticular hydrocarbons profiles of newly emerged, nurse and forager workers. The results showed that the gland cell's ultrastructural features are diverse in the studied classes of workers and, as well as the mandibular gland secretion composition differ among the worker's task groups, while the cuticular hydrocarbon profiles are similar between newly emerged and nurse workers, but distinct from foragers. When the composition comparison is made, similarity was found between cuticular and secretion hydrocarbon types in all classes of workers, however when a statistical analysis was made taking in account the amounts of each type, the profiles are distinct between both cohorts of newly emerged and foragers workers. Only in the nurse workers the hydrocarbons profiles of cuticle and glands are similar. These results are discussed in order evaluate if the mandibular gland secretion contribution for the composition of the surface hydrocarbons.

14-8 ERGONOMICS OF LOAD TRANSPORT IN THE MEDITERRANEAN SEED HARVESTING ANT *MESSOR BARBARUS*

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Worker caste polymorphism plays an essential role in the organization of foraging in ants. In the species transporting loads individually in particular, it allows to extend the range of food items that can be brought back to the nest. Because of the allometric relationships that can exist between the different parts of the body however, the net transport efficiency (measured as the product of the speed of the worker by the weight of the load they carry, divided by the weight of the worker) is not necessarily the same for workers of different sizes and this may have important consequences for caste ergonomics optimization.We studied the ergonomics of load transport in workers of the polymorphic Mediterranean seed harvester ant *Messor barbarus* while transporting pieces of pasta of various sizes (2.8-128.8mg) in the field. The speed of the workers decreased significantly with increasing values of the load (measured in units of ant body weight) they carry, in the same way for different sized workers. The probability to carry or drag a load did not vary significantly with worker size but depended significantly both on the value of the load and on their higher linear dimension, but not on the interactions between these two characteristics. Net transport efficiency initially increased with the weight of the item transported, reached a maximum and then declined faster for major (>10mg) workers than for both media (5-10mg) and minor (<5mg) ones. Net transport efficiency did not vary significantly among workers of different sizes transporting loads equal to up to 12 times their own weight. Above this value however it was significantly higher in both media and minor workers than in major workers. This was not due to a any adaptation in the morphology of the former workers but rather to the fact that, when confronted with items >10 times their own weight, major workers had difficulties to move them, while both minor and media workers readily dragged them back to their nest.

14-9 Functional significance of and genetic mechanisms underlying extreme worker size polymorphism in *Pheidole* ants

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Almost all *Pheidole* ants have a dimorphic caste system consisting of only one minor and one major worker caste. Only seven New World species have a worker caste system consisting of minor workers and a broad range of major worker sizes (soldier-polymorphic). A detailed study of two of the seven soldier-polymorphic species (*P. obtusospinosa* and *P. rhea*) and the dimorphic *P. spadonia* revealed that these three species had drastically different worker size frequency distributions. Further investigation of the foraging and defensive roles of major workers showed that each species maintained a specific set of major worker sizes for different purposes. All majors of the three species were involved in processing large food items into smaller particles. Small majors of *P. obtusospinosa* carried small pieces of food back to the nest while none of the majors of the other two species exhibited this behavior. Also, small majors of both *P. obtusospinosa* and *P. rhea* showed much greater involvement in food processing than their large majors. However, field observations of *P. obtusospinosa* revealed that their large majors were the only workers involved in head-blocking at the nest entrance to combat army ant raids. In contrast to the two soldier-polymorphic species, majors of the dimorphic *P. spadonia* were the most involved in guarding food sources and the nest entrance. A genetic component involved in the production of the different worker sizes needed for colony function was also investigated in two species: *P. rhea* (broadest size range) and *P. spadonia* (one of the most narrow size range). Results showed that 90% of the queens of the dimorphic *P. spadonia* were singly-mated while 90% of the queens of the soldier-polymorphic *P. rhea* mated two to four times. In the multiply-mated *P. rhea*, there was potential for patriline and/or matriline bias towards the production of particular worker sizes, which could contribute to the maintenance of worker morphological diversity.

14-10 Structure and composition of trap-jaw ant mandibles

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Ant mandibles have evolved to perform a wide variety of functions, including foraging, food processing, defense, brood care and nest excavation, which is reflected in their morphological diversity. Some of the most remarkable mandible adaptations are possessed by trap-jaw ants, whose elongated mandibles rapidly snap shut to catch fast or dangerous prey and experience forces 500 times the ant's body weight. To explain the structural stability of trap-jaw mandibles, we examined the cuticular organization and elemental composition of *Odontomachus brunneus* using light and scanning electron microscopy. We found that the distal mandibular teeth of *O. brunneus* have high amounts of sclerotized exocuticle and are reinforced with the metal zinc. For comparison, we also examined the mandibles of ants with radically different mandible morphology: the herbivore, *Atta texana*, and the omnivore, *Linepithema humile*. While both of these species also have zinc-reinforced teeth, preliminary data indicate that *O. brunneus* and *A. texana* have relatively thicker mandibular teeth than *L. humile*.

14-11 MORPHOMETRIC STUDY OF INTERCASTES IN THE ANT *TEMNOTHORAX NYLANDERI*: VARIABILITY OF QUEEN-WORKER MOSAICS

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In ants, winged queens and wingless workers are distinct castes based on the coherent expression of characters such as ocelli, flight thorax and reproductive organs: queens must fly, mate and lay many eggs, unlike workers. Despite development being highly canalized, mosaics of winged queen and worker, called "intercastes", appear irregularly across species. They are wingless but segments of their thorax are not fused as in workers, and their reproductive organs can be queen-like. Plateaux (1970) described in *Temnothorax nylanderi* a large series of intercastes exhibiting great variability in the expression of ocelli, thoracic structure, ovariole number, and spermatheca. All these were viable adults, but lacked a specific function. Little is known about the characteristics of intercastes, so we performed a morphometric study on 109 of these specimens, together with 48 dealate queens and 35 workers. Principal Component Analysis based on 9 measures of external morphology (head, thorax and gaster) indicated that most intercastes fall into either worker-like or queen-like categories. Similarly, size of thoracic traits tended to show bimodal distribution. In general, larger body size was correlated with the expression of queen characters such as ocelli, minute wings and thoracic segmentation. However, 9 % of specimens fell into "intermediate intercastes" whose morphologies greatly deviate from workers and winged queens. These intermediate intercastes reveal a loss of coherence among caste-specific traits. In intermediate-intercastes and queen-like intercastes, correlations between traits were low, suggesting that some traits became more independent in these categories. The morphological variability of intercastes gives insight into the rearrangement in caste-specific traits that may give rise to morphological novelties.

14-12 NEW STATISTICAL METHODS FOR THE STUDY OF MORPHOLOGICAL EVOLUTION IN THE ANT WORKER CASTE

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The evolution of ant worker castes has been a topic of interest for over a century. A crucial step in uncovering the factors driving caste proliferation is to understand the changes in trait covariation associated with the evolution of distinct worker castes. These changes have been traditionally investigated using simple biplots, in the tradition of Huxley and Teissier laid out in the 1930s. In this presentation, we introduce a set of statistical methods based on graphical modeling and the concept of conditional independence. These methods are valuable not only to graphically display differences in trait covariation, but also provide a statistically sound framework for hypothesis testing. We illustrate these methods in two contexts: the evolution of the dimorphic worker caste in the hyperdiverse *Pheidole* and the intracolony variation in worker morphology in the leafcutter ant *Atta sexdens*. Our results indicate that evolution of distinct worker castes is associated with an intricate interplay between dissociation and integration (i.e. increasing or decreasing trait covariance) that is much more complex that one could envision simply using traditional methods. Moreover, the obtained independence graphs can serve as an important basis for further mechanistic studies of worker developmental pathways and multivariate selection.

14-13 The influence of season and larval competition on caste fate in a fission-performing ant.

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In species that undergo colony fission, queens leave their nest with a group of workers which increases greatly their survival and success with respect to species undergoing independent colony founding. As a consequence, colonies are expected to invest massively in workers and produce only a few queens. This occurs in *Aphaenogaster senilis* in which queens are mostly produced in queenless (QL) or in large queenright nests. Although previous studies have shown that only 0.07% of the larvae develop as queens in nature; the underlying functional mechanisms that control such fine-tuning in the queen production are badly understood. We present a series of experiments that test the role of environmental conditions in determining the caste of diploid brood. First, groups of 200 QL workers were provided with an increasing number of larvae between 2 and 40. On average, 2.3±1.67 queens were produced per group, independently of the initial number of larvae. Consequently, as the number of larvae raised together increased, the probability of a given larva developing into a queen decreased, suggesting competition between the brood. However, we found that the presence of larvae already oriented toward queen development had no effect on the probability of queens production. In a second set of experiments, we showed that, interestingly, the production of queens varied greatly with the season and between colonies. Queen production in QL groups was very low in winter in spite of controlled laboratory conditions and increased in spring and summer. These data suggest that in QL condition, the production of new queens from undifferentiated larvae is influenced by numerous environmental factors, and we hypothesized, from the temporal variation of queens production, that caste determination may be affected by maternal effects.

14-14 Evolution of nesting habitats and metapleural glands in ants

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The metapleural gland is a unique and complex structure located on the propodeum of ants. Given its function in immune defence against microbes, it has undoubtedly played an important role in the ecological success of ants by enforcing colony hygiene and preventing diseases. Although this gland has an important antimicrobial function, it has been lost several times. It has been proposed that these losses are due to a shift in nesting habit, i.e. ants living in trees can afford to loose the metapleural gland because this environment harbours fewer microbes. Here we test this idea, the 'Arboreality Hypothesis', by constructing a phylogeny for 48 formicine ant species using eight markers (nuclear and mitochondrial) and Bayesian tree building methods. Character mapping for the presence/absence of the gland and nesting habitat was carried out with SIMMAP including 1000 trees from the posterior distribution of the MCMC run. Our results show several independent losses and regains of the metapleural gland across the subfamily. We also find a significant correlation between the two traits, suggesting that the 'Arboreality Hypothesis' can indeed explain the pattern of presence/absence of the metapleural gland in this group of ants.

14-15 Behavioural regulation of monogyny among ergatoid queens in *Eutetramorium mocquerysi*, an ant from Madagascar

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Winged queens have been replaced by wingless reproductives in species belonging to all lineages of ants. In various amblyoponine and myrmicine genera (e.g. *Mystrium*, *Myrmecina*, *Ocymyrmex*), 'multi-purpose' ergatoid queens reproduce. These are produced in large numbers and do not disperse, hence they can be as numerous as workers in a colony. Only a few mate and lay eggs, while the others function as labourers. In *Eutetramorium mocquerysi*, colonies (N=18) consisted of 35±18 adults that were all similar in external morphology. However, dissections (N=655 adults) revealed the existence of two castes: ergatoid queens with six ovarioles and a spermatheca, and workers with two ovarioles and no spermatheca. There were more ergatoid queens than workers in most colonies. Only a single ergatoid queen was mated in each colony, and had active ovaries with yellow bodies. All adults were marked individually, including newly emerged callows. Daily observations indicated that one of the ergatoid queens was behaviourally dominant (specific posture, and performance of antennal boxing). This individual was always found to be mated and reproductive. The virgin ergatoids were either nurses or foragers, depending on age. Similarly, young workers behaved as nurses, and later became foragers. After removal of the reproductive, virgin ergatoid queens and a few workers attacked each other by gaster curling, biting and dragging. Eventually a new dominant differentiated, and she always belonged to the caste of ergatoid queens. Furthermore she was relatively young. When the original reproductive queen was re-introduced, she quickly recognized the new dominant (and vice-versa), which may be based on a fertility signal. Given that the only other species in this Malagasy genus (*E. monticelli*) has winged queens, this dramatic modification in the reproductive caste of *E. mocquerysi* seems an adaptation for dependent colony foundation (i.e. queens disperse together with nestmate workers).

14-16 Explosive behaviour in workers of the snapping termite *Neocapritermes taracua*: Is there anything ordinary?

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The genus *Neocapritermes* includes many species making a significant part of the soil-feeding termite diversity in Neotropical region. The most astonishing characteristic of some *Neocapritermes* species is a peculiar defensive behaviour consisting in abdominal dehiscence through body-wall rupture. Here, we investigate this mechanism in a large French Guiana species, *N. taracua*. The line of weakness is located at the mid-dorsal junction of thorax and abdomen. In this anterior region of the abdomen are also located groups of secretory cells (class 3 according to the classification of Noirot & Quennedey 1974) producing "blue crystals" (hard and fragile material, sky-blue in colour, and of clove-like shape) stored in pouches formed between posterior outgrowths of the metanotum and the first abdominal tergite. The "blue crystals" contain a major protein of 70 kDa, as well as a polar substance blue in colour; these are together responsible for the toxicity of the bursting liquid. In addition to these outside-stored crystals, acini of labial glands produce proteinaceous secretion by budding off basal parts of parietal cells (filled with secretion) to the hemocoel. When faced with an opponent, the *Neocapritermes* worker can ultimately burst, and the crystals then quickly (within seconds) dissolve in the hemolymph enriched in labial gland secretion. The resulting liquid is toxic for termite competitors and at the same time works as a sticky immobilizing agent. This defensive strategy is also clearly related to worker polyethism, as accumulation of defensive secretions from both sources increases during the last (and only) worker instar, and predominantly young, unarmed workers occur in the

nest, while old chemically loaded workers go out to forage. Last but not least, all workers of *Neocapritermes* are equipped with a frontal gland.

14-17 On the male larvae of *Camponotus rufipes* Fabricius (Hymenoptera: Formicidae)

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Camponotus rufipes Fabricius is a South American ant of the subgenus *Myrmothrix*, comprising 27 described species and subspecies. It is frequently found in humanized environments in Brazil. Some species within this genus are economically important species called carpenter ants, as they damage wooden structures, while others attack bee colonies. There are too few studies and no larval descriptions with *C. rufipes*. Knowledge about immature morphology is important for taxonomic and phylogenetic studies, and can help clarify many aspects of ant biology. The present study aimed at describing the male larvae of *C. rufipes* by light and scanning electron microscopy. We detected the existence of four larval instars, thus the larval description was based on 20 specimens of each instar. The larvae of *C. rufipes* shared the following traits with others of the genus: body shape pogonomyrmecoid, presence of 'chiloscleres' on labrum, ventral 'praesaepium', labial pseudopalps, ten pairs of spiracles, antennae with three sensilla, and camponotoid mandibles. The following traits were particular to *C. rufipes*: extreme diversity of hair types (branched hairs types with as many as 6 ramifications) and mandibles with 6 teeth. Body length was: 1.10-1.95 mm for 1st instars, 1.80-3.25 mm for 2nd instars, 2.57-3.90 mm for 3rd instars, and 3.62-9.71 mm for 4th instars. Diameter of 1st thoracic spiracle was: 0.010-0.013 mm in 1st instars, 0.013-0.015 mm in 2nd instars, 0.015-0.018 mm in 3rd instars, and 0.025-0.030 mm in 4th instars. Maximum width of head capsule was: 0.38-0.40 mm for 1st instars, 0.49-0.50 mm for 2nd instars, 0.59 mm for 3rd instars, and 0.74 mm for 4th instars. Length of mandibles was: 0.080-0.100 mm in 1st instars, 0.120-0.140 mm in 2nd instars, 0.160-0.200 mm in 3rd instars, and 0.240-0.280 mm in 4th instars. All bodily dimensions proved reliable for instar separation.

14-18 Caste morphometrics and wing loading in *Dolichovespula maculata* (Hymenoptera: Vespidae)

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The demands of flight impose constraints on body form in flying animals. A key parameter is wing loading, the ratio of body weight to wing area. Interpecific variation in this has been intensively studied in birds. The few studies with bees and other insects have shown the same general compromise between geometric similarity and constant wing loading at different sizes. It remains unknown whether the same rule applies within species. *Dolichovespula maculata* is a social wasp in which females are very variable in body size, while queens and workers differ only statistically in form. Males are also relatively variable in size. We measured seven parameters in 150 females and 50 males: fresh and dry weight, length and width of the forewing, length of the hind tibia, head width, and mesoscutum length. Some ratios were isometric: a) wing length: wing width, b) wing length: tibia length, c) wing length: mesoscutum length, and d) fresh and dry weighs. In line with published interspecific comparisons, we predicted a slope of the line log wing length/log body weight between 0.33 and 0.50. In fact, the slope was significantly below 0.33. Unless larger individuals are internally denser - a very puzzling pattern - it appears that larger individuals have smaller wings in relation to overall body size. This suggests that wing length in these social wasps is constrained by the tight spaces inside the nest.

14-19 EXPRESSION AND FUNCTIONAL ANALYSES OF *DISTAL-LESS* RESPONSIBLE FOR SOLDIER-SPECIFIC MORPHOGENESIS IN NASUTE TERMITES

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Termite soldiers, the highly specialized defensive caste, show exaggerated species-specific morphologies. Soldiers of the termitid subfamily Nasutitermitinae possess a long and horn-like frontal projection (nasus) and an invaginated glandular structure in the head (frontal gland). During molt into the presoldier stage, which is the transitional stage between soldiers and workers, a developmental process specific to morphogenesis can be seen. It was shown that the structure forming the nasus (an imaginal disc-like structure) forms under the cuticle of worker heads just prior to molt into presoldiers, and the center of the disc-like structure (nasus disc) elongates in the distal direction at the time of presoldier molt. To understand the molecular mechanisms underlying nasus elongation and frontal-gland development, we focused on the roles of a regulatory patterning gene, *Distal-less* (*Dll*), during presoldier molt induced by JHA in *Nasutitermes takasagoensis*. Histological observations of the morphogenetic process showed that the nasus disc formed simultaneously with frontal-gland invagination after gut purge, comprising rapid epithelial cell proliferation. Real-time quantitative PCR analysis showed that *Dll* expression in the worker head was quite high just after gut purging, and at the beginning of the nasus-disc formation and frontal-gland invagination. Immunohistochemistry showed that DLL protein was localized in the developing nasus disc and frontal gland. *Dll* RNAi resulted in reduction or complete loss of the furrow structure of the nasus disc. Our results suggest that the regulatory patterning gene *Dll* is at least involved in nasus formation. Regulatory networks for limb formation, including *Dll*, may be co-opted to novel structures during the evolutionary process, acquiring soldier-specific morphology in termites.

Oral Presentations

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THE GENETIC ARCHITECTURE OF THE POLLEN HOARDING SYNDROME IN HONEY BEES

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The pollen hoarding syndrome is a suite of interconnected complex traits that has been discovered by studying selected honey bee strains but also applies more generally. In accordance with the reproductive ground plan hypothesis of social evolution, the pollen hoarding syndrome comprises complex social behavioral traits that are tied to reproductive physiology. We analyze a series of QTL mapping studies that have elucidated the genetic architecture of several aspects of the pollen hoarding syndrome. The resulting sets of QTL show a partial genetic overlap between all different traits that have been studied so far, supporting the reproductive ground plan hypothesis at the genetic level. In addition, the results indicate that even pleiotropic QTL for complex behavior can be robust, suggesting that the underlying genes may code for fundamental control modules such as the insulin/insulin-like signaling pathway. We describe follow-up studies of QTL candidate genes to provide examples how a further mechanistic understanding of the commonalities and differences of the various aspects of the pollen hoarding syndrome can be gained. The high recombination rate and large offspring numbers of social insects in general make QTL studies a particularly powerful approach since next-generation sequencing approaches now enable a resolution of segregating genetic variation at the single nucleotide level.

CONTROL OF SEXUAL DIFFERENTIATION AND BEHAVIOR BY THE *DOUBLESEX* GENE IN *DROSOPHILA MELANOGASTER*

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Our laboratory uses the fruit fly, *Drosophila melanogaster*, to study the genetic, developmental, and neural mechanisms that underlie sex-specific behaviours in higher animals. In particular, the elaborate courtship ritual performed by the male fly has provided remarkable insights into how the neural circuitry underlying sexual behaviour, which is largely innate in flies, is built into the nervous system during development, and how this circuitry functions in the adult. Innate behaviours refer to the actions of an animal that manifest themselves without prior experience, and thus by implication are genetically inherited. Yet how does gene expression control the development and function of the nervous system so that a gene's action influences some discernible aspect of behaviour? We are studying how the *Drosophila* transcription factor genes *fruitless* and *doublesex* act within the complex and highly organized network of transcription factors to orchestrate the developmental events necessary for sex-specific behaviours and physiology, and the broader lessons this can teach us about the mechanisms underlying the development of sex-specific neural circuitry.

FROM GENES TO SOCIETIES - HOW GENOMES CAN HELP US TO UNDERSTAND THE GENETIC ARCHITECTURE OF SOCIALITY

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The evolution and development of complex phenotypes in social insect colonies, such as colony recognition, caste determination or division of labor is best understood within an expanded mechanistic framework of Developmental Evolution. On the other hand, social insects offer a fertile research area in which fundamental questions of Developmental Evolution can be addressed empirically. I will briefly review the concept of gene regulatory networks (GRN) that aims to fully describe the battery of interacting genomic modules that are differentially expressed during the development of individual organisms and in our case societies. These hierarchical networks spanning different organizational levels from genes to societies should be integrated and incorporated into full gene regulatory network models to elucidate the evolutionary and developmental mechanisms underlying social insect phenotypes. The newly sequenced ant and bee genomes, that cover a wide range of social organization, will open a new and fertile research area to better understand the genetic and genomic changes associated with the evolution of sociality in a Developmental Evolution framework. I will present examples how the newly sequenced *Pogonomyrmex barbatus* ant genome has already helped us to identify potential modules of GRN involved in communication, caste determination and phenotypic plasticity.

GETTING DOWN TO THE NITTY GRITTY: USING RNAI TO EXAMINE THE ROLE OF SINGLE GENES IN BEHAVIOR

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In insects, the biogenic amines act as neurotransmitters, neuromodulators and neurohormones. In the honey bee (*Apis mellifera*), these molecules have been linked to behaviors such as onset of foraging, circadian rhythm, response to pheromones and learning and memory. The biogenic amines affect cells via G protein coupled receptors, and multiple receptor subtypes may respond to each biogenic amine. Thus, to understand how the biogenic amines modulate behavior requires analysis of the contributions of individual receptors. RNA interference has been used to knock down the expression of specific biogenic amine receptors via injection of dsRNA into the honey bee brain. The effects of knockdown of specific dopamine and octopamine receptors on behavior will be discussed, and the RNAi results will be compared with those of experiments using pharmacological methods to reduce biogenic amine signaling.

TRANSCRIPTOMIC AND CHEMICAL SIGNATURES OF DOMINANCE STATUS IN *POLISTES METRICUS* WASPS

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Dominance behavior in *Polistes* wasps plays a key role in the organization of these primitively eusocial societies. Using recently developed genomic resources for the paper wasp *Polistes metricus*, we investigated brain and ovary gene expression patterns associated with caste and reproductive status within the social dominance hierarchy. Via microarray analysis of dominant and subordinate foundresses, workers, and queens, we found differences in brain expression of several hundred genes related to metabolism, stress response, development, and neurotransmitter synthesis. Among the differentially regulated genes were several genes with roles in aggression in *Drosophila* and juvenile hormone signaling. In addition, a large subset of the transcriptome showed differential expression in the ovaries, with many genes exhibiting an expression pattern that tracked ovarian physiology, rather than behavioral state. Meta-analysis of our results and published studies on honey bees were used to determine the extent of overlap in gene expression patterns associated with honey bee and wasp social organization. In addition, we also investigated the potential role of chemical modulation of *P. metricus* social organization by surveying the chemical composition of cuticular hydrocarbons and three exocrine glands. Using the same individuals for which brain and ovary expression data were collected, we found differences in chemical profiles related to seasonality (cuticular hydrocarbons), dominance during colony founding (mandibular glands), and mating status (Dufour's glands). This wealth of both chemical and transcriptomic data on well-characterized set of individual wasps was also used to look for novel connections between chemical profiles and gene expression and within and between individuals.

A SYSTEMS STUDY OF WORKER BEHAVIOUR IN THE HONEY BEE

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We are utilizing a Systems approach to study the genetic basis of worker behavior in the honey bee. We crossed two honey bee subspecies that show heritable differences in several aspects of worker behavior, physiology and brain gene expression. We are identifying genetic loci that affect internal phenotypes (e.g. physiology and brain gene expression) as well as our ultimate behavioral phenotype, the age at onset of foraging. The dataset will allow us to construct causal networks indicating how genetic variation controls internal physiological and transcriptional phenotypes, which in turn affect worker behavior.

BEHAVIOUR AND GENE EXPRESSION ARE AFFECTED BY THE GENOTYPIC COMPOSITION OF SOCIAL GROUPS

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Honeybee workers can detect and remove diseased brood from their nest, thus reducing the maleficial effects of various parasites in the colony. This hygienic behaviour has a strong genetic component, as inferred by classical genetic experiments on hygienic and non-hygienic strains (Rothenbuhler 1964). Here we examine the effect of genotypic composition in social groups on gene transcription and hygienic behaviour. We established three independent bee lines of high (H) and low (L) hygienic performance and quantified hygienic behaviour by the number of opened cells harboring pin-killed larvae. We assayed group and individual hygienic performance of three genotypic compositions (100%H, 100%L, 80%L/20%H) in a group of 500 bees by infrared video analysis. The amount of opened pin cells was significantly increased in the 20%H and 80%L composition of what we have expected from pure H or L lines (chisquare test, p=0.015). This finding suggests that epistatic interaction among individual genotypes contribute to this non-additive performance of the social group. Individual behavioural analysis showed that in presence of the H bees more L bees performed this task, but not at a higher frequency (MW test, p=0.028). We also analyzed the transcription differences of hygienic worker brains in the different genotypic environments and describe the molecular differences associated with this epistatic phenotype. 76 individuals were assayed on a total of 132 whole-genome microarrays. We detected a list of 943 candidate genes that are epistatically regulated in bees performing hygienic behaviour in different genotypic environment. Combined, these findings suggest that the epistatic interaction of alleles present in different individuals strongly influence behavioural performance and the gene expression associated with it. Our findings provide direct evidence that the genotype composition of a group adds another regulatory level at which genes or phenotypes are expressed.

DUPLICATE WHOLE GENOME SCANS OF THE HONEY BEE REVEAL TWO QUANTITATIVE TRAIT LOCI ASSOCIATED WITH WORKER STERILITY

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One of the defining features of eusocial insect societies is reproductive division of labour, yet genes that limit reproduction in the worker caste - so called “genes for altruism” - have not yet been identified. In honey bees (*Apis mellifera*), a selected `anarchist' line exists, in which up to 40% of workers have activated ovaries, compared to less than 0.1% in wild-type strains. Four genetic loci linked to ovary activation have been previously identified in this line (Oxley, 2008).We will report the results of a second backcross experiment in which we characterize the mode of inheritance of anarchy, and identify candidate genes that regulate sterility in the honey bee worker. F1 anarchist x wildtype queens backcrossed to an anarchist drone produced colonies exhibiting up to 15% of workers with active ovaries, compared to 0% in the reciprocal wild-type backcross, thus indicating a recessive mode of inheritance. Workers from a single backcross to the anarchist line exhibiting either well developed ovaries (n=132), or no ovary activation (n=135) were genotyped at 193 genetic markers covering 99% of the honey bee genome. Seven loci were identified that co-segregated with ovary activation, two of which correspond with loci *OvA2* and *OvA4* identified in the previous study. Based on functional annotation, we have identified 3 and 9 candidate genes linked to *OvA2* and *OvA4*, respectively. Furthermore, one gene linked to *OvA2* was found to be differentially expressed between anarchist and wild-type workers in a previous microarray study. We therefore confirm the presence of two loci involved in expression of the anarchy phenotype, and will present the primary gene candidates for the regulation of worker sterility in the honey bee.

From genes to genomes to epigenomes: how do different phenotypes and behaviours in bees arise from the same genotype?

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Social insects offer special advantages in the analysis of epigenetic phenomena because they are renowned for their striking morphological and behavioural polymorphisms. The nutritionally controlled queen/worker developmental canalization in the honey bee *Apis mellifera* is currently the ultimate in developmental flexibility in any phylum. Despite their identical nature at the DNA level, the queen bee and her workers are strongly differentiated in their anatomical, behavioural and physiological differences and the longevity of the queen. The high level of intake of royal jelly during larval development markedly influences the epigenetic status of cells of an individual without altering any of the hardwired characteristics of the genome. As a result, two contrasting organismal outputs, fertile queens and non-reproductive workers are generated from the same genome. In addition to profound physiological and anatomical differences including brain architecture these two types of female bees also show striking behavioral divergence. Such extraordinary ability to produce contrasting morphological, reproductive and behavioural phenotypes from the same DNA genome remains one of the key, unresolved questions in biology; how do strikingly different phenotypes and behaviours occur with no conventional genetic changes? The discovery of epigenomic mechanisms in honey bees brings a fresh perspective to the study of social behaviour in this organism. I will review the recent progress in characterisation of brain epigenomes in queens and workers and their relevance to regulatory networks. I will argue that complex phenotypic traits, including behaviours, are not predetermined. Genes depend on input from the phenotype and influence behaviours in a probabilistic rather than deterministic manner. The honey bee system is poised to allow a transition from static molecular data to flexible epigenomes to sophisticated behaviours.

Poster Presentations

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15-1 Selective death among female brood of the fire ant *Solenopsis invicta*?

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Colony social organization in *Solenopsis invicta* is under strong genetic control. Colonies that contain more than 10% of workers with a *b* allele at the *Gp-9* locus are polygyne and accept multiple reproductive queens per colony, whereas colonies with only *B* workers are monogyne, headed by a single reproductive queen. Moreover, queen acceptance is associated to the queen's genotype at *Gp-9*. All polygyne reproductive queens are *Bb*, while *bb* queens die prematurely from intrinsic causes, and adult *BB* queens are culled by workers that carry the *b* allele. *Gp-9* thus is considered a rare example of a green beard gene: Workers carrying the *b* allele aid its transmission by selecting against queens that do not carry it. Genotypes of field-collected adult queens and workers have suggested that polygyne workers may also kill *BB* queen and worker brood. We test the hypothesis that a skew in *Gp-9* genotypes of female pupae is associated with the social form (and thus indirectly the *Gp-9* genotype) of nursing workers. We find significantly less than the expected percentage of *BB* queen pupae when reared by polygyne workers. This suggests that polygyne workers may be killing *BB* queen brood. Alternatively, *BB* queen brood may have higher intrinsic mortality than *Bb* queen brood, or *Gp-9* may be involved in caste determination. We also show that *Gp-9* genotype frequencies among worker brood are unaffected by the social origin of rearing workers. This suggests that the lower than expected frequency of adult *BB* workers sampled from polygyne colonies in the field may be due to factors including differences in life expectancy between genotypes, or sampling biases related to genotype specific division of labor.

15-2 Age- and behavioural-specific expression of vitellogenin genes in the fire ant *Solenopsis invicta*

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Understanding the proximate mechanisms underlying division of labour in eusocial insect societies is a major issue in sociobiology. Vitellogenin (Vg), is a yolk protein precursor that has been involved in the regulation of division of labour in honeybees. Contrasting with most oviparous species which have a single *Vg* orthologue, the red imported fire ant *Solenopsis invicta* possess three *Vg* paralogues (Vg1, Vg2 and Vg3). We tested whether these paralogues evolved caste-specific and division of labour-related functions. Our analyses revealed that the Vg genes display caste-specific expression with Vg1 being specifically expressed in workers and Vg3 in queens. Vg2 was expressed in both castes, but at much higher levels in queens. Interestingly, there was a reversed effect of age and behaviour on the expression of Vg1 and Vg2: Vg1 expression increased with age and onset of foraging, while Vg2 expression decrease with age and onset of foraging. Overall, these results suggest that that worker-specific Vg paralogues evolved specific non-reproductive functions related to division of labour in *Solenopsis invicta*.

15-3 HIND LEG DEVELOPMENT IN HONEY BEE, *APIS MELLIFERA*, LARVAE

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Apis mellifera caste differentiation is triggered by a switch in larval diets during the early stages of development, generating several morphological and physiological differences. The hind legs of workers present specialized structures for pollen collection manipulation. Herein we describe the histological changes ocurring during hind leg development in queen and worker larvae, in combination with an analysis of differential gene expression. The legs develop from imaginal discs which are ingrowths of the body wall resembling pockets of epidermis beneath the ventral thoracic cuticle. In the fourth larval instar, the imaginal disc epithelium has a multilayered appearance with cylindrical cells and spherical nuclei. The discs interior contains small myoblasts, the precursors of muscle cells. During the feeding stage of the last larval instar, the discs primarily grow, without exhibiting evident changes in epithelium structure. During the spinning stage differentiation commences, but tibia and tarsus cannot yet be distinguished as individual structures. At the end of this stage, the leg buds evert from their pockets and expands beneath the cuticle, gradually taking on the shape of adult legs. RNA extracted from the hind pair of imaginal leg discs of queens and workers in the early spinning stage was used to establish libraries of differentially expressed genes by means Representational Difference Analysis, a methodology comprising two consecutive rounds of suppression subtractive hybridizations and cloning and sequencing of differently expressed gene fragments. ESTs obtained from the respective libraries were analyzed by BLAST searches against a non-redundant database and mapped to the honey bee genome sequence. Functional information was obtained by Gene Ontology analysis.

15-4 THE EPIGENETICS OF HONEY BEE DEFENSIVE BEHAVIOR

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Selection at the group level is thought to be an important force in the evolution of social insects. In the honey bee, gene expression influences both group behavior and individual worker behavior, as well as division of labor. This has been demonstrated by the mapping of quantitative trait loci that influence the tendency of workers to perform specific tasks. Selection also acts upon individual reproductives within colonies. Differences in fitness consequences for drones versus queens for genes expressed in their progeny may select for imprinted gene expression – the silencing of alleles of particular genes depending on whether they were maternally or paternally inherited. Honey bees are promising models for looking at this “intragenomic conflict” because they have a working CG methylation system. Honey bees also are polyandrous and haplo-diploid, traits that may increase selection for imprinting. For example, a gene that increases the probability that an individual will become a reproductive should be more likely to show a paternal effect because it could provide an advantage to a particular subfamily in competition to be the next queen; paternally imprinted “sting genes” could influence the result of inter-queen combat. We found a strong paternal effect on honey bee colony stinging behavior, and also for individual stinging responses of worker bees in laboratory assays. We conducted a genome scan for allele-specific expression in reciprocal F1 workers to provide the first look at the extent of imprinting in the honey bee. These results provide a way to study the extent of epigenetic effects on the evolution of social behaviors and to identify the genes involved.

15-5 Sociogenomics of the ant circadian clock

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The circadian clock has emerged as an important molecular mechanism influencing the behavior of social insects. Previous studies have shown that the features of the circadian clock are associated with rhythmic behavior and division of labor in both honeybee and ant societies, species that represent two distinct evolutionary origins of eusociality. Here, we analyze the newly sequenced fire ant genome and describe the ant circadian molecular clock. The fire ant genome encodes orthologs to all of the core circadian clock genes (Period, Cryptochrome-m, Cycle, Clock, Vrille and Pdp1) with the notable exception of Timeless (Tim1). In addition, the C-terminal end of the ant ortholog to Cycle includes a highly conserved transactivation domain that is present in *Apis* and mammalian Cycle proteins, but is absent in *Drosophila*. A number of stability genes integral to clock function are also described for ants. Expression analyses of the core clock genes across a typical 12:12 light/dark cycle reveal correlations between fluctuations in clock gene expression and the daily behavioral rhythms of ants. Based on these findings we propose that the molecular clockwork in the ant is similar to the honeybee and *Nasonia*, suggesting that Hymenopterans may have circadian clocks that are more similar to mammalian clocks than to *Drosophila* and other insect clocks. Comparisons of the structure and function of molecular clocks from eusocial species will improve our understanding of how evolution has shaped properties of circadian clocks in social insects.

15-6 RNA interference in the termite *Reticulitermes speratus*: silencing of the hexamerin gene using a single 21 nt siRNA

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Termites express polyphenism, in that nymphs can differentiate into either the alate or the nymphoid form, which is one of the reproductive caste phenotypes (neotenics). Using RNA interference, we identified hexamerin in the termite *Reticulitermes speratus* Kolbe (Blattodea: Rhinotermitidae) by cDNA and N-terminal protein sequencing. We used a single 21 nucleotide siRNA fragment to silence the hexamerin gene in order to avoid off-target effects that could be caused by using long dsRNA or a mixture of siRNAs of various lengths (15 – 25 bp) prepared by digestion of dsRNAs (~500 bp). The siRNA injection treatment used to silence hexamerin caused moderate suppression of hexamerin gene expression in workers for 8 days after the injection, while hexamerin gene and protein expressions rose significantly for 2–9 days in the body section of nymphs. Promotion of nymph differentiation to nymphoid occurred with the siRNA injection treatment. These findings suggest a unique differentiation mechanism for the development of nymphs to the reproductive caste phenotype, nymphoid, caused by the elevated expression of the hexamerin protein.

15-7 ALTERNATE SPLICING OF CP2 TRANSCRIPTION FACTOR HOMOLOGUE CORRELATES WITH REPRODUCTIVE DOMINANCE IN HONEYBEES

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In eusocial insects female reproduction is typically restricted to the queen caste that is usually the only female caste with fully activated ovaries. The evolution of worker sterility has been plausibly explained by kin selection theory, and many traits have evolved to prevent conflict over reproduction among the females in an insect colony. In honeybees (*Apis mellifera* spec.) worker reproduction is controlled by pheromones of the queen and the brood as well as worker policing. However, workers of the Cape honeybee, *A. m. capensis*, can evade both queen and worker control and establish themselves as parasitic pseudoqueens in foreign colonies. This trait is facilitated by the ability to parthenogenetically produce diploid female offspring (thelytoky), to rapidly activate ovaries and to produce queenlike amounts of queen pheromones. All of these traits have been shown to be under the control of one pleiotropic recessive locus on chromosome 13. As transcription factors are known to control several traits within an organism, we studied the CP2 transcription factor homologue *gemini*, which is located within this locus. We screened that candidate gene for differential splicing and indeed found two exons altered during mRNA processing. Exon five on the one hand was assessed to be a cassette exon, whereas exon seven, which is part of the homologous CP2 DNA binding domain, contains an alternative 5´splice site. Moreover, the splice abundance of those exons correlates with both, the ovarian status of arrhenotokous workers as well as with different modes of parthenogenesis. Therefore and due to its location within the *th*-locus we conclude that *gemini* is part of a regulatory network controlling reproductive dominance in honeybees.

15-8 THE GENETIC ARCHITECTURE OF PHEROMONE RESPONSE AND OVARIOLE NUMBER IN WORKER HONEY BEES

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Individual variation in social behavior can increase the productivity and success of a group as well as play a role in maximizing individual fitness. However, the molecular mechanisms underlying individual behavioral differences in social groups have not been broadly examined. Honey bees (*Apis mellifera*) represent one of the best-studied models of social behavior, and provide an ideal system to explore these types of questions. Queens produce a pheromone in their mandibular glands (QMP) that regulates many aspects of worker behavior and physiology, including eliciting queen attendance by the workers in the form of a retinue response. There is substantial variation in attraction to QMP in adult workers which is significantly correlated with ovariole number - a trait strongly associated with reproductive potential. To characterize some of the genetic and physiological factors associated with individual pheromone response and ovariole number, we exploited several of the genetic and genomic resources available for the honey bee. Transcriptional profiling identified hundreds of transcripts that are organized into statistically-correlated gene networks associated with this response. Linkage mapping was used to identify quantitative trait loci (QTL) underlying variation in pheromone response and ovariole number. The results of these studies help to inform our understanding of how genetic and environmental factors can act at the individual-level to produce variation in a colony-level, interacting phenotype. Furthermore, these studies provide a basis to investigate the molecular mechanisms underlying cooperation and conflict between queens and workers in honey bees and other social insects.

15-9 Gene expression analysis in different developmental stages of the Asian subterranean termite *Coptotermes gestroi*

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Termites present caste polyphenism at several developmental stages. In the species, *Coptotermes gestroi*, developmental branches split after two larval stages, with the larvae evolving into nymphs or workers. Nymphs follow the reproductive line to develop into winged and eyed alates, or neotenic reproductives. Workers can remain as workers, undergoing sequential molts, or become soldiers through two successive molts, probably triggered by juvenile hormone. To find differentially-expressed genes, subtractive cDNA libraries were constructed from mRNA isolated from whole bodies of different castes and developmental stages of *C. gestroi*. This method generated a total of 3,749 sequences of appropriate standard quality that were clustered and assembled, revealing important genes. Even though most of the ESTs were not annotated because of the lack of sequence homology, genes that could be identified had their expression quantified by Real time PCR. Workers presented higher expression of transcripts encoding endo-β-1,4-glucanase (3.759± 0.7454; $P<0.05$), α-amylase (4.425 ±1.887; $P<0.05$), Hexamerin I (10.05±0.02612; $P<0.05$) and Ferritin (2.057± 0.5281; $P<0.05$). Vitelogenin was highly expressed by the alates (6.955±0.04880; $P<0.05$) and queens (1.537±1.135; $P<0.05$). This experiment confirmed, in part, the ESTs results, providing novel insights into previously uncharacterized aspects of *C. gestroi* polyphenism and caste-associated biology.

15-10 Characterization of two honeybee genes potentially involved in hexamerin sequestration during metamorphosis

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A remarkable event in insect ontogenesis is the storage of hexamerins in larval hemolymph for exploitation during the non-feeding pupal stage. Following metamorphic molt, hexamerins are sequestered by the fat body through endocytosis mediated by a receptor, which in some dipterans is regulated by ecdysteroids and by a 19 kDa protein, HP19. As a first step toward the understanding of the dynamics of storage and sequestration of hexamerins in *Apis mellifera*, we characterized two genes, *Amhp19*^{1} and *Amhp19*^{2}, potentially involved in this process. They were identified in the honeybee genome by BLAST searches using the *Corcyra cephalonica hp19* sequence as query. Specific primers were designed and used in RT-PCRs with RNA from fat body and gut. The complete cDNAs were obtained and confronted with the respective genomic sequences for identification of exon/intron boundaries. This *in silico* analysis revealed that both genes consist of four exons. Their predicted proteins contain the conserved glutathione S-transferase domains, 23.8 and 23.6 kDa, pI values of 8.8 and 8.4, and several phosphorylation sites. Transmembrane regions were not identified. The expression of both genes is developmentally regulated and tissue-specific, with a higher level of transcripts in the gut and visceral fat body than in parietal fat body. In the gut and visceral fat body, *Amhp19*^{1} expression is restricted to the 5th instar spinning larvae and intermediate pharate adults, with a very higher expression in the former. In both tissues, *Amhp19*^{2} is expressed from the 5th instar spinning larvae up to intermediate pharate adults, with little variation in transcript levels. Both genes were up-regulated in larvae treated with 20-hydroxyecdysone. As *Amhp19*^{1} and *Amhp19*^{2} transcriptional patterns and regulation are consistent with a function in the dynamics of hexamerin sequestration, we currently are using RNAi to investigate their roles in hexamerin mobilization from hemolymph to fat body during metamorphosis.

15-11 How ants switch jobs - Role of the *foraging* gene

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Social insects are spectacular examples of behavioral adaptations with individuals performing specialized tasks in the colony. However, little is known about the molecular basis underlying behavioral specialization and flexibility. Experimental studies in the ant *Pheidole pallidula* showed that the cGMP-dependent protein kinase, encoded by the *foraging* gene, plays a causative role in behavioral plasticity. By changing the task-specific stimulus (a mealworm to induce foraging or alien intruders to induce defense behaviors) or by pharmacologically manipulating PKG levels, we were able to change the task-specific behavioral responses. Moreover, we also found that the level of expression of the *foraging* gene is correlated with behavioral differences between young queens of the invasive ant *Solenopsis invicta*. The *for*-PKG molecule is thus emerging as a major player in regulating the flexibility of responses to social context. These results open up interesting opportunities to better understand the influence of genetic and environmental factors on social behavior and reproductive strategies.

15-12 Genomic analyses of social organization and viral infection in fire ants

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The red imported fire ant *Solenopsis invicta* was accidentally introduced into the USA in the last century from Argentina. Fire ants subsequently rapidly spread across the southern regions of the country, from Texas to Virginia. Both in their native range and in North America, fire ants have two modes of colony organization: monogyne (single egg-laying queen per nest) and polygyne (two or more egg-laying queens per nest. Colony social organization is completely associated with allelic differences at a single gene (*Gp-9*). Monogyne colonies contain only *BB* workers and accept only one *BB* queen, while polygyne colonies contain both *BB* and *Bb* workers and all queens have the *Bb* genotype.We are developing genomic resources to characterize the molecular mechanisms which regulate fire ant social behaviour. We have developed a microarray platform to probe expression of tens of thousands of genes. We are using this technology to monitor patterns of gene expression which are associated with queen presence and viral infection. Queens appear to produce a pheromone that promotes worker acceptance or aggression towards additional queens, controls sexual maturation of alates, and, in polygyne colonies, inhibits other queens' reproductive activity. Comparison of gene expression patterns across these groups will allow us to determine how the same social cue can produce such dramatically different phenotypes in individuals from different genotypes, castes, and physiological states. Thereafter, we screened for the prevalence of two viruses among queens collected in the field and compared gene expression among infected and healthy queens and workers. These studies will allow us to begin to elucidate the molecular mechanisms regulating viral pathogenicity and host-pathogen interactions. Furthermore, comparisons with gene expression profiles associated with different behavioural states will allow us to make predictions about the possible effects of these viruses on social behaviour.

15-13 AM-FRU, A GENE OF THE SEX DETERMINING CASCADE OF THE HONEYBEE, IS SEX-SPECIFICALLY EXPRESSED IN THE MALE PUPAL BRAIN

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With the help of genome sequence we identified the *fruitless* orthologue in *A. mellifera*, *Am-fru*. In *Drosophila* the *fru* gene encodes BTB-Zn finger proteins that function as a male-specific transcriptional regulator that specifies male sexual behaviour. We isolated from the honeybee a cDNA fragment spanning the BTB domain and extended this fragment by RACE experiments using RNA preparations derived from male and female pupae. Using 5' RACEs we isolated from males exon 1-2-4 sequences and from females exon 1-3-4 sequences suggesting that the transcript produced from the same promoter P1 is sex-specifically spliced. Our 3' RACEs revealed three non sex-specific 3' ends which are generated by the combination of different 3' exons to a common upstream exon through alternative splicing. The P1 derived male mRNAs encode , like in *Drosophila*, a male specific protein that contains a predicted BTB and alternative Zinc-finger domains. The female splice product includes exon 3 that introduces a translational stop codon terminating translation prematurely. Immunostaining shows sex-specific expression of Am-Fru protein in about 1800 neurons of the male pupal honeybee brain. Given the considerable similarity in structure and in sex-specific regulation of transcripts as well as sex-specific expression of Am-Fru protein in brain we speculate that *fru* is a conserved regulator specifying sexual behaviour in insects.

15-14 UNRAVELING THE PLEIOTROPY EFFECTS BEHIND THE GREEN BEARD

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The “green beard effect” requires that a single locus encode for three traits: a perceptible phenotype, the capacity to recognize this phenotype in others, and discrimination in favor of individuals with this phenotype. One of the few green beard loci described so far is the non-recombining chromosomal region (with haplotypes *B* and *b*) around the putative odorant-binding protein encoding gene *Gp-9* of fire ants. This locus determines the workers’ behavior: when the percentage of *Bb* workers is smaller than ~10%, workers always kill additional queens (monogyne form), whereas when the percentage is greater than 10% they readily accept additional queens but only those carrying the *b* allele (polygyne form). *Bb* and *BB* queens differ in many traits, including odour, amount of fat accumulated before mating flight and behaviour. To understand how the *Gp-9* region produces such varied pleiotropic effects, we used cDNA microarrays to compare gene expression between *Bb* and *BB* queens at different ages: 1 day, 11 days and >1 year post eclosion. In total, >1000 genes were differentially expressed between *Bb* and *BB* queens. Interestingly many genes encoding odorant binding proteins, chemosensory proteins, regulatory proteins, and transposons were over-expressed in *Bb* individuals. This study thus shows widespread differences between queens in monogyne and polygyne colonies associated with their *Gp-9* genotype.

15-15 THE FOUR HEXAMERIN GENES IN THE HONEY BEE: STRUCTURE, MOLECULAR EVOLUTION AND FUNCTION DEDUCED FROM EXPRESSION PATTERNS

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Hexamerins are hemocyanin-derived proteins that have lost the ability to bind copper ions and transport oxygen; instead, they became storage proteins. The current study aimed to broaden our knowledge on the hexamerin genes found in the honey bee genome by exploring their structural characteristics, expression profiles, evolution, and functions in the life cycle of workers, drones and queens. The hexamerin genes of the honey bee (*hex 70a*, *hex 70b*, *hex 70c* and *hex 110*) diverge considerably in structure. Bioinformatics search for motifs in the respective UCRs revealed six overrepresented motifs including a potential binding site for Ultraspiracle (Usp), a target of juvenile hormone (JH). The expression of these genes was induced by topical application of JH on worker larvae. The four genes are highly transcribed by the larval fat body, although with differences in transcript levels, but only *hex 110* and *hex 70a* are re-induced in the adult fat body in a caste- and sex-specific fashion, workers showing the highest expression. Transcripts for *hex 110*, *hex 70a* and *hex70b* were detected in developing ovaries and testes, and *hex 110* was highly transcribed in the ovaries of egg-laying queens. A phylogenetic analysis revealed that HEX 110 is located at the most basal position among the holometabola hexamerins, and like HEX 70a and HEX 70c, it shares potential orthology relationship with hexamerins from other hymenopteran species. Striking differences were found in the structure and developmental expression of the four hexamerin genes in the honey bee. The presence of a potential binding site for Usp in the UCRs, and the results of experiments on JH level manipulation in vivo support the hypothesis of regulation by JH. Transcript levels and patterns in the fat body and gonads suggest that hexamerins serve as storage proteins for gonad development, egg production, and to support foraging activity. A phylogenetic analysis revealed independent radiation in insect orders.

15-16 ANNOTATED TRANSCRIPTOME AND DRAFT GENOME ASSEMBLY FOR THE ARGENTINE ANT, *LINEPITHEMA HUMILE*

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The Argentine ant (*Linepithema humile*) is a global pest that affects native arthropod biodiversity and protects agricultural pests, yet few molecular or genomic resources are available to aid in its study. Here we report the first annotated transcriptome and draft assembly of the *L. humile* genome. The genome assembly has ~12x coverage, was generated using 454 and Illumina technologies, and represents 86% (215 Mb) of the complete genome (251 Mb). Preliminary analyses reveal that 98% of the core CEGMA protein functional domains are present in the genome, indicating that the current assembly covers most of the essential metabolic genes. The genome appears to encode complete RNA interference and de novo DNA methylation systems and numerous chemosensory proteins. Unlike the only other eusocial hymenoptera that has been sequenced (honeybee), *L. humile* has a wide diversity of retroid transposable elements similar to those found in the solitary jewel wasp genome. This resource provides the basic genomic information necessary to study gene pathways involved with pesticide resistance, aggression, kin recognition, and other facets of ant biology that lend to its extreme success as an invasive pest.

15-17 AGE AND TASK DEPENDENT *FORAGING* GENE EXPRESSION IN THE BUMBLEBEE *BOMBUS TERRESTRIS*

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The *foraging* gene, encoding a cGMP dependant protein kinase, has been found to be involved in age dependant division of labour in social insects. In contrast, task allocation in the bumblebee *Bombus terrestris* is mainly determined by size. Our results confirm the existence of a *foraging* homologue and a high conservation amongst insect PKGs. Quantitative real time PCR studies revealed a higher expression in foraging worker bees as compared to nursing bees. More importantly, in both nurses and foragers, *Btfor* expression decreases with age suggesting a role for PKG in memory and learning behaviour in addition to a regulatory function in division of labour. In a supplementary experiment, the possible effect of the neonicotinoid insecticide imidacloprid and the JH analogue kinoprene, both known to be harmful for bumblebees, was tested. Imidacloprid had no effect on *Btfor* gene expression, while the lower expression in kinoprene treated workers concurs with a simulation of the ovarian growth.

15-18 ROLE-DEPENDENT CHANGE IN THE STRUCTURE AND FUNCTION OF THE HYPOPHARYNGEAL GLANDS IN WORKER HONEYBEES (*APIS MELLIFERA* L.)

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Associated with the age-related role change of the worker honeybees (*Apis mellifera* L.) from nursing to foraging, some structural and functional changes occur in the hypopharyngeal glands (HPGs): nurse bee HPGs are well-developed and synthesize major royal jelly proteins (MRJPs), while forager HPGs are shrunk and synthesize α-glucosidase to convert nectar into honey. To identify candidate genes involved in the structural and functional changes of the HPGs, we used the differential display method and quantitative RT-PCR to search for genes whose expression in the HPGs depends on the role of workers. Here, we newly identified a *buffy* homolog encoding a Bcl-2-like protein, as a nurse bee HPG-selective gene, and a *matrix metalloproteinase 1* (*MMP1*) homolog, as a forager HPG-selective gene, respectively, suggesting that caspase cascade and degradation of extracellular matrix are involved in the structural and functional changes of the HPGs. Quantification of caspase-3 activity in the HPGs using caspase assay revealed that caspase-3 was not activated in the forager HPG, suggesting that the function of buffy in the HPGs is not related to apoptosis. Furthermore, to discriminate whether *buffy*- and *MMP1*-expressions depend on their age or roles, we quantified these transcripts in the HPGs of nurse bees and 'precocious' foragers derived from 'single-cohort' colonies (SCCs). The SCCs were initially composed of young workers with the same age, and, under the absence of older workers, some young workers initiated foraging earlier than usual as 'precocious' foragers. As results, *buffy*-expression was higher in nurse bees than in `precocious' foragers, while *MMP1*-expression was higher in 'precocious' foragers than in nurse bees. These results indicate that expressions of these two genes in the HPGs depend on the role but not age of the workers. *buffy*- and *MMP1*-expression might be regulated by some hormonal factors that coordinately regulate worker behavior and HPG physiology.

15-19 Building a genetic map for *Solenopsis invicta* by sequencing restriction-site associated DNA (RAD) markers

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Next generation sequencing technologies (NGST) permit quickly generating relatively inexpensive draft genome sequences for traditionally non-model organisms. Because eukaryotic genomes contain many repetitive sequences longer than NGST read lengths, *de novo* assembly of medium-to-large sized eukaryotic genomes remains challenging. While paired-end sequencing strategies can be used to bridge across repetitive sequences to build scaffolds from assembled sequence fragments (contigs), many scaffolds and contigs remain unconnected. For example, in the fire ant *Solenopsis invicta*, despite ~12x 454 and ~37x Illumina sequencing coverage, our assembly still yields ~10,000 scaffolds rather than the ideal 16 that corresponds to the chromosomes. We are using a recently developed method, restriction-site associated DNA (RAD) tag sequencing (Baird N.A. *et al*. 2008, *PLoS ONE* 3(10): e3376) that permits rapid SNP discovery and genotyping at low cost, to join scaffolds into super-scaffolds. This technique also permits mapping of genetic traits. We will present our progress on building a fire ant genetic map anchored to the assembled sequence and proof-of-principle re-mapping of the monogyny/polygyne social polymorphism that is associated with the gene *Gp-9*.

15-20 Selection for a preference in food collection is associated with proteomic and epigenetic alterations in honeybees

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Protein abundance and epigenetic marks such as DNA methylation are major driving forces in the formation and evolution of organismal phenotypes. We show that artificial selection for a preference in food-collection (either more protein or more carbohydrate-rich food) is connected to alterations of the proteome and the DNA methylome in honeybees. Proteomic analysis on individuals of the selected strains revealed differences in proteins that can influence behavior such as vitellogenin and juvenile hormone esterase. Importantly, we also detected differences in histone abundance. Honeybee histone variants are encoded in two gene clusters that locate to quantitative trait loci differentiating the two strains. This feature makes them potential key players in the selection process. Generally, histones play a crucial role in DNA packaging and their abundance levels can influence genomic accessibility to enzymes that modify the DNA sequence such as DNA methyltransferases. Thus, differential histone abundance can affect epigenetic patterns. Interestingly, epigenetic analyses revealed the occurrence of differential DNA methylation in the two strains. The effect was found to be tissue-specific and to mainly affect genes involved in protein and nucleic acid binding as well as in transcription. DNA methylation can have effects on transcript levels and on the formation of splice variants. Thus, the differential protein abundances found by proteomics analyses could also be a consequence rather than a cause of differential DNA methylation. In summary, we have collected information that suggests a dynamic interplay between protein abundance and DNA methylation, which is likely to play a crucial part in the metabolic networks that govern phenotype divergence in the two honeybee strains.

15-21 The genetic architecture of a trait underlying social conflict

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Explaining how interactions between genes and the environment influence social behavior is a fundamental question, yet there is limited relevant information for species exhibiting natural variation in social organization. The fire ant *Solenopsis invicta* is characterized by a remarkable form of social polymorphism, with the presence of one or several reproductive queens per colony and the expression of other phenotypic and behavioral differences being completely associated with allelic variation at a single Mendelian factor marked by the gene *Gp-9*. Furthermore, the *b* allele at *Gp-9* is a rare example of a “green beard gene” because *b* workers favor the reproduction of *b* queens by executing queens that do not carry *b*. This selfish allele has not reached fixation because of balancing selection: the phenotypes associated with *b* are counter-selected in certain environments, and *bb* homozygotes are lethal. Some theoretical and empirical evidence suggest that *Gp-9* is part of a genomic region with no recombination. BAC-mapping, FISH and chromosome-excision based approaches were unsuccessful at identifying the *Gp-9* region. To identify the region marked by *Gp-9*, we undertook *de novo* sequencing of the 500 MB fire ant genome. Respectively 40x and 12x genome coverage of sequence were generated by Illumina and Roche 454 technologies. These data were assembled into 10,543 scaffolds with an N50 size of 720,578 bp. Preliminary analyses reveal that the *Gp-9* region in complete linkage disequilibrium between the two social forms is a region representing approximately 1% of the genome. The *Gp-9* haplotypes do not recombine between social forms and show extensive differences in structure and gene content, similar to sexual chromosomes.

Oral Presentations

The genetic basis and consequences of social evolution

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THE INTERACTION BETWEEN A SINGLE GENETIC ELEMENT AND SOCIAL ENVIRONMENT ON SOCIAL ORGANISATION IN FIRE ANTS

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In this talk I will discuss how interactions between genes and social environment influence behavior and social organization. In particular, I will show that, in ants, worker behavior and gene expression profiles are more strongly influenced by indirect effects associated with the genotypic composition of workers within their colony than by the direct effect of their own genotype. This constitutes an unusual example of an 'extended phenotype,' and suggests a complex genetic architecture directly and indirectly influencing the individual behaviors that, in aggregate, produce an emergent colony-level phenotype. I will finally discuss of these gene by environment interactions underlie the presence of two distinct modes of social organization

SOCIAL EVOLUTION IN A SUBSOCIAL BEETLE - QUANTITATIVE TO MOLECULAR GENETICS OF PARENTING

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Social evolution involves interactions among individuals, which can complicate genetic studies by making the phenotype difficult to define in a way that permits genetic approaches. Nevertheless, we are beginning to see more genetic studies of socially relevant traits. One of the earliest phases of social evolution includes interactions between parents and offspring and parenting. In this talk I will review our work on the quantitative and molecular genetics of parenting in the beetle, *Nicrophorus vespilloides*. Patterns of quantitative genetic variation provide insights into how differences in parenting behaviour of males and females might evolve. Adopting a candidate gene approach provides additional insights into how changes in gene expression influence social behaviour evolution. We are beginning to understand how genetics influences social evolution, and our work highlights how examining non-model systems provides additional insights.

ENVIRONMENTAL AND GENETIC COMPONENTS OF A MAJOR EVOLUTIONARY TRANSITION: SOCIALITY IN SWEAT BEES

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The evolution of eusociality is one of the major biological transitions. Eusociality involves a reproductive division of labour between the members of a society, as well as other complex group-level traits that require coordination and communication between individuals. The transition to eusociality has been well analysed from a functional perspective, but the underlying mechanisms are less well understood. Nevertheless, it has been suggested that the transition might sometimes be environmentally induced. Sweat bees (Halictidae) provide a unique opportunity to understand the genetic and environmental influences involved in social transitions. They evolved eusociality more recently than other insect lineages, and include multiple transitions in both directions between eusociality and non-sociality. We carried out the first direct test of what underpins social phenotype in sweat bees using the socially polymorphic *Halictus rubicundus*. *H. rubicundus* is non-social in colder parts of its distribution but eusocial in warmer parts. Do these differences reflect genetic change, or differential expression of the same set of genes? We present results from transplanting bees between sites to test whether they are socially plastic. Our results have general implications for social evolution.

DON'T FORGET THE KING: MALES ALSO INFLUENCE CASTE ALLOCATION AND SEX RATIO IN ANTS

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Individuals in animal societies may differ in their reproductive output. In social insects, differences in reproductive success between queens may also stem from differences in their relative contribution to the three castes (new queens, males and workers). Such a partitioning of reproduction occurs between queens in colonies of the Argentine ant *Linepithema humile*. We conducted controlled crossings and established colonies headed by a single queen to investigate the genetic factors underlying these differences. The biomass produced was only influenced by the genetic background of queens. By contrast the caste fate of the female offspring was only affected by the genome of the male the queen mated with. Finally, the sex ratio was influenced both by the maternal and paternal genomes with a significant interaction between the parental genomes. The finding of complex interactions between the genomes of parents on sex allocation has important implications for life-history evolution and conflict resolution in social insects.

REPRODUCTIVE DIVISION OF LABOR IN A HARVESTER ANT: FROM GENOME TO GENES

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Reproductive division of labor is the cornerstone of advanced sociality. The queen caste specializes at reproduction while the worker caste on the day-to-day labor of the colony. In addition to behavior, the castes differ greatly in physiology, lifespan, and development. We sequenced the harvester ant, *Pogonomyrmex barbatus*, genome in order to find candidate genes involved in the developmental differentiation of castes. This system was chosen because in some populations the queens and workers develop from genetically different eggs and can be genotyped prior to the onset of developmental differences, and thus the genes involved in differentiation can be assayed in individuals whose developmental fate is known. Here we report preliminary findings from the genome, and candidate gene expression, regarding the mechanisms of caste determination in harvester ants.

GENOME SEQUENCING AND COMPARISON OF THE SOCIALLY DISTINCT ANT SPECIES *HARPEGNATHOS SALTATOR* AND *CAMPONOTUS FLORIDANUS*

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Next generation sequencing technology allows genomics approaches to the understanding of the evolution of eusociality that were not yet available even for large consortia a few years ago. We used Illumina technology to sequence and compare the genomes and transcriptomes of two ant species that represent different ends of the sociality continuum in ants. *Harpegnathos saltator* is a ponerine ant with weak queen-worker dimorphism and workers that can replace queens functionally. *Camponotus floridanus* on the other hand exhibits strong queen-worker dimorphism with polymorphic and strongly reproductively degenerated workers. This distinctiveness in social elaboration allows to compare properties of the genomes in regards to social organization and to look for genomic correlates of major properties of ant societies. Our genome size estimates vary from 250Mb for *C. floridanus* and 330 Mb for *H. saltator* leading to 90% coverage of our genomes for both species. Our current gene sets contain 17,064 models for *C. floridanus* and 18,565 for *H. saltator* 80% of which are supported by RNA-seq or EST data. A global comparison of the genomes revealed that levels of methylation in larvae and adults of *C. floridanus* are about twice as high as in *H. saltator*, potentially suggesting that increased methylation may be associated with increased morphological and physiological specialization. We also found that telomerase and sirtuin genes are strongly upregulated in reproductive workers in *H. saltator*. Since these genes have been associated with increased longevity, the pattern may help to explain the differential life expectancy in reproductives and infertile workers in social insects.

MOLECULAR SIGNATURES OF SELECTION REVEAL CONVERGENT AND LINEAGE-SPECIFIC PATHS TO EUSOCIALITY

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The eusocial insects are exemplars of social complexity and ecological success. They also present a strong example of convergent evolution, with remarkably similar forms of social organization despite multiple independent evolutions of eusociality. This raises the question of whether selection has acted on similar sets of genes during independent evolutions of eusociality. To address this question we generated ~1GB of sequence for nine socially diverse bee species, from solitary to highly eusocial, representing three of the six independent evolutions of eusociality in the bees. To search for molecular signatures of adaptive evolution, we fit ~3600 alignments of orthologous protein-coding sequence from these nine species, plus from the honey bee *Apis mellifera* genome, to codon substitution models. This analysis revealed two striking patterns of sequence evolution. First, 17% of all genes analyzed are evolving differently between eusocial and non-eusocial lineages, with 65% of these genes evolving more rapidly in eusocial relative to non-eusocial bees. This set is enriched for genes involved in brain development, oogenesis, transcription, and several metabolic pathways. This result suggests unique selective pressures associated with eusociality, with common genetic changes observed in all lineages that evolved this lifestyle. However, there are large differences in the types of genes evolving most rapidly in the highly or primitively eusocial lineages. The majority of genes evolving most rapidly in highly eusocial lineages are involved in carbohydrate metabolism, especially glycolysis. The genes evolving most rapidly in primitively eusocial lineages have different and more diverse functions, including oogenesis, neuron differentiation, and response to hormone stimulus. These results suggest that the multiple evolutions of eusociality have involved a mosaic of selection on a core set of genes across all lineages as well as different sets of genes in specific lineages.

MOLECULAR BASIS FOR REPRODUCTIVE DIVISION OF LABOUR IN A LOWER TERMITE

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Wood-dwelling termite workers are totipotent individuals that can develop into soldiers and two kinds of reproductives: winged sexuals which develop via several moults and leave the nest to found a new colony or neotenic reproductives which develop via a single moult when the current reproductives fall ill/die. The distinct development of the genetically identical individuals must be regulated through differential gene expression. Using representational difference analysis of cDNA (cDNA-RDA) we compared gene expression between workers and female reproductives in the two closely related species *Cryptotermes secundus* and *C. cynocephalus*. In total, thirteen genes were identified being overexpressed in queens compared to workers. Yet, only three of these genes (Neofem1, Neofem2 and Neofem3) showed a conserved queen-specific expression pattern in both species. They, presumably code for an esterase-lipase (homologous to an odorant decreasing esterase of the silk moth), a -glycosidase (homologous to a sex-specific surface protein in the Maderian cockroach) and a vitellogenin (developmental regulator in all insects), respectively.In an experiment combining molecular with behavioural methods, Neofem2 was silenced in queens by RNA interference (RNAi). This led to an increase of butting in workers, a behaviour typical for queenless colonies and indicative for future replacement reproductives.Thus we conclude that Neofem2 is necessary for the queen to signal dominance and suppress worker reproduction.The functional significance of Neofem1 and 3 will be studied and discussed.

SOCIALITY IS LINKED TO RATES OF PROTEIN EVOLUTION IN A HIGHLY SOCIAL INSECT

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Eusocial insects exhibit unparalleled levels of cooperation and dominate terrestrial ecosystems. The success of eusocial insects stems from the presence of specialized castes that undertake distinct tasks. We investigated whether the evolutionary transition to societies with discrete castes was associated with changes in rates of protein evolution. We predicted that proteins with caste-biased gene expression would evolve rapidly due to reduced antagonistic pleiotropy. We determined if rates of protein evolution were associated with patterns of gene expression using genomic data from the honeybee *Apis mellifera*. We found that queen-biased proteins did indeed evolve rapidly, as predicted. However, worker-biased proteins exhibited slower evolutionary rates than queen-biased or nonbiased proteins. We suggest that distinct selective pressures operating on caste-biased genes, rather than a general reduction in pleiotropy, explain the observed differences in evolutionary rates. Our study highlights, for the first time, the interaction between highly social behavior and dynamics of protein evolution.

Poster Presentations

The genetic basis and consequences of social evolution

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16-1 Testing genetic hypotheses of reproductive regulation in honey bees

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A fundamental issue in sociobiology is to understand how social insect females regulate their individual reproduction to maximize inclusive fitness. The environmental cues controlling ovary activation in honey bee workers are understood at a basic level, but the underlying gene regulatory networks are not. In this study we use oligonucleotide microarrays to identify suites of pheromonally-responsive genes differentially expressed between workers with and without active ovaries. We compare genes identified from the microarray to candidate sets already implicated in reproductive regulation, and in particular, those implicated in the reproductive groundplan hypothesis. Moreover, we have used pathway analysis to reconstruct a basic molecular network that links pheromonal effects on gene expression to differences in worker reproductive physiology. So far, our gene set is consistent with aspects of the reproductive groundplan idea, and with comparable genomic screens. Nonetheless, screens yield new candidate genes not previously implicated in honey bee reproduction. A basic pathway analysis shows two distinct sets of co-regulated genes: one set associated with ovary-inhibition, and a second set associated with ovary-activation. These genes are of potential significance to sociobiology for they represent the very genes that underpin the expression of reproductive altruism in social insects.

16-2 Genes for maternal effects: short-term associations between the queen's transcriptome and her offspring's caste fate

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Individual caste fate depends not only on abiotic environment, the individual's genes, and behavioral choices of nurses, but also on queen (i.e. maternal) effects. Even male offspring have at least two possible fates (full development or early death). Mechanisms for queen effects include methylation of oocyte genomes and the provisioning of eggs with nutrients, hormones, and transcripts. Genes that mediate queen effects on caste fate are of interest because they represent likely targets for strong sex ratio selection. To identify such genes, we quantified the association between the expression of ~25000 ESTs in 30 queens from reproductive fire ant colonies (*Solenopsis invicta*); and the short-term probabilities of all possible caste fates for their fertilized and unfertilized eggs. After listing the genes that can best predict caste fate for offspring, we use their Gene Ontology (GO) classifications to infer the most likely queen effect mechanism by which they operate.

16-3 Breakthrough of sequencing technology facilitates research on social insects

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Breakthrough in sequencing technology has rapidly lead to a decrease in costs and an increase in throughput. With more and more organisms sequenced (including many social insects), a flood of genetic data is being generated worldwide everyday. Progress in genomics has been moving incrementally due to this revolution in sequencing technology. At the same time, in-depth analysis in functional genomic studies such as whole transcriptomics and epigenomics has become realistic, and have already brought us unprecedented knowledge. The Bejing Genomics Institute (BGI) is on the cutting edge of the application of genomic information in various fields of research, including social insect studies. Recently, in collaboration with other researchers, we already finished the genomes of two eusocial ant species and identified the genetic components of these two organisms. In the near future, as part of our 'Tree of Life' project aimed to sequence all scientifically important model organisms, we will continue to decode more eusocial animals, and provide functional genomic resources and more genomic tools to facilitate the studies in this area.

16-4 UNDERSTANDING FIRE ANT VENOMS BY PROTEOMICS: *SOLENOPSIS INVICTA BUREN* × *SOLENOPSIS SAEVISSIMA SMITH*

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The composition of venom proteins of fire ants is only partially known. The present investigation benefited from gram amounts of venom protein from *Solenopsis invicta Buren* and *Solenopsis saevissima Smith* that were analyzed using modern proteomic tools. Our analyses indicate that each worker of *S. invicta* contains about ten times as much venom proteins as a *S. saevissima* worker, being the venom of both species basically composed of a few neurotoxins and allergens. Additionally, traces of phospholipases, a vascular growth factor, an antioxidant and a phospholipase inhibitor were found. The results obtained are reflective of the basic functions of the venom, i.e. colony defense and subduing of prey. We are currently attempting to extend our analyses to *Solenopsis geminata* Fabricius, and trying to obtain venom peptides to be sequenced by mass spectrometry and bioassayed.

16-5 (UN)LIMITED VARIABILITY? EVOLUTION OF SEX DETERMINING ALLELES IN HONEY BEES

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Evolutionary processes lie hidden within DNA sequence data and can be uncovered by analysing nucleotide polymorphism in different alleles. In the honey bee *Apis mellifera* sex is determined by a single locus complementary sex determination mechanism. The gene *complementary sex determiner* (*csd*), the primary signal of the cascade, determines sex by its allelic composition as females are always heterozygous while fertile males are hemizygous at *csd*. High numbers of csd alleles segregating in the honey bee population are maintained over an extended period of time when compared to neutral polymorphism (balancing selection). We surveyed a panmictic sample of *A. mellifera* haplotypes to gain insight into the capability of *csd* to evolve and maintain new allelic specificities. We focused on the potential specifying domain, consisting beside an arginine-serine- and a proline-rich domain of a hypervariable region (HVR). Not only the length of the HVR can vary remarkably (between 11 and 31 amino acids) but also the composition of its repeated N(1-5)Y motifs. In the surveyed region two different haplotypes were detected which presumably have a different evolutionary history. The analysis of synonymous and nonsynonymous substitution rates in the adjacent regions indicate a fast evolution of the HVR via duplication and deletion of the repeats. Pairwise comparison of functionally distinct alleles gave insights into the minimum of variation necessary for functional allelic specificities. These complex pictures of nucleotide polymorphism have implications for understanding evolutionary dynamics and the functional differentiation of *csd* alleles in honey bee populations. This study was supported by DFG (HA-5499/3-1).

16-6 Exploring the molecular processes underlying female caste development in the honey bee

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The concept of royalty in *Apis mellifera* has fascinated scientists for millennia. In 350 B.C.E Aristotle spoke of a ruler bee which had an accelerated rate of growth during development compared to ordinary bees. In 1806 Francis Huber noted that all worker larvae have the potential to become queens when fed royal jelly. Subsequent to these observations royal jelly has become well known as the trigger for queen development in honey bees. However the underlying molecular processes that result from royal jelly consumption and lead to queen development are not well understood. The work presented here provides the most comprehensive molecular analysis of caste development in honey bees to date. Here we examine differences in gene expression between queen and worker larvae as young as six hours post hatching. A custom oligonucleotide microarray containing all known honey bee genes, expressed sequence tags and micro RNAs was used in combination with quantitative reverse transcription PCR to confirm differences in gene expression between queen and worker larvae at seven points during development. The candidate gene lists produced from this analysis reflect previous findings, such as up regulation of hexamerin proteins in workers, as well as identifying numerous genes which have not been implicated in caste development previously. This study will provide a framework for future analysis of candidate genes using *in situ* hybridisation, bisulfite sequencing and larval RNA interference. The information obtained from this analysis will provide insight into caste development in the honey bee and more generally the underlying mechanisms of polyphenisms and developmental plasticity.

16-7 The hidden cost of altruistic policing

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Kin selection is the dominant paradigm to explain the evolution of cooperation and, worker policing, one of its most convincing examples. Here, workers aggress upon laying workers or consume their eggs to increase overall colony relatedness. However, policing presents an evolutionary paradox as those workers who police are simultaneously less likely to be laying workers (i.e. the traits are negatively correlated). Consider a population where all workers lay male eggs into which a policing mutant arises. The mutant will suppress a fraction of the laying workers and their eggs will be replaced by queen-laid ones. If the queen has multiply mated, the mutant will gain fitness by this action. Some proportion of eggs, however, will still be worker-laid and the mutant has functionally self-sterilized with regards to this pool of offspring. Such self-policing could be a strong selective force against the evolution of policing others. Here, we present several simulations to examine this dynamic and at what levels of selection policing is selected for.

16-8 Gene networks linked to vitellogenin mRNA disruption

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Vitellogenin (Vg) is a yolk precursor protein of nearly all oviparous species. In honeybees, Vg has also been hypothesized to control lifespan and behavior. To identify target genes that respond to Vg pleiotropic effects, we performed dye-swap microarrays from larval and adult stages of queens and workers fat bodies treated with of double-stranded RNA for Vg (dsVg) or for green fluorescent protein (dsGFP, used as control). Total RNA from four developmental time points (5th larval instar-queens (QL5) and -workers (WL5), newly-emerged queens (QNE) and 7-day-old workers (W7d) was used to synthesize aRNA probes for slides hybridization. Changes caused by dsVg treatment were reported. Although many genes were inhibited in all analyzed situations, these changes never exceeded 0.7-fold. The variation of upregulated genes ranged from 1.4 to 99-fold. QNE exhibited the largest number of upregulated genes (63), followed by W7d (38), WL5 (23) and QL5 (15). The most pronounced effects of dsVg were observed in W7d, in which 19 genes were altered more than 10-fold, as *headcase* (related to morphogenesis) and *ice* (caspase precursor). Some larval upregulated genes are involved in signal transduction (cytochrome C, *windbeutel*), gamete generation (singed), and lipid metabolism (*CG7367*, *CG3523*). A senescence marker upregulated in both QNE and W7d (alpha-glucosidase) indicated that Vg regulates longevity. The upregulation of *malvolio* in W7d, a gene that triggers transition to foraging tasks, reinforced that Vg knockdown changes behavior. Enzymatic genes exhibited antagonistic regulation as a result of Vg silencing: *methyltransferase*, *carboxypeptidase*, *thioredoxin-reductase*, *farnesyl pyrophosphate synthase*, *delta-9 desaturase*. This is the first time that transcriptional changes resulted from a yolk silencing is observed. Our data showed crucial biological processes and genes under Vg regulation, contributing with new information about mechanisms controlling the social organization.

16-9 The mitochondrial genome of *Acromyrmex echinatior*

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Mitochondrial genome sequences offer insights on phylogenies, molecular evolution, and genome evolution. Even though ants comprise the largest animal biomass in tropical ecosystems, only few complete mitogenomes from this important group are available. Here we present the complete mitochondrial genome of the Panamanian leafcutterant *Acromyrmex echinatior* (Hymenoptera: Myrmicidae), obtained by a combined 454 and Illumina (Solexa) sequencing approach. Though the sequence could be obtained by either method, the combination aided in resolving ambiguities in areas of low nucleotide entropy. Our results show that the *A. echinatior* genome has a typical insect mitochondrial gene set and gene order, but with 20kb of sequence, is longer than most insect mitogenomes. The additional length is due to expanded regions between genes. The AT content is approximately 80%, and thus consistent with the range observed for other invertabrate mitogenomes. The alpha and beta strands show a marked difference in G and C content.

16-10 IMPORTANCE OF THE MAJOR ROYAL JELLY PROTEIN IN THE EVOLUTION OF SOCIALITY

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A fundamental problem in evolutionary biology is explaining how sociality evolves. Recent advances in genomic techniques mean that we can now study social evolution in the social insects at the molecular level. The candidate gene approach, where specific 'genes for sociality' are examined, has proved successful and enlightening method to study social evolution. Here we propose to determine the extent to which Major Royal Jelly Protein (MRJP) underlies important transitions for sociality. MRJPs play a crucial role in the social behaviour of honeybees, specifically in nutritional control of caste determination. Until now, honeybees were the only animals known to express true MRJPs. Consequently, MRJPs were thought to have evolved uniquely in this group, playing a key role in the evolution and maintenance of the honeybee's complex social behaviour. However, the recent finding of a MRJP-like EST in the primitive eusocial paper wasp *Polistes canadensis* suggested that the widespread importance of MRJPs in social evolution has been underestimated, and their role may be more ancient and fundamental in the evolution of sociality that previously though. Using 454 transcriptome sequencing, we have identified several MRJPs in *P.canadensis* and found several differences in isoform expression amongst worker, queen and foundress castes. Comparative analysis of *Polistes canadensis* transcriptome with other insect genomic data clarify the phylogenetic context of MRJP evolution for sociality.

16-11 THE INJECTION OF IN VITRO SYNTHESIZED MRNA AS A METHOD FOR TRANSIENT GENE EXPRESSION IN THE HONEYBEE

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Molecular investigation of functional processes in honeybees requires reliable tools for genetic manipulation *in vivo*. We present a method to introduce in vitro transcribed mRNA into early developmental stages of honeybee embryos (*Apis mellifera*) resulting in equally distributed transient expression of the respective protein. We injected mRNA encoding the red fluorescent MARS protein, whose expression we observed for several days using conventional and confocal fluorescence microscopy, while the corresponding transcripts could still be detected three days after injection. This technique is a versatile tool for the functional study of early developmental processes in honeybees, because the injected mRNA is instantly available for translation, and the time consuming procedure of promoter selection, e.g. for plasmid based gene expression, is completely excluded. Since the mRNA is transcribed by a viral T7 polymerase, the cDNA of the desired gene simply has to be cloned in an appropriate plasmid vector carrying a T7 promoter. We will present the complete procedure of transient gene expression in the honeybee, including mRNA generation, sample preparation and in vivo injection.

16-12 THE EXPRESSION OF DIFFERENT SET OF GENES CHARACTERIZES THE PUPAL AND ADULT CUTICLE DEVELOPMENT IN *APIS MELLIFERA*

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Insect integument consists of an external cuticle, or exoskeleton, that overlies the epidermis. Cuticle is a complex material made of chitin filaments embedded in a proteinaceous matrix, and provides protection against desiccation, patogens and predators, besides conferring structural and mechanical support to the organism. Insects produce a new cuticle every time they molt, and this requires activation and repression of specific cuticle protein genes. To understand metamorphosis at the molecular level, we investigated gene expression changes in the honeybee thoracic integument during pupal/pharate-adult transition at three specific time-points (immediately before, during and after apolysis), which are marked by low, high, and very-low ecdysteroid titers, respectively. Using cDNA microarrays constructed from 13,440 oligos, we detected a total of 333 differentially expressed genes in the integument of the honeybee. Among them, 21 genes were recognized as structural cuticular proteins (CPs) belonging to CPR, CPF and apidermin protein families. Among the 21 CPs genes, 2 have not been found in the published honeybee genome. The majority (15) of the cuticle protein genes, including AmelCPR3, AmelCPR14 and apd-3, exhibited a significantly higher expression following apolysis, when ecdysteroid titer decays. This is a developmental period characterized by synthesis and differentiation of the adult cuticle, thus suggesting that these genes are involved in shaping the definitive exoskeleton. In contrast, some cuticle protein genes, like AmelCPR21, apd-2 and AmelCPF1, showed a significantly higher expression before apolysis, suggesting that their products have a role in pupal cuticle structure. All together, our data highlight significant differences among expression patterns of cuticle protein genes, thus indicating that they are regulated by distinct levels of ecdysteroids, and have specific roles in the construction of the pupal or adult exoskeleton.

16-13 A SECOND GENERATION GENETIC MAP OF THE BUMBLEBEE, *BOMBUS TERRESTRIS*

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The bumblebee *Bombus terrestris* is a key pollinator in natural ecosystems and for many commercial crop plants. Apart from this *B. terrestris* is an important model system in ecology and evolutionary biology, including studies on social behavior, host-parasite interactions, immunology, plant-pollinator interactions and genetics. We here present a high resolution *B. terrestris* genetic (meiotic) linkage map as a basic tool for quantitative trait loci (QTL) mapping, analysis of the structure and organization of the genome, as a framework for genome sequence assemblies and to address evolutionary questions. Using a mapping population of *Bombus terrestris* and novel microsatellite markers obtained from BAC library end sequences we created a linkage map comprising 18 linkage groups of 21 to 146 cM representing all chromosomes (n=18). The total recombination genome length is 1724 cM with an average marker distance of 4.7 cM. With a genome size of ~ 275 Mb, this results in an overall recombination rate of 6.3 cM/Mb. Sequence homologies with the honeybee genome allowed to homologize many *B. terrestris* and *Apis mellifera* chromosomal regions. Synteny blocks and rearranged regions could be used to illustrate chromosome evolution in a comparative approach.

16-14 TRANSCRIPTOME ANALYSIS OF PHENOTYPIC PLASTICITY IN A TROPICAL PAPER WASP

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Understanding how different phenotypes arise from the same genome is a fundamental problem in biology. The queen and worker castes of social insects are excellent examples of alternative phenotypes. Those of the *Polistes* paper wasps are especially interesting as they provide insights into the origins of sociality. Queens and workers are highly plastic phenotypes whose expression can change in response to a changing social environment. It is clear that plastic phenotypes are produced through differential expression of shared genes (Sumner et al. 2006, Toth et al 2010). However, until recently the techniques available for genomic analyses in non-model organisms have been largely restricted to analyses of pre-selected (and hence, potentially biased) genes, e.g. based on homologies with genes associated with certain behaviours in the honeybee or other previously identified genes. Here, we present the results of a transcriptome study of plastic phenotypes in the tropical paper wasp *Polistes canadensis*. Using next generation sequencing, we sequenced the brain transcriptomes of four different phenotypes: queens, foragers, foundresses, and newly emerged females. We contrast the assembled transcriptomes of these four groups to compare global gene expression, identify phenotype-specific genes by classifying them into putative functional categories, and use similarity analyses to describe phenotypes in molecular terms. We use these data to test key hypotheses on the evolution of castes and phenotypic plasticity, and discuss our results in the context of other species.

16-15 GENOMIC APPROACHES TO UNDERSTANDING KARYOTYPE EVOLUTION IN THE *MYRMECIA PILOSULA* SPECIES COMPLEX IN AUSTRALIA

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A notable feature of ants is high variation in both genome size and especially chromosome number, even at low taxonomic levels, suggesting an important role for karyotype evolution in speciation. A remarkable example is the Australian *Myrmecia pilosula* complex of “jack jumper” or "bulldog" ants, whose five species have chromosome numbers from 2n=2-32, with notable variation in karyotype within species as well. Surveys of the *M. pilosula* species complex have identified numerous colonies derived from apparent hybridizations between species, and within species between colonies with different karyotypes. We aim to address the question of karyotype evolution in this species by first sequencing the genome of the 2n=2 form of *M. croslandi*. In itself, this would be an impactful genome sequence because of the highly unique karyotype. We then propose to use this first genome sequence as a platform to align additional genomes from the species complex, while at the same time mapping the scaffolds obtained onto chromosomes. This approach will provide the most complete data possible on the evolution of karyotype in this species complex.

Oral Presentations

The beneficial use of ants and termites in agriculture and nutrition

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PROSPECTS AND FUTURE DIRECTIONS FOR USING *OECOPHYLLA* ANTS AS BIOCONTROL AGENTS IN HORTICULTURE AND FORESTRY

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As predators of pests, about 15 species of ants from 8 genera are recognized as being beneficial for horticultural crops and forest trees. Among these, *Oecophylla* ants have been used more extensively than other genera of ants due to their efficiency in controlling numerous pests. From 304 to 1994 (1690 years), 47 pest species were recorded as prey of *Oecophylla* ants. The ants were initially used on citrus to control its main pests, and later extended to coconut and cocoa. During this long period, the ants were used without considering the colonies from which they came, resulting in difficulty in maintaining the ant populations at stable and high levels. Therefore, frequent and labour intensive transplantation was necessary. In the latter 1990s, the *Oecophylla* ant technology was developed, and it consisted of separating and transplanting the ant colonies, managing queen ants, creating a mixed-cropping system and reducing competitive ant species. With this technology, the ant populations can be kept stable and high for a long period. Ant pheromone, exocrine compounds and the relationship between extra-floral nectar and the ants were also found to play an important part in controlling pests. These developments have accelerated the use of the ants. From 1995 to 2009 (15 years only), an additional 56 pest species were controlled by the ants. The use of the ants also extended to cashew, mango and African mahogany. Eight IPM programs using *Oecophylla* ants as a key element have led to increases in farm net incomes of >100% on coconut, >70% on mango, 13-50% on cashew and 40% on African mahogany compared to conventional management. Having considered farm income, farmers' health, food safety and environmental pollution associated with pesticides, we suggest that existing IPM programs be modified to use the ants as a key element where possible and that further study is needed on the potential of the ants to control pests on additional horticultural crops and forest trees.

MATING STRATEGY AND COLONY REARING OF *OECOPHYLLA SMARAGDINA*

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With the increasing use of weaver ants, *Oecophylla sp*. in biological control, the availability of wild colonies may become a problem in many regions. Therefore, rearing of colonies in artificial nurseries could be a possibility in the future. Very little is known about their mating strategy, so it is crucial to investigate where and when they mate in order to find newly fertilized queens, or to develop methods to mate the queens in captivity. Field observations and experiments were carried out in Darwin NT, Australia to elucidate the mating strategy. Further, an array of artificial nesting sites was tested in order to find the most efficient method to collect the fertilized queens when they search for a place to found a new colony. Rearing colonies from fertilized queen to colonies with thousands of workers is not problematic, but it requires relatively long time. We have tested several methods to boost colony growth in order to reduce the time to mature colony. The following successful methods have been tested: 1) Use multiple queens to start the colony. 2) Add pupae to the founding queens when they get larvae.3) Add extra brood to the colony frequently during the growing phase. It is our hope to make nurseries where *Oecophylla* colonies for use in biological control can be reared fast and efficient, so they can be produced and sold for a reasonable price. The advantages of artificially reared colonies are: 1) No need for expert knowledge to obtain colonies. 2) Stable supply. 3) No impact on natural populations and ecosystems. 4) The age of the queens is known.

CONSERVATION OF AN ECOSYSTEM-ENGINEER - THE MOUND BUILDING TERMITE *MACROTERMES BELLICOSUS*

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Almost everyone knows termites as pest that causes a yearly damage of millions of dollars by destroying buildings and crops but only few people think about their crucial importance for different tropical and subtropical ecosystems. Termites are important ecosystem-engineers because they mineralize high amounts of carbon and enhance the soil's infiltration rates. Furthermore their relevance as prey for other animals, ranging from small arthropods like ants to big mammals like aardvarks, is striking. Especially the African fungus-growing species *Macrotermes bellicosus* (Isoptera, Macrotermitinae) provides with its huge mounds starting points for island forests and shelter for animals like snakes, mangooses etc. Investigations on the socio-cultural and socio-economic role of termites and the mound-related plants revealed their high importance for local communities. Multipurpose utilization comprises the use of i) termites as chicken fodder, ii) soil of mounds for fertilization and construction, iii) fungus and plants for nutrition, and implicates applications in iv) traditional health care, and v) cultural and religious ceremonies. Recently, mound abundance decreased in Northern Benin (West-Africa) at an alarming rate, probably due to an increased human impact including pesticide application in cotton cultivation. In order to mitigate this development an integrative research approach is applied. This includes population genetic analyses and ecological studies to understand population dynamics and identify crucial parameters, like dispersal distances, to elaborate the minimum number of mounds necessary for providing ecosystem services and allowing sustainable use. The results of this long-term study are presented.

TERMITES AND ANTS BOOST WHEAT YIELD IN DRYLAND CROPPING

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A soil-insect exclusion experiment was conducted in a low-till, controlled traffic, dryland winter wheat paddock near Geraldton Western Australia from 2006 to 2008. Exclusion was with bifenthrin (a registered insecticide) and tillage (used for summer weed control) was applied as a second treatment in a split plot design. Wheat yield did not differ between treatments in 2006, but differed significantly in 2008, 55% greater in plots with most ants and termites (water control + not tilled) compared with those where they least abundant (biflex + tilled); grain quality was unaffected by the treatments. The lag indicated the manipulation took effect over time. The mechanism behind the yield increase was increased water and nitrogen availability in the control plots. Rainfall and water infiltration was similar in all treatments in 2006, but was ~30% higher in control plots compared with exclusion plots in 2008. This was related to the number of insect tunnels in the soil: the same in all treatments in 2006; but three times the number of insect tunnels in control plots compared with biflex plots in 2008. Soil nitrogen was higher in biflex plots in 2006, probably due to the decomposition of the dead insects post bifenthrin application, but it was higher in water control plots in 2008. The higher nitrogen in 2008 was probably from termites; all termite species collected from the paddocks were found to have nitrogen fixing genes in their gut microbes. There were double the numbers of *Salsola* tumbleweeds, and four times the numbers of mature (flowering) plants, in biflex plots compared with water control plots, inversely corresponding with a lower number of seed-eating ants. This study suggests that there is large potential for termites and ants to boost production in sustainable dryland farming.

INDUCING SEED HAULING BY ANTS TO ENHANCE THE RECOVERY OF DEGRADED PASTURES IN COLOMBIA

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Ants play outstanding ecological services in agroecosystems such as predation, soil build up and pollination. Different ant species are attracted to seeds with structures such as elaiosomes and arils, which they carry to their nests. Many seeds are predated by the ants, but a proportion may be abandoned or moved to the nest trash. Germination can occur if seeds are placed in suitable microhabitats, i.e. inside ant nests with higher humidity, organic matter or oxygenated soil. In this study we evaluated whether the movement and dispersal of ecologically valuable seeds for the restoration of degraded pastures in southwestern Colombia can be induced or enhanced in local ants. First, an artificial aril (AA) was designed to induce myrmecochory by generalist tropical ants. Second, AA was evaluated under field conditions in six open cattle pasture farms. Twenty experimental units (EU) i.e. a paper disk containing four *Senna spectabilis* (Fabaceae) seeds, were set up, separated 10m from one another, along linear transects at each of the farms, in four treatments: (1) seeds with AA, (2) seeds smeared with a drop of honey (3) seeds smeared with a drop of tuna-oil (4) seeds alone (control). A mesh was used to prevent vertebrate disturbances. Seed removal was recorded five times in 48h. A total of 340 out of 480 seeds were moved from the EUs by ants. The mean number of removed seeds was statistically different among the treatments. Tuna oil and artificial aril were removed more frequently than honey and control, which was moved half as frequently as the tuna oil. Seeds were secondarily dispersed up to 30cm. Three (out of seven) ant species, *Ectatomma ruidum*, *Solenopsis sp*. and *Pheidole sp*. were responsible for removing almost all seeds. This study suggests that inexpensive techniques could be developed to enhance the rehabilitation of degraded pastures by inducing seed movements by generalist ants.

CONVERTING PEST INSECTS INTO FOOD: THE HARVEST OF EDIBLE WEAVER ANTS (*OECOPHYLLA SMARAGDINA*) FROM PLANTATION CROPS

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Canopy dwelling weaver ants (*Oecophylla* spp.) are used to control a variety of pests in a number of tropical tree crops. What is less familiar is the existence of commercial markets where these ants and their brood are sold for (i) human consumption, (ii) pet food or (iii) traditional medicine. In Thailand, for example, weaver ant brood is harvested in vast amounts as a delicacy and sold for twice the price of beef. As these markets provide a basis for commercial ant farming, the possibility of using plantations patrolled by weaver ants as catchment areas for ant harvest, was examined. Depending on management, 32-115 kg ant brood (mainly new queens) was harvested per ha per year without detrimental effect on colony survival and worker ant densities. This suggest that ant biocontrol and ant harvest can be sustainable integrated in plantations and double benefits derived. As ant production is fuelled by pest insects, problematic pests are converted into food and additional earnings. To assess the profitability of providing additional food for the ants, *O. smaragdina* food conversion efficiency (ECI) was estimated in the laboratory. This estimate suggests the feeding of weaver ants in ant farms to be profitable.

Poster Presentations

The beneficial use of ants and termites in agriculture and nutrition

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17-1 Successful maintenance of a stingless bee population despite a severe genetic bottleneck

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Stingless bees play an important ecological role as pollinators of many wild plant species in the tropics and have significant potential for the pollination of agricultural crops. Nevertheless, conservation efforts as well as commercial breeding programmes require better guidelines on the amount of genetic variation that is needed to maintain viable populations. In this context, we carried out a long-term genetic study on the stingless bee *Melipona scutellaris* to evaluate the population viability consequences of prolonged breeding from a small number of foundress colonies. In particular, it was artificially imposed a genetic bottleneck by setting up a population starting from only two foundress colonies, and continued breeding from it for a period of over 10 years in a location outside its natural area of occurrence. We show that despite a great reduction in the number of alleles present at both neutral microsatellite loci and the sex-determining locus relative to its natural source population, and an increased frequency in the production of sterile diploid males, the genetically impoverished population could be successfully bred and maintained for at least 10 years. This shows that in stingless bees, breeding from a small stock of colonies may have less severe consequences than previously suspected.

17-2 Antibacterial activity by *Apis florea* and *A. andreniformis* honey

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Honey is supersaturated liquid with sweet taste and is modified from flower nectar by honeybees. Not only, it is useful in consuming, but it also acts as an antibiotic agent. It can provide this activity since it is very acidic and sugary. Thus, in this research, it was aimed to determine the basic properties of honey from *Apis florea* and *A. andreniformis* which are native to Thailand. In addition, antibacterial activity from both types of honey was also observed. The results presented that there were proline contents in *A. florea* and *A. andreniformis* honey at 1.8 and 2.4 mg/ml, respectively. Furthermore, by Bradford assay, the concentration of total protein in *A. florea* and *A. andreniformis* honey was 20.5 and 21.5 mg/ml, respectively. The pH of *A. florea* honey was 4.14 while of *A. andreniformis* honey was 3.87, respectively. Considering a protein pattern after SDS PAGE, two major protein bands of 89 and 123 kDa were revealed in both types of honey. By the analysis of MALDI TOF MS, the obtained amino acid sequences were similar to nuclear migration protein nud C in *Nasonia vitripennis* and MGC82998 protein in *Xenopus laevis*, respectively. Considering the antibacterial activity, each honey at various dilutions provided the different efficiency in inhibiting the growth of chosen bacteria statistically significantly ($p \leq 0.05$). Neat honey (100%, v/v) provided the best efficiency. In addition, with the same data, *Staphylococcus aureus* (gram + bacteria) was more sensitive to both types of honey than *Escherichia coli* (gram - bacteria). Considering the same dilutions, *A. florea* and *A. andreniformis* honey did not provide the statistical significance on the antibacterial activity ($p \leq 0.05$). Due to the above data, it indicates that not only both types of honey contain nutritional supplements, but they also provide the antibacterial activity.

17-3 ANT (HYMENOPTERA: FORMICIDAE) COMMUNITY DIFFERENCES ASSOCIATED WITH ORGANIC, NO-TILL, AND CHISEL-TILL CROPPING SYSTEMS.

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As part of a larger study of epigeic arthropods, we compared ant species richness, abundance, and community composition in organic, no-till, and chisel-till cropping systems of the United States Department of Agriculture Farming Systems Project in Maryland, USA. Study sites were planted to maize (*Zea mays*) in 2001 and soybean (*Glycine max*) in 2002. Inorganic fertilizers and herbicides were used in the non-organic plots, however insecticides were not used in any of the plots. The organic cropping system used during this study included tilling, poultry litter, and winter cover of vetch (*Vicia villosa*). Ants were sampled using pitfall traps. Trapping resulted in 9,023 ants from 19 species. All species were common to the Mid-Atlantic region of the United States and old field habitats. The most common species in traps were *Lasius neoniger*, *Solenopsis molesta*, and *Tetramorium caespitum*. Non-native species included *Anergates atratulus* and *T. caespitum*. Ants from 'tramp' species accounted for 8723 of the ants caught. The ant community in this study was dominated by 'tramp' ants rather than species which normally dominate old field samples such as *Aphaenogaster rudis*, *Prenolepis imparis*, and *Paratrechina faisonensis*. We also found during 2001 the organic plots (corn) had significantly fewer ants from 'tramp' species and total numbers of ants in traps compared to no-till and chisel-till corn plots. During 2002 the organic plots (soybean) again had fewer ants from 'tramp' species and total numbers of ants in traps compared to no-till and chisel-till soybean plots, although the numbers of ants in the organic plots rose significantly. The mean overall species richness was very low: 2 species per pitfall trap over two years with a maximum of 6. Our results demonstrate a difference in pitfall trap catches of ants between organic farming and farming practices which include fertilizer and herbicides.

17-4 IMPACTS OF LAND USE TYPES ON ANT COMMUNITIES IN A TROPICAL FOREST MARGIN (OUMÉ - CÔTE D'IVOIRE)

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Land use and particularly agriculture is a leading cause of below ground biodiversity loss. However this fauna is known to play a key role in soil fertility and to offer many other ecosystem services. In order to better understand the effects of land use on ants which are major component of the tropical soil fauna, these insects were surveyed in the eight main land use types in Oumé (Côte d'Ivoire). These included primary forest, secondary forest, multispecific tree plantations, 10-years old teak plantations, 4-years old teak plantations, food crops, cocoa plantations and fallows. Modified versions of the ants of leaf litter protocol and monolith method were used to sample the ants. Species richness, abundance, diversity and composition of ants varied among these habitats. Food crops and 4-years old teak plantations were the less species rich land use types. However they diverged in term of species composition. Forests habitats were the most species rich, reflecting their relative integrity. Ant subfamily Myrmicinae and genus *Tetramorium* were surrogate for indicating the pattern of species richness change between land uses. These results illustrated the sensibility of ants to changes in land use types and practices and encourage their inclusion in conservation orientated bio-monitoring.

17-5 Maximum challenge bioassay of the pathogenicity of EPF strains to *Lasius niger* (Hym.: Formicidae) workers and larvae

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The pathogenicity of 33 strains of entomopathogenic fungi (EPF) including *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces farinosus*, *P. fumosoroseus*, *Verticillium fusiformis*, *Lecanicillium lecanii*, *L. longisporum* and *L. muscarium species*, to *L. niger* adults and larvae were tested in laboratory bioassays at East Malling Research (EMR) in 2009. *L. niger* workers held in formicaria in optimum conditions for infection were forced to walk over EPF colonies on agar plates to reach their food source. Subsequent fungal infection of the ants and behavioural changes were observed. The overall aim was to identify species/strains of EPF that were not pathogenic to the ant so that the ants could be exploited for passive transmission to aphids for biocontrol. For each bioassay replicate, 20 worker ants and 10 larvae, from one field colony, were used. An agar plate bearing the test EPF colony, were introduced between the nest and the feeding chamber, which ensured ants had to walk over the EPF colony in order to reach food. The formicaria were held at 23 ˚C and 12L:8D photoperiod. Counts of live and dead workers and larvae were made at 3 days intervals for up to 3 months. Dead ants were removed every 3 days and incubated on damp filter paper at 23 ˚C to check for sporulation. Ant behaviours including location, feeding, cleaning, movement, contacts between individuals, were observed. Initial results showed that all strains of *B. bassiana*, *M. anisopliae*, *P. farinosus* and *P. fumosoroseus* examined were pathogenic, but at different levels and causing high levels of ant mortality over different time scales. One strain of *B. bassiana* and one strain of *M. anisopliae* showed less pathogenicity than the other strains of these species. The strains of *L. muscarium* and *L. lecanii* also showed some pathogenicity, but less than the previous species. There were large differences between strains. Of the strains examined *V. fusiformis* and *L. longisporum* seemed to be safe for *L. niger*.

17-6 Novel Low-Budget Method for Observing Honeybee Behaviour in Temperature Fields

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Temperature is one of the most important factors inside of a honeybee colony: A rather constant temperature in the brood nest area is necessary to rear brood. The adult bees of the colony have to regulate the temperature to compensate environmental dynamics. These behaviours of adult bees involve adapting water collection, collective coordination of airflows through the nest in warm conditions and collective heating and clustering behaviour in cold conditions. The reaction on colony-level to such environmental changes are the result of individual behaviours. We developed an automated 2D arena to enable experiments with animals in complex temperature gradients to investigate changes of individual honeybee behaviour. We use a set of ceramic heat lamps to establish the 2D temperature gradient. The status of the temperature gradient is observed by a matrix of 64 fast-reacting temperature sensors which are controlled by 2 multiplexers (8 channels). The observation of animal behaviour is facilitated by a standard IR-sensitive surveillance camera. For optimal illumination, sets of low-power IR-LEDs are used. The activity of the heat-bulbs is controlled by a off-the-shelf I/O-Board in a standard desktop PC, which analyses the temperature data and adjusts the powering of the heat-bulbs, as well as it records the images from the IR-camera. This setup allows to develop complex and computer controlled spatial and temporal temperature gradient fields. Please notice that all hardware components (except the IR-sensitive camera) are low-budget and the experimental setup can be built with little knowledge of electronics and soldering. Building plans will be made available online on our homepage (http://zool33.uni-graz.at/artlife). All software components – including the control software for the I/O-board – were developed by ourselves and will also be made available online. In the future we plan to test the applicability of our novel experimental setup to other (social) insects.

Oral Presentations

Optimization in natural systems: designing nature-inspired algorithms using social insects

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18. *Optimization in natural systems: designing nature-inspired algorithms using social insects.* Oral

COLLECTIVE VERSUS INDIVIDUAL COGNITION IN THE DECISION-MAKING ALGORITHMS OF ANT COLONIES

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Decision-making by social insects is marked by a tension between group and individual cognition. Choices can emerge from the interactions of many poorly informed individuals, none of which directly compares all options and chooses the best. At the same time, colony members have much in common with solitary insects that regularly make decisions on their own. This creates ambiguity about whether a collective decision is truly an emergent property, or instead results from the choices of one or more well-informed individuals. Using the model system of nest site selection in *Temnothorax* ants, I show that individual workers are fully capable of making the decisions normally carried out by the colony as a whole, provided they have timely access to all options. However, individuals typically lack this full information, and so are more likely to contribute a small part to a highly distributed decision. Furthermore, fully informed individuals are more susceptible than colonies to certain systematic errors that lessen their ability to consistently make the best choice. These results indicate that sophisticated collective decision-making algorithms do not necessarily yield wholesale new cognitive abilities lacking in individual ants. Instead, they expand the scale of problem that can be solved, and refine the efficacy of the solution.

DISCOVERING INSECT ALGORITHMS

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Engineers cannot fail to be impressed by the capabilities, both individual and collective, of insects. But the interaction in bio-inspired engineering is not just one way. We cannot adopt a biological mechanism for use in our technology unless we know what it does and how it works. Consequently, we often gain substantial new insight into what we understand - or have yet to understand - about biological systems from the attempt to re-engineer them. This can be particularly true when we try to build systems that have real-world constraints, such as robots, which can highlight key problems, such as the temporal and physical constraints that limit the plausible solutions. I will discuss these issues drawing on several examples where work in robotics has directly contributed to understanding of biology, focussing particularly on insect navigation behaviour.

SELF-ORGANISED TRANSPORTATION NETWORKS IN THE ARGENTINE ANT (*LINEPTHIMA HUMILE*)

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In most human societies, transportation networks are vital for the movement of resources, people and information. Biological systems, such as ant trail-networks, have been shaped by natural selection, and so may display many of the properties (robustness, efficiency, cost effectiveness) sought after by engineers; consequently, there is increasing interest in biologically inspired solutions to network design. Here we examine the shape, efficiency and construction process of inter-nest networks in the Argentine ant (*Linepthima humile*). Argentine ants use trail networks to redistribute food, larvae and workers among their multiple nest sites. Travelling along trail networks takes energy and also exposes workers to potential danger from predators. Ants in the lab built trail networks that balanced efficiency (in terms of creating `shortest paths' between nests), and cost. Networks formed through an iterative process where an initially inefficient network (with many redundant trails) was gradually pruned down into an efficient final network. Ant density had a significant effect on the shape and efficiency of inter-nest networks. Understanding how biological systems create efficient networks in the absence of centralized control can inform the design of our own transportation networks.

FROM HONEYBEE BEHAVIOUR TO SWARM ROBOTICS

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When they perform alone in a flat temperature gradient (30°C-36°C), a majority of young honeybees are unable to position themselves in a region of their preferred temperature (36°C). However, in a group (6 bees or more) they are perfectly able to cluster at a thermo-induced aggregation site. We investigated this collective ability of honeybees in a series of empiric experiments, realizing that a group of bees is able to solve difficult search problems in a cooperative manner: Bees distinguish local optima from global optima, react to changing environments by reverting collective decisions they have made before and they also show the phenomenon of symmetry breaking whenever they are confronted with more than one global optimum. In addition to empiric studies, we created mathematical models which investigate the collective system from a rather macroscopic point of view. In contrast to these models, we also implemented very specific multi-agent simulations that closely reflect the sensor model and the behaviour of honeybees. These models allowed us to translate the collective behaviour observed in honeybees into a simple robotic algorithm that acts in each individual of a robotic swarm. We showed that such a robotic swarm is able to perform similar collective decision making as honeybees do. Our swarm robotic algorithm was evaluated using a multi-agent simulation of a Jasmine-III swarm that had to aggregate in a luminance gradient field, in a real Jasmine-III swarm and in a swarm of Hemisson robots, which aggregate – similar to honeybees – in a temperature field. In future research, we plan to "extract" a more detailed behavioural program from honeybees and to reflect the morphology and sensor model of bees in more detail in our robotic agents.

BEES AND THE TRAVELLING SALESMAN PROBLEM: HOW TINY BRAINS SOLVE COMPLEX ROUTING TASKS

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Animals collecting resources that are fixed in space and replenish over time, often visit patches in significantly predictable sequences called 'traplines'. Despite the widespread nature of this foraging strategy, we still know little about how long-term spatial memory develops and helps individuals to decide on suitable routes to follow. Here, we investigate whether flower visitation sequences by experienced bumblebees, *Bombus terrestris*, simply reflect the order in which flowers were first discovered, or whether they result from more complex cognitive processes which allow bees to optimize overall flight distances. We conducted laboratory experiments in a large flight room and recorded how bees adjusted their flight movements in a four-artificial flower array maximizing interfloral distances. Starting from a single four-flower patch, we added three new patches so that if bees visited them in the order they originally encountered them, they would follow a long (sub-optimal) route. Bees' tendency to visit patches in their discovery order rapidly decreased with experience. Instead they tended to minimize total flight distance by re-arranging the order in which they visited flowers and selected nearly optimal routes. This resulted in the development of a primary route (trapline), followed by one or two less used secondary routes. Bees consistently used their trapline after overnight breaks, while occasionally exploring completely novel routes. We discuss how spatial scale may influence the navigational strategies used by traplining animals to cope with such highly complex routing tasks, analogous to the well-known travelling salesman problem.

OPTIMIZATION IN NATURAL SYSTEMS: ARGENTINE ANTS SOLVE THE TOWERS OF HANOI

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Many algorithms for modern optimization problems, such as how to construct complex machinery while keeping time and costs to a minimum, receive their inspiration from biological systems, where millions of years of evolution has driven constant refinement. Most cases however only approach solutions for static problems, and the biological inspiration is only superficial. Little is known about how biological systems solve dynamic problems, yet most of these systems evolved under constantly changing conditions, while such dynamic scenarios prove challenging for modern artificial algorithms. We used the Towers of Hanoi puzzle to test whether Argentine ants can solve a potentially NP-hard problem, and whether they can adapt to dynamic changes in the problem. We mapped all possible solutions to the Towers of Hanoi on a single graph and converted this into a maze for the ants to solve. We show that the ants are capable of solving the Towers of Hanoi, and are able to adapt when sections of the maze are blocked off and new sections installed. We show that increasing colony size increases the total network length and number of paths constructed. We further investigated the effect of exploration pheromone on the ants ability to solve the Towers of Hanoi and to adapt to changing conditions. The presence of exploration pheromone greatly increased the efficiency of the resulting network and increased adaptability to changing conditions. Our results are interesting because previous studies have suggested that mass-recruiting ant species such as Argentine ants are incapable of adapting to changing conditions. Our results also suggest that novel dynamic optimization algorithms stand to benefit greatly from stronger biological foundations.

Poster Presentations

Optimization in natural systems: designing nature-inspired algorithms using social insects

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18-1 STABILITY AND VARIABILITY OF THE FOOD-FLOW INSIDE THE NEST ACCORDING TO STARVATION

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Food sharing is interlocked with the development of elaborated social structures. The emergence and the stability of these structures heavily depend on the sharing of information, material and energy. Although food exchanges are crucial to the survival of a society, very few works focused on its dynamics and structure. We monitored the spreading of a radiolabelled sucrose solution inside a nest of *Formica fusca* and looked at the influence of the starvation degree (1, 4 and 7 days starvation) on the stocks and the food flow's characteristics. The stocks exhibited highly stable spatial patterns with heterogeneous load repartition among workers which suggest a division of labour, even in rather homogeneous groups, with few ants holding most of the reserves. The aggregation index borrowed from landscape ecology demonstrated a strong spatial aggregation of the stocks when the colony has been starved for 4 or 7 days. The stocks aggregation is not as visible for 1 day starved colonies. The flow's dynamics are characterized by a decrease proportional to the harvest quantity that acts as negative feedback. This rule applies for all three conditions. Still, the food-flow dynamics and the spatial patterns of the stocks are amazingly stable not only during the experiments but also between colonies and conditions. This new insight on the food sharing process raises many questions on how the colony manage, use and maybe also transform the aliments it harvested. We propose a set of algorithms able to reproduce the robustness and the stability of the food-flow dynamics and the spatial patterns of the stocks. These could be applied to regulate the activity of swarms of micro-reactors.

18-2 SOCIAL INSECTS AS MODELS FOR BIOMIMETIC DESIGN

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Social insects are emerging models for biomimetic design, with applications ranging from optimization to architecture. The recent conference "Social Biomimicry: Insect Societies and Human Design" at Arizona State University brought together biologists, designers, engineers, computer scientists, architects, and businesspeople, to explore how social insects can stimulate design and how biomimicry can enrich social insect biology. We will highlight several examples of social-insect-inspired design and share some of the conference's major insights, including different approaches to biomimicry and ways to promote interdisciplinary collaboration and education. We stress that biomimicry can benefit biology as well as design.

18-3 HONEYBEE NEST-SITE SELECTION AS AN OPTIMISATION PROCESS

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Swarm intelligence is a subfield of biologically inspired computation that applies concepts underlying the collective behaviour of social insects to various problem domains. Bee inspired optimisation is a new trend in this field. Current bee inspired algorithms are based on the bees' foraging and mating behaviour. We introduce a third possible class of optimisation algorithms based on nest-site selection. This class is interesting from an optimisation point of view as it corresponds to a decision-making process (i.e., choosing a single nest-site) in an uncertain and dynamic environment. We designed a detailed model based on the honeybee's nest-site selection process to study the optimisation potential of nest-site selection. Several optimisation experiments have been performed to test a swarm's decision-making ability in noisy and dynamic environments. In addition, the possible application of an iterative nest-site selection scheme to function optimisation has been tested by using simple benchmark optimisation functions.

18-4 EVOLUTION OF SELF-ORGANIZED DIVISION OF LABOR

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Division of labour is a key feature for the ecological success of social insects. Several theoretical models have been developed to explain how complex patterns of colony organization emerge from interactions of individuals obeying to simple behavioural rules. Fixed response threshold models argue that individuals have internal thresholds for responding to external stimuli associated with certain tasks. Division of labour emerges from individuals having different thresholds for different task-associated stimuli. However, an evolutionary perspective that integrates this mechanistic view is largely lacking. We developed an evolutionary version of the fixed threshold model, where thresholds are treated as genetically coded traits and are allowed to evolve. Results indicate that evolution of threshold values that promote specialisation is possible in the presence of costs of task switching. However, under the task choice algorithm used, evolution of specialisation requires a rather large genetic variation, either in initial conditions or as a result of high mutation rates. Our evolutionary analysis deepens our conceptual understanding of the conditions necessary for evolution of division of labour and calls attention to the importance of considering the consequences on colony efficiency when studying mechanistic models of division of labour.

18-5 PHEROMONE COMMUNICATION SYSTEM OF SWARM ROBOTS INSPIRED BY ANT TRAIL FORAGING

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We introduce a pheromone communication for swarm robots. We have developed an indirect communication system of swarm robots, both real and simulated, that is inspired by pheromone-mediated trail foraging of ants. Instead of pheromone and antennae, the robots use ethanol and detect it by two alcohol sensors. The algorithm of trail forming mimics that of ants: when the robots find a prey, they go back to the nest site, leaving the pheromone trail that attracts other robots. The robots exhibits similar patterns to those of ants, such as the relationship between robot number and foraging efficiency, and the transition from individual- to group-foraging associated with vaporization speed of the pheromone. Our system can help understand the collective behavior of social insects in that it can track the robots individually and manipulate the parameters separately, which are often difficult in real social insects.

18-6 Honeybee foragers increase the use of waggle dance information when using private information becomes unrewarding

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Social insect foragers often have access to both social and private information about the locations of food sources. In honey bees (*Apis mellifera*), foragers can follow waggle dances (social information) to obtain vector information about the location of profitable food sources or they can use route memories (private information) acquired during previous foraging trips. The value of vector information is poorly understood and currently debated. Social information should be favoured when using private information has a low benefit. To test this we trained foragers to a high quality sucrose feeder which subsequently became unrewarding. Our results show that as the use of private navigational information became unrewarding, the trained foragers increased the time spent following waggle dances advertising an alternative food source with the same odour. A significant proportion of foragers successfully switched to the food source indicated by dances (13.5% after 1 day, 25% after 2 days). However, the trained foragers also showed a strong attachment to the known but currently unrewarding feeder (4.9 visits in 4 hours over 2 days following the cessation of reward), even after repeatedly following dances advertising a profitable alternative (11.6 dances). Successful recruits to the food source advertised by the waggle dances had more social information about the novel food source, i.e. they followed dances for longer. Our data suggest that honey bee foragers follow a strategy that is quite conservative in terms of switching from one food patch to another, presumably because carbohydrate food sources have a high chance of becoming rewarding again after periods of being unrewarding.

18-7 Collective decision making in honeybees: Environmental attraction factors versus socially driven aggregation

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It is known from literature that bees younger than 24 hours have a temperature preference near 36°C. We showed that they collectively locate themselves at their preferred temperature and almost neglect local optima. Thus they discriminate two different temperatures spots and select the best one. Experiments were conducted whether or not a social gradient can influence this behaviour. We constructed a circular arena and generated a global (36°C) and a local (32°C) optimum with ceramic heat lamps. We used an acrylic cage with fence walls and caged bees inside at the local optimum to generate a 'social gradient' (control: empty cage in both optima). We tested groups with 6 (n=11) and 28 (n=13) bees. After 30 minutes we counted the bees at the global and the bees at the local optimum. Our results show that there is a social factor involved in cooperative thermotaxis of honeybees: In both group sizes the proportion of aggregated bees in the local optimum is higher than in the global optimum, whenever bees were confined to cages there. The proportion of aggregating bees was higher in the experiments with caged bees than in the control experiments. In our control experiments with empty cages, we showed that the empty cages had no influence on the aggregation behaviour of the bees. In conclusion we interpret our findings in the following way: In a temperature gradient (30-36°C) there exists a significant influence of the social context onto the aggregation behaviour of bees. In our experiments we influenced collective decision making of bees and we showed that the thermotactic behaviour of young honeybees is affected not only by temperature but also by a social gradient.

18-8 Spatio-temporal characteristics of honeybee, *Apis cerana japonica*, in shimmering behavior

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It was well-known that Japanese honeybee, *Apis cerana japoinca*, causes a characteristics behavior called shimmering by turbulence, such as air puff or vibration. This behavior is considered a threat behavior against predator. In this research, we have observed collective and individual behavior during shimmering by using high-speed video camera. Spatio-temporal properties are analyzed by image analysis of wing movement in individuals. In the beginning phase, a small number of sensitive workers start to flap their wings, and then workers around them generate same behavior, leading to form a small group of flapping workers. A sensitive workers distribute uniformly on a comb, thus several groups of flapping workers constitute a global behavior of shimmering. Most of workers maintain their flapping only once in certain periods during one shimmering behavior, but there are workers that conduct flapping and stopping their wings repeatedly. Each worker has its period of flapping, mainly ranging from 0.15[s] to 0.30[s]. It is shown that start delay of behavior depends on the distance from the early bird workers and the angle with them, i.e. a worker starts flapping earlier when the distance from an early bird worker is smaller and the angles of their heads are closer.

18-9 Finding home: Inbound searching behaviour in the Australian desert ant *Melophorus bagoti*

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Australian desert ants *Melophorus bagoti* return home after foraging by means of path integration and visual navigation. If these mechanisms do not deliver them exactly at the inconspicuous nest entrance, they engage in a systematic search. We look at the structure of this search pattern in detail. The search pattern consists of loops and is centred on the position where the nest is most likely located. At first, it covers a rather small area, but then gradually extends outward to cover a larger area. The search density is also adapted to the preceding foraging distance, with longer distances leading to flatter, wider search distributions. The search strategy is well described by a double exponential model, lending support to a theoretically optimal Composite Brownian Walk.

18-10 COOPERATIVE THERMOTAXIS OF HONEYBEES IN A COMPLEX AND DYNAMIC THERMAL ENVIRONMENT

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In a thermal gradient ranging from approx. 10°C to 40°C young bees locate their preferred temperature of 36°C, according to the temperature in the brood nest. In a temperature arena with a flat thermal gradient (30°C - 36°C) the majority of single bees exhibits just a slight uphill bias in the gradient and only one third of them are found at 36°C. In contrast groups of bees aggregate collaboratively at 36°C in such a gradient. We studied the flexibility of group behaviour of young bees in a dynamical thermal gradient with temperatures ranging from 30°C to 36°C. We performed the experiments in a circular arena and generated the complex thermal gradient with two heat lamps. Each experiment lasted for 105 minutes. During the first part of the experiment the gradient consists of a global optimum at 36(±1)°C, a local optimum at 32(±1)°C and an ambient temperature of approx. 30°C. We turned off the heat lamp above the 36°C zone after 30 minutes. After a cooling phase the 32°C zone became the new global optimum. We tested groups of 64 bees (N = 20).Within the first 30 minutes the bees aggregate collectively in the 36°C zone. After the heat lamp above this zone has been turned off they realign collectively and aggregate at the new global optimum with 32°C.There is a competition for the free bees between the two zones and bees choose the respective global optimum collectively. With this work we show that groups of bees are able to react flexible on changes in their environment.

Oral Presentations

Behavioral syndromes in social insects: the evolution of behavioral variation between individuals and colonies

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PERSONALITIES, BEE-ALITIES AND BANALITIES

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In recent years, the study of 'personality' has become fashionable in vertebrate behavioural ecology, but there is a risk that this implies a trivialisation of the concept of personality in psychology. Viewing animals as 'persons' is neither conceptually adequate nor likely to generate any scientifically valuable insight. Once we remove the anthropocentric terminology, what's really at issue is individual consistency in behaviour, and its causes and consequences. In these terms, insect behaviour science has been several decades ahead of the work on vertebrate behaviour. Especially in work on social insects, there is a long tradition of how behavioural trait configuration affects specialisation within the colony, and how sensory response thresholds might determine the particular task an individual is most likely to engage in. However, (almost) nothing in animal behaviour makes sense except in the light of learning, and here I review recent work on how individual experience influences the specialisation in insect colonies, and also differences in individual, colony and population learning ability and their ecological and evolutionary significance.

BEHAVIORAL SYNDROMES IN SOCIAL INSECTS: LESSONS FROM AND FOR OTHER SYSTEMS

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In recent years, numerous studies have shown that animals often exhibit behavioral syndromes (aka personalities) where, for example, some individuals are consistently more bold, active, aggressive, social or exploratory than others. Behavioral syndromes are heritable, are associated with neuroendocrine or physiological profiles, and have fitness consequences. My work has focused on implications of behavioral syndromes for a broad range of issues from sexual selection, sexual cannibalism, predator-prey interactions to ecological invasions, including work on insects, spiders, fish and amphibians. Here, I discuss issues and implications of the behavioral syndrome concept for social insects, going well beyond the obvious notion that different castes or individuals with different major tasks (e.g., nurses vs foragers) exhibit different behavioral profiles. Drawing from parallel literature in other animals, I address several potentially important, relatively unstudied questions about the patterns of individual variation within social insect colonies, along with fundamental issues like - why do individuals exhibit consistent behavioral types, what aspects of personality should be correlated, and how does an individual's behavioral type relate to its performance? I then consider the fascinating issue of differences between colonies in personality. How might this be assessed, and how might it be related to emergent issues like colony performance or species interactions? Finally, I discuss ideas on connections between the behavioral types of individuals within the colony, and the colony's overall behavioral type.

TASK REVERSION IN WASTE WORKERS OF *ATTA SEXDENS RUBROPILOSA* FOREL (HYMENOPTERA: FORMICIDAE)

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In leaf-cutting ants, a specialized group of workers is involved in waste management. Since rejected material is hazardous to the colony, it is expected that older workers perform this task, according to the Evolutionary Theory of Senescence. Furthermore, waste workers should be prevented entering the colony, thus reducing risks of pathogen transmission. This scenario is based on age polyethism and does not consider task reversion or flexibility. The central question addressed here was whether the waste workers were capable of performing other tasks in the absence of specialized nestmates. Three *Atta sexdens rubropilosa* colonies of 9-10 L of fungus garden were used; each gave six sub-colonies. These received either waste workers or non-waste workers in addition to pupae and 50mL of fungus garden. Fungal and brood care, foraging and waste manipulation were observed daily until colony death. Survival analyses showed that the waste workers died earlier. Average survival times were time 13.92 and 22.66 days for waste and non-waste workers, respectively. Waste workers cared for the brood ($F= 3.52$, $P= 0.08$) and foraged ($F= 0.03$, $P= 0.87$) as non-waste workers did. However, they were not as efficient in caring for the garden ($F= 4.87$, $P= 0.04$). Activities related to waste manipulation were more frequently performed by waste workers ($F= 11.71$, $P<0.01$). These results show that task reversion does occur in leaf-cutters. Although the colony is not so well maintained, workers still can switch tasks as the social environment demands.

BUMBLE BEE SPACE USE, BODY SIZE, AND RESPONSE THRESHOLDS: VARIATION IS THE KEY TO COLONY SUCCESS.

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Previous studies have shown that variation in worker space use, body size, and/or response thresholds may contribute to the overall division of labor observed in social insect groups. However, although theories of organization in social insects rely on the assumption that such types of variation increase group fitness, there is little supportive empirical evidence. To determine whether worker variation affects group performance, I restricted the range of two types of variation in a bumble bee colony – body size and probability of responding to air temperature changes – by removing those individuals that display extreme phenotypes of either side of the mean. Colony performance was measured as the number of individuals that responded and the time it took the colony to return to a baseline state after disturbance. Two different disturbances were administered to the colony multiple times: temperature increase and debris introduction. Results suggest that variation in response thresholds, and not necessarily body size, have an effect on colony performance with regards to these two tests compared to the control. Furthermore, the results suggest that different response thresholds are not correlated with one another, or with particular body sizes of workers. However, it is possible that individuals have consistent differences, implying possible behavioral syndromes. In conclusion, this study provides the first empirical evidence that variation among workers, in terms of their inherent tendencies to respond to stimuli, positively affects colony performance.

TANDEM RUNNING: WHO TAKES THE LEAD?

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How flexible is the division of labour in social insect colonies? Do the most experienced workers conduct the most demanding and dangerous tasks because they are older and more expendable, or are they the only workers that are equipped, physiologically or through experience, to do such tasks? Alternatively, are young, inexperienced workers able and willing but socially inhibited from such tasks? To discriminate between these hypotheses, we conducted comparative emigrations with specially constructed colonies of workers that were either experienced or inexperienced in a certain task. We evaluated the relative participation and efficiency of these two types of worker in the one-to-one recruitment behaviour known as tandem running; a very sophisticated communication behaviour that has been identified as a form of teaching. Our results show that the inexperienced workers can lead effective tandem runs. However, when experienced and inexperienced workers co-occur the former are much more likely to lead tandem runs than the latter. Overall our results suggest that inexperienced ants are willing and able but usually excluded from leading tandem runs in natural colonies by their more experienced nest-mates. Such flexibility in the division of labour should make the most of experienced ants, when they are available, but should still enable a colony to function when they are not.

REPRODUCTIVE INHIBITION AS A FUNCTION OF GROUP-SIZE IN *B. TERRESTRIS*: α-WORKER TRIES HARDER WHILE β-WORKER RUNS FASTER

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The balance between cost and benefit is a powerful factor affecting decision making in animal societies. Since individuals are striving to maximize their fitness, they are predicted to evolve to take the decision that will best suit their interests under the ecological and sociobiological constrains they are facing. Social insects are good model for testing this balance since their reproduction is skewed, so that the sterile caste is constantly facing the conflict regarding if and when to reproduce. In this study we investigated the effect of group-size on aggression, reproduction and sterility-signal production in small groups of *Bombus terrestris* queen-less workers. We found that aggression by the α-worker towards her nestmates increased with the increase in group-size, although the overall aggression in a group remained constant irrespective to its size. Furthermore, α-worker in small groups (3- and 5-workers) was found to equally partition her aggression between her nestmates while in larger groups (10-workers) she was directing higher aggression towards the top-hierarchy workers. Despite this compensation, lower amount of aggression was directed towards the β-worker and hence the β-worker in larger groups was able nevertheless to develop ovaries. Sterility signal production by the sterile caste in 10-workers groups, but not in smaller groups, was found to be similar to the proportion in full-size colonies. Thence, while small groups reflect interaction of competition, larger groups reflect division of labor in which reproduction is monopolized by a small group of workers while others focus on nest maintaining, pronouncing themselves as non-threatening in order to avoid aggression.

DIVISION OF LABOR SCALES WITH COLONY SIZE IN THE SEED-HARVESTER ANT *POGONOMYRMEX CALIFORNICUS*

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Just as body size has profound consequences for the structure and function of organisms, properties of social groups can be influenced by group size. Social scaling appears to play a critical role in the organization of insect societies, particularly eusocial colonies, which are tightly integrated, adaptive units that vary tremendously in size during development and across species. We examined how the organization of work scales with colony size in the monomorphic seed-harvester ant *Pogonomyrmex californicus*. First, we observed that colonies exhibited higher levels of division of labor (i.e., individuals were more specialized) after 1 year of growth, with 160-337 workers, than when they were 3 months old, containing 10-30 workers. Next, we quantified task performance by 1-year-old colonies that ranged from 30 to 390 workers due to differences in growth rate; again, division of labor increased with colony size, in this case independently of colony age. In neither context was colony size associated with per capita workload, which was normally distributed across workers in most colonies. Finally, we will report results from a complementary experiment in which we manipulated colony size and controlled for other sources of between-colony variation. Our findings thus far indicate that scaling relationships influence the organization of work during colony growth; we are investigating potential underlying mechanisms. Social scaling may yield important insights into the development and evolution of insect societies and other social groups.

BEHAVIOURAL SYNDROMES AND ACTIVITY LEVELS IN ANT COLONIES

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Individual variation and behavioural types in insect societies underpin one of the defining features of extreme sociality: namely the division of labour. Task allocation schemes can range from specialization (“jacks of few trades”) to elitism (“hyper-active jacks of many trades”). Here, we test the hypothesis that elitism can correspond to a behavioural syndrome with multiple positive correlations across the performance of diverse behaviours that persist over time. Activity distributions are typically right-skewed so only a few individuals exhibit high performance (the elites) and the majority perform much less work. Such distributions could occur as the result of positive-feedback mechanisms or simply through mutual exclusion in time or space. For example, threshold models of division of labour demonstrate that more sensitive individuals with lower thresholds for a particular task may exclude others by reducing stimulus levels below the thresholds of their nestmates through task performance. Such a mechanism could give rise to the transformation of many normally distributed biological characteristics into skewed activity distributions that are stable over time. Last but not least, we will suggest that such activity distributions might translate into behavioural syndromes at the colony level and hence may have important evolutionary consequences.

THE LINK BETWEEN THE OVARY, SUCROSE SENSITIVITY, AND FORAGING DIVISION OF LABOR IN THE HONEY BEE, *APIS MELLIFERA*

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There is increasing evidence that, for workers of the honey bee, *Apis mellifera*, ovarian status influences behavioral phenotype. Our work demonstrates that the size of the ovaries has modulatory effects on foraging division of labor. It has been previously shown that European honey bee workers that have been selectively bred to store larger amounts of pollen (High strain) also have a higher number of ovarioles per ovary than workers from strains bred to store less pollen (Low strain). To determine if this relationship was fully reciprocal, i.e. if variation in ovariole number affects foraging behavior, we investigated foraging behavior of African-European hybrid strains selectively bred for variation in ovariole number. The results from this study demonstrated similar relationships to those previously described, supporting the proposed interlinkage between reproductive development and behavioral phenotype. Links have also been demonstrated between the ovary and sucrose sensitivity, the ovary and foraging bias, and sucrose sensitivity and foraging bias. These links suggest a mechanism where the ovary influences foraging by modulation of sucrose perception. We used a flow-rate controlled artificial sucrose delivery system and a proboscis extension response assay to investigate the relationship between the ovary, sucrose sensitivity and carbohydrate collection under highly controlled conditions and in the absence of pollen (protein source). In this study, we found an interaction effect between ovary size and sucrose sensitivity on the quantity of carbohydrates collected. This result supports a proposed mechanism where the ovary impacts carbohydrate collection through modulation of sucrose sensitivity. This in turn may impact protein collection.

Poster Presentations

Behavioral syndromes in social insects: the evolution of behavioral variation between individuals and colonies

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19-1 Queen behavior in *Metapolybia sp.* (Hymenoptera: Vespidae) related to colony cycle

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The tribe Epiponini is mainly characterized by the foundation of colonies by swarm, a variety of morphological caste syndromes, polygyny and cyclical oligogyny during the colony cycle. All these features vary across taxa and may have an important influence in colony life and females' behavior. The study of female's behavior in colonies is of great interest in order to understand the interactions between females and how it can be related with different aspects of colony life. We studied the behavior of queens in five colonies of *Metapolybia sp*. in Brazil performing direct and indirect observations by removing the envelope and observing individually marked females. We classified as queens those females who laid eggs, had their ovaries developed and presented sperm cells inside their spermatheca. Colony 1 (C1) was at an initial stage of worker emergence, C2 at a male producing stage, C3 and C4 were at a pre-emergence stage, and C5 was at a mature stage of worker emergence. Females showed one behavior "dance display" (DD) that could be a test of dominance performed by workers and queens. Queens showed one display of dominance behavior, "bending the abdomen" (CA). DD and CA behaviors were less frequent in C3, C4 (initial stages) and C5 than in C1 and C2 (later stages). This reflects that at initial phases of colony cycle, a stage when workers are needed, females' oviposition may be more permissive than in later phases, were workers population is larger enough to maintain colony needs. In C5 (mature stage) behavior tests were less frequent because queen number could be stabilized during this phase. Interactions between females of studied colonies show that smaller societies could have interactions as complex as those reported for larger societies. In the case of the Epiponini, complexity of female's interactions could be more related with other colony features such as colony cycle and cyclical oligogyny, rather than colony size itself.

19-2 Non-invasively observing social insect behaviour using diagnostic radioentomology

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X-ray Computerised Tomography is effective for non-invasively visualising internal and external detail of social insects and their behaviour. Research continues on MacroCT and MicroCT for entomologists, collectively termed Diagnostic Radioentomology (DR). For MacroCT, human body scanners were used to assess *Trigona carbonaria*, *Amegilla holmesi*, *Bombus terrestris* and *Apis mellifera*, *Nasutitermitinae Spp*., and *Formica Spp.* behaviour. Castes were accurately identified. Evidence of a fluid level meniscus in natal cells, not previously reported, incidence of parasitism by *Miltogramma Spp*. and outbreaks of *Ascosphaera Spp*. were identified. DR enabled visualisation of egg laying, juvenile development and cell provisioning in *B. terrestris* and tracking of small hive beetle in *T. carbonaria*. Segregated foraging/feeding was evidenced for the first time in *A. mellifera* using radiographic contrast. For MicroCT bench-top and synchrotron systems were used to assess internal/external morphology and physiology of *T. carbonaria*, *A. holmesi*, *A. mellifera* and *Proplebeia dominicana* (~20MA trapped in amber). External morphology of coxae, trochanters, tibiae, tarsi of each leg including broadened hind basitarsi, antennal scapes and the various parts of the tongue including the proboscis and labium were visualised. Individual facets of the eye were barely discernable but they and fine detail of hairs on the body and legs were better detailed with synchrotron methods. Passage of nutrients from gut to haemolymph was visualised for the first time using labelled food. DR has advantages over traditional methods for non-invasively observing social behaviour of insects, for visualising scientifically valued specimens trapped in amber or museum specimens and for following insect lifecycles from egg to imago.

19-3 Context-dependent eavesdropping by a dominant stingless bee species

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Stingless bees (Apidae, Meliponini) are important tropical pollinators that display complex and diverse communication mechanisms. Species in several genera mark high-quality resources with recruitment pheromones. Because these marks are in the public domain, they are susceptible to eavesdropping by both conspecific and heterospecific competitors. Stingless bees thus provide a unique system for studying interceptive eavesdropping between non-cooperating competitors. We investigated the responses of a dominant species, *Trigona hyalinata*, to the recruitment pheromone of a subordinate species, *Trigona spinipes*. These species have overlapping geographic ranges, floral preferences and pheromone chemistries. Our results suggest stingless bees' eavesdropping responses are complex behaviors that vary with dominance, pheromone strength and location. *Trigona hyalinata* switches between attraction and avoidance of *T. spinipes* pheromone in a context-dependent manner. This behavior is consistent with the hypothesis that stingless bees eavesdrop optimally, maximizing net benefit of scouting trips by minimizing costs due to conflict and prolonged search. We present empirical and theoretical evidence to support this hypothesis.

19-4 Biogeochemistry and the life histories of ant colonies

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On ecological and evolutionary scales, the structure of social insect colonies responds to environmental conditions and limiting resources. Colonies may express differential colony sizes, caste ratios, reproductive investment, growth rates and trophic positions. Presenting the findings from a community of ants throughout a Costa Rican tropical rain forest landscape, I demonstrate that limitations of macronutrients and micronutrients are broad clear predictors of the life history strategies of the litter-dwelling ant community. A series of experiments were conducted to manipulate available resources and predictions were tested across naturally occurring environmental gradients. I have found that the properties of the resource base account for much of the site-specific differences in colony life histories across a single landscape.

19-5 DECISIONS AND ADAPTATIONS: SYMPATRIC CONGENERIC AUSTRALIAN ANTS OCCUPY DISCRETE TEMPORAL NICHES

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We discovered a closely related group of four sympatric ant species of the genus *Myrmecia* that range from being exclusively diurnal, diurnal-crepuscular, crepuscular-nocturnal to being exclusively nocturnal. We are trying to understand the factors that restrict them to these temporal niches and identify the associated costs. We find that temperature and competition between *Myrmecia* species are not the factors restricting them to their respective activity periods. At this stage, we cannot rule out predation pressure, competition from other ants and the temporal availability of food resources as potential drivers. We do find that visual system properties of the four species are finely tuned to cope with the ambient light levels the ants experience at their particular time of activity. This is even true for the different castes / sexes within a single species that in some cases are active at different times of day. We tracked the paths of foragers with cm accuracy using Differential GPS. We show that both diurnal and nocturnal ants navigate visually: ants that are displaced within 10m of their nest re-orient quickly and head straight for the nest of for their food trees. When displaced outside their familiar landmark area, displaced ants head into the direction indicated by their path integration system. The time at which nocturnal ants begin activity throughout the year is independent of temperature, but strictly determined by the ambient light intensity around sunset time. This suggests that whatever factors have caused the evolution of temporal niche partitioning in these ants, their activity patterns are now determined by their visual system properties that appear to restrict them to foraging at particular light levels.

19-6 NO TRADE-OFF BETWEEN LEARNING SPEED & ASSOCIATIVE FLEXIBILITY: A REVERSAL LEARNING TEST WITH MULTIPLE BUMBLEBEE COLONIES

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Potential trade-offs between learning speed and memory-related performance are thought to be important factors in the evolution of learning. Here, we test whether rapid learning interferes with the acquisition of new information using a reversal learning paradigm. Initially, individual bumblebees (*Bombus terrestris*), from multiple colonies, were trained to associate yellow as a predictor of floral reward (initial phase). The association between colour and reward was then reversed, meaning bees then had to learn to visit blue flowers (reversal phase). We demonstrate that variation in learning speed among bumblebee colonies in the initial and reversal phases of this task are positively correlated. Hence, colonies which were fast to learn the association between yellow and reward also reversed this association rapidly. These results are inconsistent with a trade-off between learning speed and the reversal of a previously made association. On the contrary, they indicate that colony differences in learning performance and flexibility could reflect more general differences in colony learning ability. As such, results from this study provide additional evidence to support the idea that rapid learning has adaptive value.

19-7 Using metabolic scaling to examine how ant colonies work: the case of *Pheidole* majors

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Ant colonies are superorganisms with emergent traits that, for some species, reflect the combined activity of physically distinct worker castes. Although larger castes have high production costs, they are thought to save their colonies energy by efficiently performing specialized tasks. However, because workers are generally idle until sensing specific stimuli, their maintenance costs may be an important component of colony-level investment. I used metabolic scaling to examine the maintenance costs of dimorphic major and minor *Pheidole* castes across levels of colony organization (e.g. individual, group, colony). Majors from three species had lower mass specific metabolic rates than minors because of allometries at both individual and group levels, and subsequently lived longer when starved. Thus, large major castes may offset their production costs in both their idle and active states. The slope scaling metabolic rate from incipient to reproductive colonies of *P. dentata* (~colony mass$^{0.89}$) fell between the slopes for minor groups (~group mass$^{1.04}$) and major groups (~group mass$^{0.79}$) and thus appears to reflect developmental shifts in subunit mass and number and their offsetting effects on per-capita energy demands. These results highlight how metabolic scaling may help visualize the energetic correlates of emergent behavior and unravel the mechanisms governing colony organization.

19-8 Queens or workers - who decides about reproductive dominance in the ant *Leptothorax acervorum*?

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Ants of the species *Leptothorax acervorum* are distributed over large parts of the northern hemisphere. In southern Europe its distribution is limited to the mountains. The species exhibits a conspicuous social polymorphism, e.g., in the number of reproductive females per colony and reproductive skew. In contrast to colonies from Central and Northern Europe, colonies from Central Spain are known to be functionally monogynous (only one out of several queens is laying eggs). Establishing dominance hierarchies among queens is one way to overcome competition for reproduction. Here, we show that aggression between queens as well as between workers and queens contribute to the formation of reproductive hierarchies.

19-9 The Winner Takes It All, The Loser Standing Small: First Evidence of Winner and Loser Effects in a Eusocial Species

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The earliest documented study on dominance hierarchies in animals dates almost a century back. Since then a lot of work has been done to understand the costs and benefits associated with position of an individual in the dominance hierarchy. In many species, a dominant individual has preferential access to food, mates and other important resources. What makes certain individuals more dominant than others? In answering this central question, studies have mostly focused on properties inherent to an individual. In the last few years, however, a substantial pool of work has come up to suggest that social interactions can influence how an individual perceives its own strength versus others' strength and this in turn may affect the final outcome of a contest. Most of these studies have looked at the effect of prior experience of winning and losing a contest. These effects have come to be recognized as winner and loser effects. Simply put, winner and loser effects mean greater chance of winning or losing a contest owing to a previous win or loss, respectively. We carried out a study on Indian paper wasp, *R. marginata* to see whether winner and loser effects exist and affect a contest outcome. Each contest was staged between a naive individual and an individual with a prior experience of winning or losing. It was found that previous winners won more and previous losers lost more, suggesting that both winner and loser effects exist in *R. marginata*. Moreover, there was no significant difference between the relative magnitudes of winner and loser effects. Preliminary results also suggest that winners are more prone to initiate new interactions and losers are more prone to avoid interactions. To our knowledge, this is the first such study reported in a eusocial species, and may give us further insight to the determinants of behavioural dominance and how dominance hierarchies are built in group-living animals.

19-10 Activity pattern and metabolic cycle of a South American leaf-cutting ant, *Atta sexdens rubropilosa* (Formicidae)

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The purpose of this study was to analyze the correspondence between activity patterns and metabolic cycles of ants under the working hypothesis that maximum activity matches with maximum whole-animal energy cycling. To test it, we investigated activity cycles of a South American leaf-cutting worker ant, *Atta sexdens rubropilosa* (Hymenoptera: Formicidae). Activity levels were monitored among foragers of a natural colony and two laboratory colonies at each 3rd of 24h cycle. Additionally, we measured sVo_2 [ml O_2 /(min x mg)] of forty individuals at each hour of 24h period. Activity patterns were comparable among colonies with a characterized bi-modal action over 24h under natural photoperiod. Colonies appeared to have one peak at afternoon with duration of 3hrs and another with 4hrs at night. Both colony types exhibited longer descend phase as well, starting out-of-then-nest activity earlier in the morning up to late mid-day. During this bout, their foraging activity was about to even. On metabolic events, they had higher sVo_2 during night than daytime. A progressive increase of sVo_2 built up from afternoon to midnight and thereafter turned to fall gradually up to morning. Their physical activity cycle was approximately synchronized with their metabolic events. All results said about their nocturnal behavioral orientation. A main assumption of this study is that temporal pattern of activity may have adaptive and evolutionary significance and are shaped by natural selection to allow for better task specialization and proper integration of individuals into society. Under this adaptive scenario, our data would suggest that physiological cycles allow for enhanced activity at times of day in which out-of-the-nest activity is not necessarily maximum. A simple relationship between maximum foraging and maximum metabolic rates would do not explain our data. A better understanding of daily cycles of activity, outside and inside the nest, may help to understand these patterns.

19-11 Behavioral syndromes on an individual and colony level in ants

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Personalities or behavioral syndromes are found in many animal species, in which individuals behave consistently through time and across contexts. These suites of correlated behaviors describe different behavioral types of a population. In social insects, individuals of different morphology and age were known to behave differently. We investigated whether behavioral types can also be found among similarly-aged ant workers of the same caste and colony. *Leptothorax acervorum* workers were subjected to three tests (explorative, aggressive and social behavior) at day 30 and 60 of their life. In addition, we analyzed division of labor by observing the position and behavior of focal ants in their nests at two different time periods. The cuticular hydrocarbon profiles, the body size and ovary development of workers were determined subsequently. Ant workers behaved consistently over time and across contexts, independent of age. Exploration on day 30 correlated positively with exploration on day 60. Brood care behavior was associated with exploration. Observations within nests gave similar results. Albeit workers generally progressed to more outside tasks with age, ants, which were found close to the brood at day 30 were more often close to the brood at day 60. Cuticular profiles were associated with different behaviours and larger workers performed more brood care and had more developed ovaries. In a second trial series we asked whether different behavioral types can be also found on the colony level. Using the same three experimental set-ups, we tested 10 workers each of 39 *Temnothorax longispinosus* colonies. We found consistent differences in behaviour between workers of different colonies in all three behavioural categories. Besides both on the individual and the colony level explorative colonies and workers showed less brood care behaviour, but more aggression. These findings indicate that the behavioural syndrome approach can be successfully applied to social insects.

19-12 Ultramorphology aspects of hairs and sensilla present in female wasps *Trypoxylon rogenhoferi* (Hymenoptera: Crabronidae)

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Most Hymenoptera form colonies those can be temporary or permanent. The solitary wasps of *Trypoxylon* genus capture their prey (spiders) for supplying their nests. *Trypoxylon rogenhoferi* presents a wide geographical distribution, occurring from Brazil to Argentina, and nesting exclusively in pre-existing cavities with subdivisions in cells which are provisioned with spiders captured and paralyzed. To perform this work, nests containing eggs and larvae were collected and were subsequently sent to the Laboratory of Entomology, Federal University of Acre, Rio Branco, Acre, Brazil, where the individuals reached the adult stage. After this adults were transported to the laboratories of UNESP, IB, Rio Claro, SP, Brazil where they were processed for observation under a scanning electron microscope Philips SEM 505. The ultrastructural analysis revealed a total covering of the body with a large number and variety of hairs and sensilla, distributed throughout the insect body. They have morphology, size, and diameter highly differentiated, varying according each region. Interspersing them were seen drops of secretion, including in the openings of cuticular pores, suggesting that many glands must be present in epidermis of these insects showing the need of large amounts of secretion to maintain its physiology and behavior. The antennae were fully covered with hairs and sensilla, extremely short and with a diameter greater than those found distributed throughout the body. Along the hairs a great amount of droplets of secretion was found, indicating that in the antenna are also active glandular cells in synthesis and secretion of chemical substances, probably of pheromonal action. In the terminal region of the abdomen there was a tuft of long hairs, suggesting that they are responsible for directing the poison at the moment that it is released to the outside (in the situation of predation).

19-13 Division of labour by division of risk

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Solitary and eusocial animals for balancing safety and food requirements adaptively allocate foraging effort in patches that differ in potential risk. Additionally, colony members of social insects can increase colony fitness by minimizing the effect of a forager's death. “Division of labour by division of risk” hypothesis predicts that foragers with short life expectancy have a small future input into energy gain of the colony, thus their loss for colony fitness is less harmful than the loss of foragers with long life expectancy. There is some evidence of life expectancy dependent transition from nest tasks to foraging, but it has remained unclear whether foragers' decision to perform foraging in risky circumstances is also directly dependent on their life expectancy. We tested this hypothesis by using laboratory colonies of the ant *Myrmica scabrinodis* and creating food patches for them differing in level of risk. To do this, we manipulated the distances, temperatures or the presence of competitors on foraging patches. Foragers' life expectancy were shortened by treating them with carbon dioxide or by removing their propodeal spines. Both treatments significantly shortened worker life expectancy in comparison to untreated control ants. Next, we showed that foragers with an experimentally reduced life span undertake food collecting in risky circumstances more frequently than control foragers. This implies that ant workers adjust their threshold engaging in foraging under risky conditions according to their life expectancy.

19-14 "Personality" in bumblebees: individual consistency in response to novel colours

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It is now recognised that many vertebrates and a few invertebrates do show individual-specific consistency in their behaviour across time and context, sometimes in ways that can be paralleled with human personality. Our work aimed at assessing behavioural consistency in a social insect: the bumblebee *Bombus terrestris*. We focused on a behavioural dimension commonly used in personality studies: the response of an individual to novelty (neophilia/neophobia spectrum). We used a foraging paradigm to quantify individual bees' response to novel flower colours and to assess the repeatability of this response over time. As for vertebrates, most individual bumblebees respond to a novel stimulus by increasing the time they spent investigating it compared to known stimuli. Using a new statistical approach, the Consistency Model, we found that individual bees tended to be consistent in their response to novelty over a few hours but were not consistent in their behaviour over three days. We conclude that for the neophilia/neophobia paradigm used here, bumblebee foragers do not fulfil the criteria for animal personality in the common sense of the term. Instead their behavioural response to novelty appears to be plastic, varying on a day to day basis.

19-15 ROAD TO REPRODUCTIVE CASTES: FACTORS AFFECTING OVARIAN DEVELOPMENT IN THE PRIMITIVELY EUSOCIAL WASP *ROPALIDIA MARGINATA*

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Colonies of the primitively eusocial wasp *Ropalidia marginata* consist of a single fertile queen, and several sterile workers. Queens are the sole egg layers in a nest and are characterized by developed ovaries, consisting of several mature and developing oocytes at various stages, whereas worker ovaries seldom have developed oocytes, and most workers show no ovarian development. When freshly eclosed virgin females were isolated in laboratory conditions, about half of them founded nests and became egg layers, but we have no information on the rate of ovarian development for egg layers and non egg layers. Our current study includes ovarian development in a similar experimental setup. To study factors affecting ovarian development, we isolated a total of 703 freshly eclosed females from 37 nests and maintained them in solitary conditions, with ad libitum food and resources. Animals were randomly sampled at predetermined fixed ages ranging from 0 to 200 days. Data was collected on females *viz*. ovarian development, body size, rate of feeding, nest founding (egg laying) ; natal nest parameters *viz*. total number of cells, number of females, proportion of empty cells and environmental parameters *viz*, day (month/season) of eclosion and mean monthly temperatures at eclosion. Non linear least square modeling for curve fitting was used to compare four distinct models of rates of ovarian development. The best fit model amongst them indicates increase in ovarian development from zero day old to 60-80 day old females, with a slight decline thereafter. Multiple regression and Logistic regression analysis reveals age, rate of feeding, proportion of empty cells and season as significant predictors of ovarian development.

Oral Presentations

Insect societies as complex systems

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ANT COLONIES AS COMPLEX SYSTEMS

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One of the fundamental challenges in complexity sciences is to study systems that allow controlled experimental manipulation in order to develop testable models and new theories. Ant colonies are a prime candidate for complex systems allowing controlled laboratory experiments as they exhibits all the characteristics of a complex system: Ant colonies are self-organised adaptive systems whose macroscopic (colony-level) emergent properties are induced from the interactions on the microscopic level among the individual ants and their interactions with the environment. Studying spatio-temporal dynamics of these super-organisms yield insight into how the complex system functions. Movement is the observable signature of behaviour. In social insects, studying the movement of individuals within the confines of the colony nest is crucial for understanding their social organisation. Here we show our first results from studying the movement patterns of individual workers within an ant colony. We manipulated population density by offering each colony to occupy in a temporal sequence of nests with increasing or decreasing area. We found that the distribution of individual average moving speed is continuous at both high and low population density. There is also evidence of some constraint on individual movement at high density. Instantaneous speed is lower when the nest area per ant is smaller. Our results also show that path lengths follow a broad distribution and that movement bout duration has a fractal character. These two signatures of a complex system pose the question whether the observed individual movement patterns are due to interactions or to individual ant behaviour.

COMPLEX FORAGING PATTERNS IN ANTS: THE KEY ROLE OF WORKERS' DENSITY AND SOCIAL CONTEXT

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The ant society is a complex dynamic network in which several nestmates interact at a local scale and make a huge number of decisions at every moment. These individual decision rules as well as the resulting collective behaviour are subject to changes that make insect societies adaptive and functional systems. Relationships between system agents are mainly non-linear and founded on both negative (damping) and positive (amplifying) feedbacks – both contributing to the emergence of a diversity of social structures. As for other complex systems, collective patterns are very sensitive to the topology of the dynamic network as well as to the density (number and/or rates) of interactions between workers. In the case of ant foraging, exploitation patterns strongly depend on colony size in a non-linear and discontinuous way: the density of nestmates' interactions influences the occurrence as well as the transition from one system state to another. However, ants do not passively undergo such a density-dependent structuring effect but instead, can play a proactive role in pattern formation. We discuss the ways workers can "assess" nestmates' density and "act upon" some parameters of the system in order to invoke the onset of qualitatively different collective structures. Moreover, since ant societies are dynamical systems that change over time, they exhibit hysteresis with prior states having an influence on present states. We shall see how ant individuals could keep track of prior states and accordingly tune their individual behaviour. Special attention will be given to the role of area marking as a cue for ants 1) to assess current and past nestmate density, 2) to shape their foraging behaviour and communication and 3) to orient the way through which the whole system can evolve.

INTERACTION NETWORKS CONTROL INFORMATION FLOW AND ENERGETICS IN ANTS

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Most complex systems are made of far less complex components, but achieve high functionality. Larger systems are also usually more energetically efficient. How is this possible? Many have hypothesized that simple components with local senses and small memories can establish communication pathways that enable efficient information flow and resource transfer. Ant colonies are a model system to approach this question: larger colonies are more energetically efficient, and workers must succeed at resource gathering and brood care without any centralized control. However, very little is known about how real ants actually interact. I have analyzed the complete set of time- and space-resolved interactions between all individual ants in twelve filmings of *Temnothorax rugatulus* colonies. The resulting communication and resource transfer networks have structures very different from those in any human or human-engineered system. I test two models of network dynamics and find that ant interaction is reciprocal and regulated in ways that give the queen a central role but are not yet fully explained. I also speculate that the interaction network controls metabolism by mediating resource transfer. Thus the scaling of network topology may be the origin of allometric metabolic scaling, and so explain the increased energetic efficiency found in larger colonies.

EMERGENCY NETWORKING: FAMINE RELIEF IN ANT COLONIES

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There is increasing recognition, in a wide range of disciplines, that the local and global structure of interaction networks can tell us a great deal about a system of interacting agents. There are now many examples of vertebrate animal social networks, which tend to sample interaction and association patterns. There are rather fewer examples of networks constructed by following a process through a population, and most of these are for invertebrate species. We have constructed the complete network of liquid food transmission (trophallaxis) between individuals after 48 h of starvation in four colonies of *Temnothorax albipennis* ants with individually marked workers. We analyse the feeding pathways in terms of the timing, order and duration of events, the space-use of individuals within the nest, and the different roles played by individuals during the first 30 minutes of famine relief.

THE STATISTICAL PHYSICS OF DECISION-MAKING IN INSECT COLONIES

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The stochastic methods of statistical physics provide tools to derive the emergent macroscopic behaviour of a complex system from a microscopic description. We apply these techniques to analyse collective-decision making in social insect colonies, allowing us to derive the colony-level behaviour from an individual-level model. This contrasts with the traditional approach where a differential equation model, with or without arbitrary noise terms, is assumed. Social insect colonies vary in size from on the order 100 to 10,000,000 individuals, and such a statistical physics approach allows us explicitly to derive equations for both the average behaviour and the noise in the system, across this entire scale. We develop such a framework by building upon an existing stochastic model of opinion formation to model the decision-making processes in emigrating ant colonies. This new model is both driven by and evaluated against results from experiments with the rock ant *Temnothorax albipennis*. We begin with a microscopic master equation description of relevant individual-level interactions in the colony. For biologically realistic colony sizes, we then derive equations describing the emergent macroscopic behaviour of the colony as a whole, including the important stochastic fluctuations about this average via a Fokker-Planck equation. This allows us to elucidate rigorously the role played by the individual-level phenomena of direct switching in the colony-level decision-making process, which optimality theory has predicted to be of crucial importance, and which we compare with our experimental results. This illustrates the power of the stochastic methods of statistical physics for understanding social insect colonies as complex systems.

SPATIAL DISTRIBUTION AND SOCIAL NETWORKS IN THE ANT *ODONTOMACHUS HASTATUS*

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The rate of interactions and information transfer among individuals play a key role in the organization of animal societies. Graph theory has provided valuable insights into the functional organisation of several complex systems where interactions among their components are central. Social insects display key network attributes appearing consistently across biological complex systems. The network approach is thus expected to significantly increase our understanding of the organization of insect societies. Using RFID technology, we designed an original experimental set-up which allowed to associate the identity and spatial coordinates of each individual for several consecutive days. Experiments were performed on colonies of the ant *Odontomachus hastatus* containing fifty or more individuals. We examined how the removal of the queen influenced the networks of social interactions among workers. Ants were individually marked and their spatial position in the experimental nest was sampled every 8 minutes during one week. From the data sets collected, we obtained the social networks and used mathematical tools developed in the framework of graph theory to describe the structure and functional properties of networks with a particular emphasis on the spatial distribution of workers.

TIME IN AN ANT NETWORK

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In ant colonies workers perform various tasks some of which are performed around-the-clock (e.g. nursing) whereas others are executed at specific times of the day (e.g. foraging), hence requiring the colony to keep track of time. But how an ant colony keeps track of time is puzzling because only a fraction of workers, those leaving the nest, have access to temporal input through sunlight. Here, we used our newly developed method based on automatic video-detection of individually labeled ants to track the movement and interactions of all individuals in 4 ant colonies continuously for 20 days. We then inferred the activity patterns and social network for all individuals. First results reveal that the activity rhythms of individuals vary greatly. By contrast, the colony rhythm approximates 24 hours. Similar results were found when considering subgroups of workers. Altogether our results indicate that rhythms at the group level differ from those of its constitutive members suggesting that time keeping might be a group feature in social insects.

COMPLEX STRUCTURE FORMATION: FOUR-DIMENSIONAL ANT NEST ARCHITECTURE

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The nests of social insects are examples of complex structures produced by self-organisation. Understanding the form and development of such structures has implications for our knowledge of other complex phenomena such as foraging trails, neural networks, plant roots, transport systems, and computer and online social networks. The dynamics of ant nest excavation has proved difficult to study. We combined Micro-CT scanning and time series experiments to provide the first high-resolution four-dimensional reconstructions of individual ant nests. We also tested the hypothesis that features within the sediment can influence the architecture of ant nests. Our results demonstrated that the growth of nests was logistic. The ants also exploited and excavated horizontal tunnels along the planes between layers of sediment. Such layering within natural sediment profiles could therefore explain the conspicuous feature of regularly spaced horizontal chambers observed in some ant nests. This demonstrates that other self-organised biological or engineered structures may derive some of their sophistication from capitalising on larger scale patterns within their environment.

GROUP DYNAMICS AND RECORD SIGNALS IN THE ANT *TEMNOTHORAX ALBIPENNIS*

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Multi-component systems in which rates of change decrease rapidly over time (specifically, according to the inverse of time) indicate that strong interactions between component parts are important in governing the observable events. Systems that display such dynamics are termed 'glassy'. We have recently provided the first experimental evidence of glassy dynamics in a biological system. By studying ant colonies, as complex systems, we have strong evidence that interactions among the populations within the nest organize departures of workers from it. In this talk I will describe two experiments that manipulate the demography of the society, and hence also the pattern of interactions between the individual components- the ants. As the glassy characteristics are degraded in both manipulations, we conclude that the specific pattern of interactions produced by the 'wild-type' demography is a key component in this phenomenon. Our work provides a new foundation for understanding what properties of the interactions produce the glassy behaviour. For this reason, we believe that our work is not only pioneering, but will be seen to be of generic importance in biology way beyond the realms of social insects.

Poster Presentations

Insect societies as complex systems

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20-1 SPATIAL ORGANIZATION OF WORKERS OF *POLISTES DOMINULUS* ON THE NEST IS NOT REGULATED BY AGE

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By using an ecological analytical approach (home range analysis), we demonstrated that the positions of wasps on *Polistes dominulus* combs are spatially structured. Each active wasp has its own small fidelity area on the comb. Areas patrolled by workers varied in shape and size, with no relation to time spent on the comb, wasp density or position of immature brood. However, this analysis is limited to a single day of activity on the nest and with no information about the workers' age. We could expect that each individual is able to change its own spatial pattern during its lifespan and over the colony cycle. Here, we assessed the existence of a "temporal spatial polyethism" among workers by evaluating whether at workers age changes in their spatial preference occur, and, whether workers of different ages belonging to the same colony patrol areas different in shape, size or position on the comb. After marking all the workers, each colony was video-recorded four times, every 15 days, from May to July. Each recording session consisted in 7 hours in a single day with a time laps of 1 second every minute. The area occupied by each wasp on the comb was obtained using 95% (home range) and 50% (core area) kernel density isopleth estimates, calculated according to its occurrences in all the frames. Statistical analyses show that workers' age do not regulate nor influence their use of space (i.e size, shape and position of both core area and home range) on the nest and the grade of superimposition with other workers. In contrast with our expectations, we did not find any temporal spatial polyethism among workers of *P. dominulus*. Our data shows that workers fidelity area changes during the colony cycle. Thus, the results suggest that, among workers, the mutual influence in the use of space may be based on a daily self-organization process not affected by workers' age.

20-2 HONEY BEE POPULATION DYNAMICS - FINDING THE INTERACTIONS BETWEEN HIVE AND LANDSCAPE FACTORS

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There have been substantial declines in the number of honey bee (*Apis mellifera*) colonies in Europe and the USA over the last 10 years. No single factor has been identified to cause these losses, but instead the interactions of a variety of stressors is believed to weaken honey bees such that colony health is severely compromised. Factors considered potentially important are parasites and diseases, availability of forage, beekeeping practices and exposure to pesticides. To assess the relative impact of these factors, we will develop a colony model that integrates honey bee and *Varroa* dynamics, transmission of viruses and is flexible to include further factors like beekeeping activities and pesticide exposure. Nectar and pollen availability will be derived from a spatially explicit landscape model. The colony dynamic model and the landscape model will be connected via an individual-based foraging procedure. Data for parameterization will be obtained from the literature and from our own experiments. In this empirical part of the project we will focus on differences in development and foraging performance between healthy and diseased colonies. For validation of the model we will take advantage of the considerable datasets already existing (e.g. *COLOSS*, German Bee Monitoring Project).

20-3 ANT COLONY PHENOLOGY: THE IMPLICATIONS OF ENDOGENOUS VERSUS EXOGENOUS REGULATION OF SEASONAL CYCLES

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Most temperate ants exhibit seasonal cycles in development and physiology, and while these annual rhythms are largely determined by factors endogenous to the colony, they can also be modified by environmental conditions. In contrast to many other insects, ants rely less on photoperiod and more on temperature as the primary environmental cue for regulating seasonality, thus there is strong potential for significant climatic change impacts on ant phenology. Moreover, the degree to which ant colonies modify their endogenous rhythm in accord with environmental temperature depends on the species, thus global warming may also cause large perturbations to ant communities as a whole. To explore the consequences of endogenous versus exogenous control over phenology, particularly under a warming regime, we construct an ordinary differential equation model. We then use this model to test the hypothesis that a higher degree of exogenous control can provide a selective advantage in terms of adapting to a novel climate. In contrast with expectation, however, model analysis suggests that the advantages and disadvantages of exogenous control are more subtle, and depend strongly on the details of the novel climate to which the ants must adapt. More specifically, we find that exogenous control benefits a species when summers become warmer or winters become colder, but can be catastrophic when summers become colder or winters become warmer. The implications of our study will be discussed with special emphasis on the optimal use of temporal information sources in a changeable environment. In particular, we will highlight the importance of seasonal information processing as a fundamental determinant of species specific sensitivities towards climatic change.

20-4 SOCIAL ORGANIZATION OF PONEROMORPH ANT COLONIES: NEW INSIGHTS FROM RFID AND DATA MINING

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Animal societies are dynamic systems characterized by many interactions between individuals. Such dynamic structure stems from the synergy of these interactions, the individual capacities in information treatment and the diversity of individual responses related with division of labour. We investigated the relative roles of social information, individual experience in the organization and colony performance during migrations (imposed moving nest). We were able to monitor automatically the ants present in different nest room and passing through the nest entrances individually using passive radio-frequency identification (RFID) technology, a new procedure as applied to social insects. This method allowed the individual measurements to activity patterns of large numbers of individuals over several weeks. This makes the technology most suitable to study areas which can be addressed with data on individual ants passing a point or points, such as foraging dynamics or division of labour between spatially separated tasks. The analysis of the collected data uses modern methods of data mining, based on a new unsupervised learning algorithm (DS2L-SOM) which is an effective neuro-inspired clustering tool to find and simply represent a significant amount of information about the structure of data. The interpretation was also completed using graphical model methods. Very encouraging results have been obtained improving our understanding of the dynamic of the colony moving. These results encourage us to explore other similar phenomena where the control of individual abilities as well as their integration at the collective scale are needed, like in the study of flux regulation in social group.

20-5 Group-living and complex dynamics enhance individual resources discrimination

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In patchy environment, when choosing habitat, group-living species are confronted with a choice between many resting sites differing in their intrinsic quality. In the light of previous works showing the role of the interactions between conspecifics in cluster formation, decision-making and resting site selection, we identified the mechanisms leading to the collective discrimination between identical sites. Here we report a study of the cockroach *Periplaneta americana* confronted with a choice between shelters of different light intensity. The main issue is how population size influences the individual discrimination ability between shelters. We show that social information obtained through inter-individual interactions (i.e. the presence of conspecifics provides a local social cue) allow cockroaches to discriminate between shelters although they are ineffective while isolated. Indeed, cockroaches in groups are more likely to settle under shelter and to select the darker one. Our theoretical results highlight that this collective discrimination emerges from competing amplification processes relying on the modulation of the individual sheltering, without any shelter comparison or communication modulation. The sheltering time increases with shelter darkness and the number of settled conspecifics. Such collective discrimination, governed by nonlinear dynamics, is a by-product of gregarious behavior, the most basic and widely spread social behavior. We postulate that similar group-enhanced discrimination capability should be shared by many taxonomic groups, including eusocial insects, and in various environmental situations.

20-6 Biomass relationships during colony development in the desert leafcutter ant *Acromyrmex versicolor*

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Leafcutter ants harvest leaves to grow a symbiotic fungus, which serves as the ants‘ direct food source. However, little is known about growth dynamics within leafcutter ant nests, which must be regulated at the level of the fungus as well as within the ant population itself. To characterize growth, we established laboratory colonies by collecting newly mated queens, and measured worker population sizes and fungus volume once a week for the first six months of colony development. In a second experiment, we quantified foraging rates and waste generation across a period of two months, in conjunction with measurements of fungus production, worker production, and larva and pupa numbers. Colonies grew exponentially during early development. During the same period, the relationship between standing fungal biomass and worker biomass scaled isometrically. Foraging rate and worker production were also positively correlated. Interestingly, the standing fungal biomass, but not worker biomass, predicted larval and pupal population sizes. These findings suggest the need for further studies of how nutritional and behavioral feedbacks between the ants and fungus shape colony development.

20-7 THEORETICAL STUDY OF FOOD RECRUITMENTS IN ANTS: A GENERALIZED MODEL

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Foraging patterns displayed by socials insects are well-known examples of complex systems in which simple behavioral rules between individuals lead to complex patterns. In those societies, individuals belonging to the same nest cooperate for collecting food resources. In ants, this cooperation relies on the recruitment of nestmates by two main ways: the direct leading towards the food source by recruiting individuals or the laying of a recruitment trail. In this context, we propose a generalized model describing food recruitments in ants: mass recruitment based on a common chemical trail, tandem-running in which informed leader ants guide recruits one by one toward the food source and group recruitment during which leaders guide a group of nestmates. The model for one food source shows that for pure mass recruitment, a non linear response of recruits to trail pheromone allow ants to save signal emission: once the trail is well established, the quantity of pheromone laid by each ant can decrease without reducing their foraging efficiency. This result is supported by several studies highlighting a decrease of trail laying intensity over time. For tandem-running species, our model confirms the crucial importance of leaders' efficiency: the loss of recruits by a small number of leaders is highly detrimental to the launching of collective food exploitation. The situation is quite different for group recruitment: larger groups of recruits can compensate for the previous loss of recruits, what is not the case in tandem running. This is in agreement with experiments showing that group leaders do not care about their recruits while there is a close relationship between tandem leaders and their recruit. Thus, this generalized model for food recruitment brings new hypotheses to account for well-known behaviours observed during foraging.

20-8 COMMUNAL NUTRITION IN ANTS

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Studies on nonsocial insects have elucidated the regulatory strategies employed to meet nutritional demands. However, how social insects maintain the supply of an appropriate balance of nutrients at both a collective and an individual level remains unknown. Sociality complicates nutritional regulatory strategies. First, the food entering a colony is collected by a small number of workers,which need to adjust their harvesting strategy to the demands for nutrients among individuals within the colony. Second, because carbohydrates are used by the workers and proteins consumed by the larvae, nutritional feedbacks emanating from both must exist and be integrated to determine food exploitation by foragers. Here, we show that foraging ants can solve nutritional challenges for the colony by making intricate adjustments to their feeding behavior and nutrient processing, acting both as a collective mouth and gut. The amount and balance of nutrients collected and the precision of regulation depend on the presence of larvae in the colony. Ants improved the macronutrient balance of collected foods by extracting carbohydrates and ejecting proteins. Nevertheless, processing excess protein shortened life span – an effect that was greatly ameliorated in the presence of larvae.

20-9 Within supercolony differences in aphid source sharing among nests in *Formica exsecta* Nyl. (Hymenoptera: Formicidae)

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Food-for-protection type mutualistic interactions are quite common between ants and other insects. Mostly homopterans, e.g. aphids are involved in such myrmecophilous relationships. Ants usually profit by enriching their diet with carbohydrate-rich by-products of these insects, while aphids benefit mainly from protection against predators and competitors. Generally the abundance of food sources, e.g. aphids, among other factors, allows the formation and persistency of large polydomous systems, so-called supercolonies in many ant species. Such systems imply the lack of aggression among workers from different nests, high exchange rate of foragers and the overlap of feeding grounds. The sharing ratio of aphid sources within a supercolony at sites with different ant nest abundance can offer us insight in the degree of integration of ant nests in such a system. Two areas with different ant nest abundance were studied in a large *Formica exsecta* supercolony in Romania in two consecutive years. The high density site (HD) contained more and larger aphid colonies overall, than the low abundance site (LD). In addition ant nests tended more aphid colonies at the HD site, but also shared these colonies, while ant nests generally monopolized aphid colonies at the LD site. Consequently overlaps among the feeding areas of ant nests were very frequent at the HD site, whereas these were almost completely lacking at the other site. We concluded that while the HD site indeed reflected the integrative features of such a system, the LD site could be viewed as a disintegrating part of the supercolony.

20-10 Shifting from individual to collective behaviours in ants

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Group-living animals display numerous collective behaviours as foraging, defence and nest construction. Many of these behaviours are considered to be self-organized i.e. they emerged simply from local information and interactions between group members. In social insects task allocation among workers could also be understood as an emergent phenomenon. One way to test this hypothesis is to search the mechanisms underlying individual decisions and the factors relevant to the shift from individual to collective behaviours. Here we studied the hunting behaviour as a function of the environmental conditions (i.e. colony size, hunting area size) and prey parameters (i.e. prey number, prey size) in the ant *Ectatomma tuberculatum* (Ectatomminae). In laboratory ants were able to catch prey alone (individual task), by group with no specialized workers (group task) or by group with distinct specialized workers, "the immobilisers" and "the transporters" (partitioned task). We first established that task organization was determined by the number of ants in the hunting area and the localization of the first prey seizure, independently. This suggested an important role of social context and individual capabilities. Prey number and size also affected hunting task probably by modifying local stimulations and/or access to the prey. From these empirical data we then developed a model to explain the emergence of collective and partitioned tasks in ants.

20-11 THE ROLE OF DANCE COMMUNICATION AND INSPECTORS IN TRACKING CHANGES IN A DYNAMIC ENVIRONMENT BY HONEY BEES

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How do honey bees track changes in their dynamic foraging environment? Previously, two complementary mechanisms have been identified by which bees can effectively switch between food sources when their relative quality changes. Firstly, an increase in profitability of a food source elicits an increase in waggle dances for that source. Secondly, bees which have retired from foraging at a food source make occasional inspection visits to that food source and resume foraging if its quality improves. Here we investigate, using both field experiments and a mathematical model, the relative importance of these two mechanisms. By experimentally manipulating dance information available to the bees we find that when food sources change quality frequently inspector bees provide a rapid response to changes, whereas the waggle dance contributes to a response over a longer time period. These results show how individual memory and collective communication can interact to allow a social insect colony to adapt to changes on both short and long time scales.

20-12 AN AUTOMATED MEASUREMENT SYSTEM FOR BEHAVIORAL ANALYSIS IN THE OBSERVATION HONEYBEE HIVE

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Characteristics of honeybee colony are generated by results of individual activities. On the other hand, animal behaviors are affected by environmental condition in the colony. To investigate the relation between various environmental factors and animal behaviors, continuous observation and measurement should be very important. In this study, we present an experimental setup for continuous measurements of an observation honeybee hive. Quantitative ambient and inside factors, temperature, humidity, concentration of carbon dioxide and so on in the hive are collected and registered in a database server, automatically. Video images of the frame in the hive are also recorded in AVI format. The database is build up with content management system (CMS), which is a widely used software tools for constructing web portal site. Observation data are managed by hierarchically defined directory structure based on the experimental date and file format. Another computer link with the server executes several preliminary processing, such as enhancement of image data, graph plotting of time series data and so on, at certain interval. Results of the processing are stored into the database as additional information of content. In the hive image, the grey scale level of each image pixel reflects objects on the frame, e.g., honeybee body, wax, pollen and so on. The activity index of the hive is derived by space-temporal changes of grey level in the image sequence. It is also registered as additional information to determine the target period for more detail analysis, such as the time to walk many workers actively. As the first trial to use our new system, it is shown that the activity caused by individuals' walking is significantly influenced by the environmental factors among the hive. The system can't be used only for behavioral studies of honeybee, but also applied to the education with broadcasting and sharing image data and measured values to the Internet.

20-13 THE COMMON STOMACH AS A CENTER OF INFORMATION SHARING FOR NEST CONSTRUCTION IN SOCIAL WASPS

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Construction of wasp nests is a self-organized process that requires building materials, pulp and water foragers, and builders to cooperate. Based on our field study, we have constructed a simple model to examine how the society of wasps as agents use a social crop, or common stomach, to store water that also provides a mechanism for worker connectivity, which in turn regulates building behavior. Our model predicts that via the common stomach usage, medium sized colonies enjoy the benefit of having highly effective foragers and this in turn means that the colonies need only endanger a few foragers to ensure steady construction. When pulp foraging becomes more costly than water foraging, the colonies adjust via recruiting more pulp foragers and less water foragers, but keep high numbers of common stomach wasps on the nest. The common stomach provides an adaptable platform for indirect worker connectivity and a buffer for water storage. We speculate that the size of the interaction platform where material transfers happen could be a consequence of evolutionary pressures that prefer to keep most individuals on the nest.

20-14 A BEHAVIORAL TRACKING SYSTEM FOR MULTIPLE HONEYBEES ON A PLANE SURFACE

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Social insects, such as honeybees, ants and termites, do not behave only as single individuals, but are also influenced by their nest mates. To reveal their social interactions, it is important to observe individuals in a social context and analyze them in detail. Various kinds of recording methods have been used to obtain behavioral data. It is a quite effective way to do detailed observation, but a lot of labor and time are needed to make quantitative analyses without automated tracking methods. In this study, we propose a new method to track multiple honeybees moving in a plane surface arena. Our method consists of following three processes: First, the principal component image (PCI) was extracted from the original image by principal component analysis. The pixels representing the honeybees' body were detected by the threshold processing in the PCI. Then, the individuals were extracted by using information about the bee's body size and shape. Detected bees were assigned to unique numbers in this step. At last, numbered honeybees on every image plane were associated with time. In parallel with this process, larger regions, containing more than one honeybee, were assigned to associating individuals by both the context of the temporal change of bee's region and the prediction of its movement. We applied our method to a movie showing 16 walking bees in an arena illuminated with red light that they are unable to see. The individual locations of most of them could be extracted, traced and their movements could be calculated from start to end. This result show our system is an efficient means of tracking the movements of individual honeybees in groups.

20-15 MOVING HOME: NEST SITE SELECTION IN THE RED DWARF HONEYBEE (*APIS FLOREA*)

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The process of nest-site selection is probably the best studied form of collective decision-making within the social insect world. It is a crucial stage of the life cycle of all insect colonies as the choice of a future nest site has a direct effect on the fitness of the colony. Different species have different needs. Thus comparing the means by which different species select a new site provides valuable insight into the ways natural selection has shaped the decision-making process to account for species' specific requirements. We studied nest-site selection in the Red Dwarf honeybee (*Apis florea*). *A. florea* is one of two basal species in the genus *Apis*. *A. florea* differs from the well-studied Western Hive bee (*A. mellifera*) in that it nests in the open rather than in cavities. This fundamental difference in nesting biology has profound implications for the nest-site selection process. In *A. mellifera* workers show a series of characteristic behaviours that allow the swarm to select the best nest site possible. To a large extent these behaviours are absent in *A. florea* due to its more simple nest-site requirements. In particular we found that two fundamental aspects of the behaviour of *A. mellifera* scouts – the process of dance decay and the process of repeated nest site evaluation – do not occur in *A. florea*. We also found that the piping signal used by *A. mellifera* scouts to signal that a quorum has been reached at the chosen site, is performed by both dancing and non-dancing bees in *A. florea*. The piping signal therefore most likely acts as a general activation signal as is described in pre-swarming *A. mellifera* colonies. Our results suggest an evolutionary pathway from a simple nest site selection process in the open-nesting species to the more complex system in cavity-nesting bees that requires more accurate evaluation of potential nest sites.

20-16 FORAGING BEHAVIOR AND COLONY CYCLE OF *AGELAIA VICINA* (HYMENOPTERA: VESPIDAE; EPIPONINI)

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The neotropical *Agelaia vicina* has the largest nest built among social wasps, yet little is known about nest construction, growth and structure. In this work, the development of two nests of *A. vicina* was followed. Studies were done through analysis of images to estimate the growth of nests. The material collected below the nests was examined to estimate colony productivity. Nests were collected to analyze their architecture and structure. Colony cycle was similar in the two colonies. Colonies increase in size throughout the dry season and into the rainy season, with a sudden drop in production at the end of the rainy season. The colonies doubled in size in about six months.

20-17 FORMATION OF EXPLORATORY NETWORKS BY ARGENTINE ANTS: FROM INDIVIDUAL BEHAVIOUR TO TWO-DIMENSIONAL PATTERNS

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A central problem in complex systems is understanding how large scale spatio-temporal patterns result from local interactions between elements at a smaller scale. In insect societies, one such example of pattern formation is provided by the exploratory patterns of Argentine ants, *Linepithema humile*. When Argentine ants are given access to an empty arena, they soon start forming characteristic patterns of trails. The trails result from a positive feed-back process: individual ants mark their own path through the arena with chemical compounds; the concentration of chemicals in turn influences the path chosen by the same or other ants at later times.By tracking a large number of ants over short time periods (more than 700 000 tracking events) we could obtain a statistical description of the behaviour of individual ants: number of ants in the arena, speed and direction of movement. In particular, we derived an expression for the changes of ant direction as a function of the (inferred) concentrations of marking they were encountering.We then implemented the same individual rules of behaviour in an agent based model to simulate the formation of the large scale patterns. By comparing the output of the model at the large scale with the real patterns it is possible to validate the appropriateness of the chosen parameters of behaviour at the individual level of description.

20-18 EFFECTS OF INTERACTION NETWORK STRUCTURE ON INFORMATION FLOW IN SOCIAL INSECTS

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Social insect colonies exhibit coordinated behaviors with no central control. Local interactions among individuals determine their behavior and result in the overall activity of the entire colony. Interactions among nestmates, in the form of brief antennal contact, influence the activity of individuals. Here I investigate the effect of variation among individuals in their interaction probability on the dynamics of information transfer. Using computer simulations, I examine whether the structure of the interaction network among colony members affects the rate at which workers receive information. I simulate three scenarios differing in the interaction probability of ants, and empirically describe the interaction network observed in lab-housed harvester ant colonies. The simulation results show that skewed interaction networks, containing few individuals with a very high interaction rate, and changing networks in which ants increase their probability to interact when becoming informed, lead to faster information transfer than interaction networks with a uniform interaction probability. Thus, individual variation in interaction probability can influence the rate at which information is transmitted in the colony either by changing the network structure or by increasing the number of overall interactions. The empirical observations support the existence of a skewed interaction network in harvester ants.

20-19 PROBLEM SOLVING IN COMPLEX SYSTEMS: COLLECTIVE CHAIN FORMATION AND GAP BRIDGING IN THE WEAVER ANT *OECOPHYLLA SMARAGDINA*

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The collective building activities of green tree ants is considered one of the most complex behaviours exhibited by social insects. Individuals join together to form chains to bridge gaps in the canopy, which can then be shortened to bring together foliage in order to construct their arboreal nests. Here we demonstrate that groups of green tree ants are capable of collectively discriminating gap distances and 'choosing' to bridge the shortest gap. We discuss the individual behavioral mechanisms that potentially underlie this group problem-solving capability and the relationship between behavioral subgroups and overall group decision-making efficiency. We also discuss a previously undetected collective group behaviour: column formation, in which ants combine to form living columns in order to bridge vertical gaps.

20-20 DIGGING BEHAVIOUR AND THE CONTROL OF NEST SIZE IN LEAF-CUTTING ANTS

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Leaf-cutting ant nests consist of numerous underground chambers, each housing a fungus garden. It is largely unknown what are the rules used by workers to enlarge the nest, and to what extent both the nest size and the size of a single fungus chamber are regulated. We investigated the dynamics of collective digging and nest enlargement in laboratory colonies of the leaf-cutting ant *Acromyrmex lundi*. Groups of ants varying in number, or provided with different fungus volumes, were allowed to establish a nest in a chamber of insufficient size, and their digging activities quantified. The rationale of the experiments was to create a situation in which workers need to relocate a fungus garden into a pre-given nest chamber of insufficient size. Under such conditions, workers need to enlarge the existing chamber so as to generate enough space for the fungus garden to be relocated. The experimental setup thus reproduced a situation that recurrently occurs during nest ontogeny, when an existing chamber is enlarged because of fungus growth, or new ones are excavated. Ants were observed to dig tunnels and to enlarge the pre-given chamber. The presence of a fungus garden significantly stimulated digging activity. Digging rates were maximal at the beginning of the experiments and continuously declined over time. Total digging activity, and the resulting nest size, positively depended on the number of ants in the group; this relationship could be described with the power law (exponent = 0.5). The chamber size, however, was independent of the number of ants, and was adjusted to the available fungus volume. It is argued that the shape of the fungus garden acts as a dynamic three-dimensional template that determines the final chamber size. New chambers are expected to be excavated around a fungus piece previously relocated in a tunnel, and to be enlarged as soon as the distance between the fungus and the chamber wall becomes reduced because of fungus growth.

20-21 (IR)RATIONAL DECISION-MAKING IN ANTS

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Rational decision-making theory predicts that animals maximize their fitness only if their decision-making follows certain consistency principles. Despite this, violations of rationality have been found repeatedly in humans and in some animals. The significance of this irrationality is controversial, but one explanation is that limited cognitive resources prevent animals from adequately processing the large quantity of information required for rational decision-making, leading them to rely on error-prone heuristics. Many animal groups, such as social insects, also make decisions, but the rationality of their choices has barely been tested. An interesting possibility is that groups evade cognitive limitations by distributing their information processing over many individuals. Here we show that individual ants, but not colonies, strongly violate rationality when presented with a challenging nest-site choice. Specifically, individuals irrationally switched their preference between two alternatives based on the presence of an unattractive decoy. Colonies showed consistent preferences regardless of the decoy's presence. Many studies have noted prominent advantages of collective decision-making in different animals. Our results point to avoiding irrational errors as a previously unrecognized advantage of collective decision-making. Furthermore, they accord with the notion of ecological rationality, which holds that animals follow decision rules that only reliably yield fitness-maximizing outcomes in their natural environment. Ants are obligately social, hence the most important context in the evolution of their decision-making behavior was probably the collective one. These results further suggest that individual behaviors should be considered in terms of the collective context, especially when those individuals are social, like humans.

20-22 MAKING A DECISION IN SPITE OF THE NOISE: NEST-SITE SELECTION BY *APIS FLOREA*

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Superficially the nest-site selection process of the red dwarf honeybee, *Apis florea*, seems very similar to that of the western honeybee, *Apis mellifera*. However, recent research has shown that the two processes are different. Unlike *Apis mellifera*, *Apis florea* scouts do not leave the swarm to re-evaluate a nest site in between dance episodes, on average there is no decay in the number of waggle runs performed per distinct dance episode and many sites are still advertised by scouts' dances immediately before the swarm departs. Many *Apis florea* scouts follow dances, but many fewer individuals become dancers. Generally *Apis florea* scouts are inaccurate, or at least inconsistent, in following and giving directions. In short, the nest-site selection process is extremely noisy. We will present several computational and mathematical models and use these to explore how the noise and inaccuracy of an *Apis florea* colony affects their ability as a collective to make a decision to leave their current location and travel to a new nest site.

20-23 MAPPING SOCIAL NETWORKS IN HOUSE-HUNTING ANTS

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Network analysis is a promising tool for insight into the collective behavior of social insects. How does a network structure emerge from individual behavior, and how does it contribute to the solution of a colony-level challenge? House-hunting by ants in the genus *Temnothorax* is a leading model of collective behavior that is well suited to network analysis. Nest site selection is of clear adaptive significance to the colony, and the entire process is readily viewable in a laboratory setting. Furthermore, each individual can be paint-marked and tracked, and each pairwise interaction can be observed. We videorecorded repeated migrations by paint-marked colonies of *Temnothorax rugatulus*, paying particular attention to the recruitment communication that organizes the emigration. We then described each emigration as a network with nodes consisting of individual ants and edges marking recruitment of one ant by another. Preliminary analysis shows elements typical of small world networks. Motif analysis further suggests intriguing structural similarities between house-hunting and gene transcription pathways, possibly reflecting their common information-processing role. Comparing multiple emigrations by the same colony reveals highly connected individuals that consistently perform the bulk of recruitment. Further analysis will focus on the network's robustness to removal of these key ants. This study marks the initial steps in applying a potentially powerful tool for understanding complex collective behavior.

20-24 HEAT, CARBON DIOXIDE, AND HUMIDITY GENERATED BY HONEYBEES JOINTLY ACT TO KILL HORNETS

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We found that giant hornets (*Vespa mandarinia japonica*) are killed in less than 10 min when they are trapped in a bee ball created by the Japanese honeybees *Apis cerana japonica*, but their death cannot be solely accounted for by the elevated temperature in the bee ball. Once hornets are captured within the bee ball, the temperature, CO_2 concentration, and humidity in the bee ball are increased rapidly by honeybees' respiration. Within 5 minutes after capture, the temperature reaches 46 °C, humidity 90% or above, and CO_2 concentration 4%. Normally, the hornet dies within about 10 minutes in the bee ball. To investigate conditional changes within the bee ball, i.e., the main causes of hornet mortality, we determined the lethal temperature on the 10-minute exposure of four species of hornet in various conditions of the temperature, humidity, and CO_2/O_2 concentration. In expiratory air (3.7% CO_2), the lethal temperatures were 2 °C or more lower than that of the normal atmosphere. All four hornet species were killed at 44-46 °C. Death was not caused by oxygen deficiency because the lethal temperature shows no change even if oxygen is supplied to compensate for the reduced oxygen due to increased CO_2.The Japanese honeybees and also the European honeybees *Apis mellifera* adapt to a high CO_2 and high humidity environment because they normally cluster. Thus, the lethal temperature of honeybees is 50-51 °C in such an environment, almost the same as that in the normal atmosphere. Japanese honeybees generate heat, a high CO_2, and high humidity environment by intense respiration to lower the lethal temperature of hornets. European honeybees are usually victims of genocide by hornets without our protection in Japan, but Japanese honeybees kill the predator without sacrificing themselves using heat and all by-products of respiration.

Oral Presentations

Communication and the integration of multiple information sources in colony organisation

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TRIGGERED BY PERIL: A NEGATIVE FEEDBACK SIGNAL CURBS COLLECTIVE ACTION IN A SUPERORGANISM

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Decision making in superorganisms such as honey bee colonies often uses self-organizing behaviors, feedback loops that allow the colony to gather information from multiple individuals and achieve reliable and agile solutions. Honey bee recruitment is a classic example. It uses positive feedback from the waggle dance to allocate colony effort among differently rewarding resources. However, the use of negative feedback signals by superorganisms is poorly understood. Here, I show that conspecific attacks at a food source lead to the production of 'stop' signals, communication that has been shown in multiple studies to reduce waggle dancing and recruitment, but lacked a clear natural trigger. Signalers preferentially targeted nestmates visiting the same food source, based upon its odor. During aggressive food competition, attack victims significantly increased signal production by 43 fold. Foragers that attacked competitors or experienced no aggression did not alter signal production. Biting ambush predators also attack foragers at flowers. Simulated biting of foragers or exposure to bee alarm pheromone also elicited signaling (88 fold and 14 fold increases, respectively). This provides the first clear evidence of a negative feedback signal elicited by foraging peril to counteract the positive feedback of the waggle dance. Stop signals from multiple actors regulated colony foraging to a specific location when danger exceeded threshold levels. As in intra- and inter-cellular communication, negative feedback may play an important, though currently underappreciated, role in self-organizing behaviors within superorganisms.

INDIVIDUAL SMARTS AND GROUP SMARTS: BOTH WORKERS AND COLONIES ARE SKILLED INTEGRATORS OF INFORMATION

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Recent findings in honey bees have shown that both individual workers and whole colonies are capable of integrating multiple streams of sensory input when making decisions. At the individual level, we now know that a worker bee is not a 'dumb insect' but instead is an alert individual who is able to acquire and integrate information from numerous sources when making a decision. For example, after returning to the hive, a nectar forager decides how long to perform a waggle dance based on a constellation of inputs used to calculate the profitability of her food source and on a constellation of inputs used to judge the current utility of dispatching more foragers to this food source. At the group level, we now know that a whole colony is able to acquire and integrate information from numerous locations in the surrounding environment to make good decisions, such as the allocation of its foragers among food sources and the choice of a new dwelling place. I will review what has been learned recently about how a colony creates inside its hive a sensory representation of the outside world and how it makes use of this sensory representation to choose a suitable course of action. I will conclude by considering some of the fundamental similarities between bee swarms and vertebrate brains in how they process information in making decisions.

KEEP ON TRUCKIN' - A NOVEL ROLE FOR ANT TRAIL PHEROMONES AS A 'KEEP ON WALKING' SIGNAL IN THE ANT *LASIUS NIGER*

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Ant trail pheromones are traditionally seen as signals directing ants towards food sources. However, on established trails most ants will have previously visited the food source and have memory of the route. Experiments with several ant species have demonstrated the dominant role of individual memory over pheromone trails at trail bifurcation. Do experienced foragers use trail pheromones at all? We studied foraging ants varying levels of experience, and showed that the presence of trail pheromone may be used by experienced ants as a reassurance that they are on the right path, and thus allow them to make journeys to and from the food source more rapidly. The presence of trail pheromone increased walking speed, reduced the amount of U turns performed, reduced walking path sinuosity and caused ants to maintain further pheromone deposition. This is an all-or-nothing effect, the strength of the pheromone trail having had no effect on the ants' behaviour. We also tested whether chemical cues left passively by walking ants, 'home range markings', rather than trail pheromones could be responsible for these effects. Only path sinuosity was affected by home range markings alone, although trail pheromones had an even stronger effect on path sinuosity. The experience level of ants also influenced the studied behaviours. These results suggest a novel role for ant trail pheromones: the presence of pheromones signals to ants that they are on the right track. When an ant leaves the marked trail the lack of trail pheromone triggers slower, more sinuous walking with more U turns, which may assist the ant in relocating the lost path. Moreover, ants that have strayed off the path will stop laying trail pheromone, thereby maintaining the integrity and directness of the existing trail. By reducing path sinuosity and the number of U turns performed, trail pheromones allow ants to make rapid, more direct journey to and from a food source and thus may increase their foraging efficiency.

SOCIAL LEARNING AND WAGGLE DANCE BEHAVIOR USED AS TOOLS TO GUIDE HONEY BEES TO A SPECIFIC CROP

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Floral odors experienced by honey bees inside their colonies can be conditioned through social interactions or via the food stores. It is known that associative memories shaped within the hive can affect preferences of foragers even several days after acquisition. These olfactory memories can be also retrieved when inactive foragers perceive the known scent carried by dancers. Our hypothesis is that memory retrieval within this informational context improves the decoding of the spatial information contained in the waggle dance and thus would promote a faster recruitment to these floral types. Beekeepers use to condition honey bee colonies by feeding them with syrups containing crushed flowers of the species that want to be pollinated. We developed a synthetic complex of a few volatile compounds that laboratory bees cannot discriminate from the natural fragrance of the sunflower, *Helianthus annuus*. Then, foraging behaviors of colonies with different treatments [sugar solution within the hive containing either (i) the synthetic-sunflower complex, (ii) the synthetic-jasmine complex; or (iii) the unscented solution] were compared by decoding waggle dances of their foragers. The observation hives were placed 600m SE from a sunflower field. Dance maps showed that the colony treated with the synthetic-sunflower complex presented lower delays to find the sunflower field, while its number of waggle dances indicating the right location increased exponentially during the first experimental day. The jasmine-treated hive showed longer delays and the number of right dances increased linearly. Even a lower rate of these behavioral measurements has been observed for the control hive. Results suggest that specific in-hive associative memories not only improve the acquisition of the location information transmitted but also open the possibility to manage honey bee colonies to specific field crops in a simple way.

THE FUNCTION OF EGG VOLATILE PHEROMONES IN TERMITES: MECHANISMS OF EGG ORIENTATION AND RECOGNITION.

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Eusocial insects rely heavily on pheromone communication to maintain their sociality. Egg protection is one of the most fundamental social behaviours in eusocial insects. In termites, workers recognize the eggs laid by queens and pile it in nursery cells to care for them. Workers recognize their eggs by using the chemical compounds on the eggs (the termite egg-recognition pheromone; TERP) and their morphology (size, shape and surface texture). It was known that TERP consists of *beta*-glucosidase and lysozyme, which strongly evokes the egg-carrying and -grooming behaviours of workers (Matsuura et al. 2007, 2009). Both enzymes are major salivary compounds in termites and are also produced in the eggs. On the other hand, we found workers of *Reticuliterumes speratus* aggregate around their eggs enclosed in a wire mesh cage, raising a hypothesis that workers can detect the existence of egg by using the egg volatiles. Headspace-GC-MS analysis of the egg volatiles showed that the eggs volatiles consist of two chemical compounds. To elucidate the function of the egg volatiles, we investigated how the artificial blend of egg-volatiles influence on the worker's egg protection behaviour. Egg protection activity can be tested by a simple bioassay using dummy eggs made of glass beads. Glass beads are only carried into and piled with the true eggs when they have been coated with a chemical sample that causes the workers to recognize them as eggs. The piling rate of dummy eggs coated with both TERP and the artificial volatiles was significantly greater than those coated with TERP, while workers didn't accept dummy eggs coated only with the artificial volatiles. These results demonstrated that the egg volatiles act as an orientation cue for workers. This is the first identification of egg volatile pheromones in termites.

ORGANISATION OF WORK IN A CHANGING ENVIRONMENT - WHAT DETERMINES WHO SWITCHES TASK?

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Flexibility in task performance is essential for a robust system of division of labour.Multiple factors may be involved in determining whether an individual social insect worker responds to a colony-level change in task demand, including physiology, experience, age, location and social interactions. We used radio-frequency identification (RFID) technology to compare the roles of corpulence, age, spatial location and previous activity (intra-nest / extra-nest) in determining whether worker ants (*Temnothorax albipennis*) respond to an increase in demand for foraging or brood care. The less corpulent ants took on the extra foraging, irrespective of their age, previous activity or location in the nest, supporting a physiological threshold model. Ants which transported the extra brood to the main brood pile were less corpulent and had high previous intra-nest activity. This supports spatial task-encounter and physiological threshold models for brood transport. Corpulence and extra-nest experience were highly correlated. We used automatic computer-controlled doors combined with the RFID system to manipulate the access of certain ants to certain tasks, to investigate the direction of causation in this relationship. Our data suggest a flexible task-allocation system allowing the colony to respond rapidly to changing needs, using a simple task-encounter system for generalised tasks, combined with physiologically-based response thresholds for more specialised tasks. This could provide a social insect colony with a robust division of labour, flexibly allocating the workforce in response to current needs.

COMMUNICATION IN DECISION-MAKING: SIMILARITIES BETWEEN VERTEBRATE BRAINS AND SOCIAL INSECT COLONIES?

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Models of the collective decision-making of social insect colonies have been shown to have remarkable similarities with models of decision-making in the primate visual cortex (Marshall et al. 2009). Drawing parallels with neural decision-making processes has allowed us to import optimality analysis techniques and apply these to house-hunting by social insects. A key prediction of this analysis is that populations of decision-making insects committed to different potential nest-sites should attempt to inhibit the build-up of scouts committed to other alternatives, similarly to the mutual inhibitory processes that characterise optimal decision-making in brains. The experimental approach we will present investigated whether individual scouts – in order to achieve optimal decision-making – directly recruited scouts committed to the alternative nest site, rather than recruiting uncommitted scouts. The experimental results will inform our current understanding of the underlying principles, and both theory and experimental design will significantly benefit from this connective approach. Due to the parallels between decision-making in social insects and that in brains, insights gathered with this approach will go far beyond being exclusively related to the species *Temnothorax albipennis* (our system of choice), but will also extend our understanding of neural decision-making in general. This talk will address both: our current model of commitment switching, and the experimental approach to test whether *Temnothorax* scouts make use of direct recruitment or not (extensively using RFID technology).

PRIOR EXPERIENCE AND NEST SITE SELECTION IN HOUSE-HUNTING ANTS: AN INTERPLAY BETWEEN PRIVATE AND PUBLIC INFORMATION

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House-hunting ants such as *Temnothorax albipennis* live in fragile nests and emigrate readily into a new home if their current nest is damaged. During emigrations, individuals may encounter more than one suitable nest; colonies must therefore choose a single, high quality nest site among several options. This is usually achieved through a combination of amplificatory recruitment processes and quorum sensing, which allow the collation of independent assessments by several individuals. Here, we show that individuals can also gather information about available nest sites prior to emigration, then retrieve this information during emigrations, which influences collective nest choice: emigrating colonies indeed avoid mediocre, familiar nest sites and favour high quality, familiar nest sites. We further show that information about familiar sites is multi-modal, involving both private and public channels. Experienced workers had a higher chance of discovering high-quality familiar sites independently, and did so more quickly, than naïve workers. Additionally, familiar nest sites were discovered earlier than unfamiliar nest sites even when all pheromone trails were moved, suggesting that experienced workers had memorised their location. Private information therefore appears to play a major role in the first steps of emigration to high-quality familiar sites. Such information can be shared with nestmates via recruitment or pheromone marking. Indeed, we found that high-quality familiar nest sites were marked with pheromones which facilitated their acceptance by naïve individuals. By contrast, low-quality familiar nest sites were marked with negative pheromones which influenced their assessment by naïve individuals. Nest site selection by emigrating colonies therefore relies on both private and public information. Individual and collective processes ensure effective sharing of information among nestmates, improving colony performance in a familiar environment.

YELLOWJACKETS USE NEST-BASED CUES TO PREFERENTIALLY EXPLOIT HIGHER-QUALITY RESOURCES

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While foraging, social insects encounter a dynamic array of food resources of varying quality and profitability. Because food acquisition influences colony growth and fitness, natural selection should favor colonies that allocate their overall foraging effort so as to maximize their intake of high-quality nutrients. Social wasps lack recruitment communication, but previous studies of vespine wasps have shown that olfactory cues influence foraging decisions. Odors associated with food brought into the nest by successful foragers prompt naïve foragers to leave the nest and search for the source of those odors. Left unanswered, however, is the question of whether naïve foragers take food quality into account in making their decisions about whether or not to search. In this study two differently scented and concentrated sucrose solutions were inserted directly into a *Vespula germanica* nest, and forager choices were monitored at a feeder outside the nest. We show that foragers choose higher-quality resources in the field using information in the form of food-associated cues obtained within the nest. By this simple mechanism the colony can bias the allocation of its foraging effort toward higher-quality resources in the environment.

THE ROLE OF THE SOCIAL ENVIRONMENT IN PROXIMATE CAUSATION OF THE SWITCH POINT IN THE BUMBLE BEE *BOMBUS TERRESTRIS*

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Many eusocial insects exhibit predictability in the timing of colony growth and development, and in some cases events in the colony cycle occur at predictable times even in the absence of variation in cues from the external environment. This is especially marked in annual species like bumble bees, in which all colonies are founded and die within a single year. Whilst the seasonal occurrence of discrete life history events is almost certainly adaptive, the proximate mechanisms regulating these events are not well understood. One example of such an event is in the commercially important and well-studied bumble bee *Bombus terrestris*. At a distinct time in colony growth, the queen stops producing diploid female eggs and starts producing haploid male eggs (the `switch point'). Previous studies have failed to predict or change the timing of the switch point as a function of the social environment. I will present the results of a study using *B. terrestris* in which the timing of the switch point is compared between eusocial queens (i.e. queens in colonies allowed to develop normally) and queens with experimentally-induced asocialilty (i.e. queens from which all adult offspring are experimentally removed). The experiment is designed to test whether endogenous queen-based mechanisms or signals from the social environment have the greatest influence on the timing of the switch point. In addition, by helping to clarify which party within the colony has greater control of the switch point, the findings should illuminate the ultimate factors that affect the timing of events in the colony cycle.

COMPLEXITY OF PHEROMONE REGULATION IN HONEY BEES: WHY SO MANY? THE CASE OF WORKER REPRODUCTION

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Honey bees successful organization relies mainly on chemical communication. The pheromone language of the colony is characterized by its great complexity and diversity. As an illustration, more than 50 substances, derived from the different colony actors, are known to be essential to the functioning of the colony. A remarkable example of this complexity is the control of worker reproduction (via the inhibition of ovary development). The queen and brood release the queen mandibular pheromones and a blend of ten esters, respectively, which partially inhibit worker ovary development. Recently, we found that worker reproduction was also inhibited by additional powerful queen pheromones and a new highly volatile brood pheromone: E-beta-ocimene (produced also by mated queen). We will report those results but also discuss the question of why such redundancy in pheromone functions? One hypothesis comes from the evidences of workers being able to reproduce despite the inhibitory presence of the queen. By emitting multiple pheromones, larvae and queen could reduce the 'risk' of having workers that bypass the control over reproduction. Another hypothesis rather highlights the complementarities of each pheromone leading to a precise chemical syntax and a fine-tune regulation of the colony unit. For example, each of these pheromones induces only a partial effect on workers ovary development. They may act synergistically and /or their efficiency might differ and depend on the context, their transmission and the variability in their production. Therefore, future studies integrating more than one pheromone promises to give a better representation of pheromone communication.

WHAT MAKES WORKER HONEY BEES WORK?

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Honey bee workers have two potential life histories: normally in a healthy colony workers are sterile and help the queen by nursing her offspring, but if a colony loses its queen or is failing workers can develop their ovaries and produce male brood of their own. Clearly the behavioural and physiological development of a worker is influenced by the condition of its colony, but what information signals colony condition to workers, and how do bees acquire and weight this information? Queen and brood pheromones are two key colony signals that influence worker development. Here we explore how these signals work independently and in combination to influence the behavioural and physiological state of workers. We tested whether experimentally varying queens' reproductive condition affected the likelihood of workers developing active ovaries. We explored how brood pheromone, queen pheromone and supplemental feeding interact to influence foraging behaviour and colony growth by experimentally varying the amount of brood pheromone, queen presence and protein supplementation between colonies in the field. We exposed workers to different levels of brood pheromone and measured brain biogenic amine levels, ovary development, and how they responded to queen pheromone. Our work will reveal how pheromone systems interact to modulate insect societies and help improve management of the honey bee for the honey and pollination industries.

DUFOUR'S GLAND IN *ROPALIDIA MARGINATA*: CAN CASTE AND COLONY SIGNALS BE EXPRESSED THROUGH THE SAME SET OF CHEMICALS?

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Ropalidia marginata is a primitively eusocial paper wasp where the queen is remarkably non-aggressive in behaviour. Despite being docile, she maintains reproductive monopoly. On removing the queen, one of the workers becomes extremely aggressive but drops her aggression if the queen is returned. If the queen is not returned, this hyper-aggressive individual will develop her ovaries, lose almost all her aggression, and become the next queen of the colony; we call her the potential queen (PQ). Using the immediate loss of aggression by the PQ upon return of the queen, we demonstrated that the queen's Dufour's gland is a source of the queen pheromone. Dufour's gland extracts of the queen mimic the queen in making the PQ drop her aggression, and, queens and workers can be correctly classified on a discriminant function based on the compositions of their respective Dufour's glands. We found that wasps can also be correctly classified according to their colony membership, suggesting that the Dufour's gland can potentially function in nestmate discrimination also. Hence we did a bioassay where after queen removal, the Dufour's gland extract of a queen from another nest was applied to check the effect of a foreign queen's gland extract on the PQ. We found that Dufour's gland extract of own queen and that of a foreign queen were equally effective in reducing aggression of PQ, suggesting that the PQ did not discriminate between nestmate and non nestmate queens. Our results therefore suggest that the ability of statistical softwares to differentiate groups based on their chemical profiles does not necessarily imply that the wasps can make such discrimination. It may thus be necessary to reexamine whether both caste and colony signals can be expressed through the same chemicals, as has been suggested from studies on other species.

Poster Presentations

Communication and the integration of multiple information sources in colony organisation

21

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21-1 CHEMICAL ECOLOGY OF EUSOCIAL GALL-INDUCING THRIPS, THEIR HOST PLANTS AND NEST PARASITES

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A monophyletic group of gall-inducing thrips species, within the genus *Kladothrips*, are among the most recently evolved eusocial insects. As for other eusocial insects, chemical signals probably play an important role for these thrips. Our study system consists of three co-evolved genera: the wattles (*Acacia* spp.) serving as host plants for the gall-inducers, the gall-inducers themselves, and the gall-invading kleptoparasitic thrips within the genus *Koptophrips*. We are interested in the chemical cues that thrips may use to locate host plants, communicate within a colony and what cues the nest parasites may use to identify host trees that have been galled. Extraction and headspace collection of volatiles from *Acacia* species revealed a very slow release of just a few substances, making the odours of different species very similar based on GC-MS-analyses and thus forming only a weak basis for host specificity. Similarly, the odour differences between intact phyllodes and those with galls are small, making the host finding behaviour of the kleptoparasites mysterious. The antennal morphology has been investigated in detail for both a gall-inducing and a kleptoparasitic species and revealed few differences between species, sex and castes (see presentation by De Facci et. al., this meeting). Electrophysiological responses (EAG) to plant odours have been recorded. The chemical content of the defence droplets varied between species, and the significance of this is under investigation. Several bioassays have been tested to detect attraction, repellence or effects on activity induced by different odours, and so far a generally higher activity of dispersers compared to soldiers has been noticed.

21-2 COMBINING DISPARATE INFORMATION TO MAKE GOOD DECISIONS: THE EFFECT OF RECEIVER-FORAGER INTERACTION ON FORAGER ALLOCATION

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In the honey bee (*Apis mellifera*) colony, different groups of bees have information about the needs of the hive and the outside environment. In particular, hive bees know about the colony's need for food while foragers know about the availability of nectar. Both these bits of information are important in making an efficient allocation of workers to the task of foraging. It has long been known that foragers are less likely to dance to recruit others if they have waited for a long time to unload their nectar to a receiver bee after arrival at the hive. If the hive's honey stores are close to capacity and there is many foragers coming in, the unloading time is high and so recruitment is less likely. If honey stores are low and there are few foragers arriving with nectar, unloading time is short and recruitment is more likely. Hence the time that a forager takes to find receiver bees and unload is important in transferring information about both forage availability and hive need between foragers and receivers. We write down a simulation model to explore the effect of information transfer via the waiting time to unload. This model suggests that different groups, either the receivers or the foragers, may control the number of foragers in the field, depending on the quality of the available nectar. It also suggests that the availability of receivers at the nest entrance, which is one factor that determines search time, may affect the colony's ability to rapidly switch from one foraging source to another.

21-3 Relative Abundance of n-Alkane Cuticular Hydrocarbons and Interaction Rate Inform Harvester Ant Foraging Decisions

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Social interactions provide local information cues to workers in the non-hierarchical regulation of worker activity in social insect colonies. In harvester ants (*Pogonomyrmex barbatus*), foragers are stimulated to leave the nest in search of seeds by the return of a different task, patrollers, to the nest. Colonies are responsive to the rate of patroller return and when patrollers are prevented from returning to nest, foraging will not begin by the colony that morning. Foragers recognize patrollers returning to the nest using cues present in patroller cuticular hydrocarbons. Cuticular hydrocarbons are a mixture of long-chain n-alkanes, methyl-branched alkanes, and n-alkenes which serve in communication, prevention of abrasion to the cuticle, and prevention of water loss. Harvester ant workers that work outside the nest, such as patrollers and foragers, have a higher amount of n-alkanes compared to workers that spend short amounts of time outside of the nest, such as nest maintenance workers. In this study, I tested the hypothesis that a high relative abundance of n-alkanes in cuticular hydrocarbons acts as a cue that allows foragers to identify patrollers during social interactions important in the regulation of colony foraging. I removed patrollers to prevent foraging and returned ant mimics to the nest, small glass beads coated with hydrocarbons, at an appropriate rate and then measured colony foraging levels. My data supported the hypothesis; when nest maintenance workers were supplemented with n-alkanes hydrocarbons, colonies responded with foraging in a similar fashion as the return of patroller hydrocarbons.

21-4 Generalisation and discrimination trail pheromones of different nests in the ant *Camponotus aethiops*

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Efficient foraging is crucial to provide food supply for animal societies. Many ant species evolved trail pheromone marking to indicate their nestmates the food sources location. We asked whether *Camponotus aethiops* ants use trail pheromones and whether these pheromones vary among societies. Freely walking ants were put on a Y-maze differentially marked: one branch previously walked by foragers (either nestmates or non-nestmates) vs. a clean branch; one branch previously walked by nestmate foragers vs. a branch previously walked by non-nestmate foragers. Ants preferred previously used branches (either by nestmates or non-nesmates) to the clean branch, proving *C. aethiops* using trail pheromones, which were generalised among societies. However, ants preferred that branch marked by nestmates to that by non-nestmates, showing that trail pheromones from different societies could be discriminated. Future work is needed to analyse the composition of trail pheromones, any cognitive process to allow ants avoiding eavesdropping by non-nestmates. Besides, physiological studies will also give light on how these socially active odours are encoded by the ant nervous system.

21-5 CHEMICAL ECOLOGY OF THE TERMITE GENUS *PRORHINOTERMES*: FROM ANATOMY TO CHEMISTRY AND FUNCTION

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The preponderant role of chemical communication and defence in the life history of social insects is obvious and well documented by the variety of described exocrine glands and their products. Societies of termites are an excellent example in this respect. First, devoid of visual orientation, they have reached a social and functional complexity comparable to that of the most advanced social hymenopterans, with chemical signals being involved in nestmate and caste recognition, trail and food marking, alarm propagation, sexual attraction, and, last but not least, in caste regulation. Second, the richness of soldier defensive chemicals identified in the unique termite defensive gland, the frontal gland, is unprecedented.We devoted the past four years to an extensive quest for chemicals used in communication and defence in the termite genus *Prorhinotermes* (Rhinotermitidae), namely *P. simplex, P. canalifrons* and *P. inopinatus*. To elucidate the chemical identity and function of these compounds we used various approaches, from electron microscopy and analytical chemistry to electrophysiology. This presentation should be a brief overview of our most important findings related to i) the role of the frontal gland of soldiers and imagoes in alarm communication and defence, ii) sexual attraction by means of semiochemicals from the tergal glands of female imagoes, iii) trail marking by means of pheromones from the sternal gland of pseudergates and soldiers, and iv) the role of proteinaceous compounds in the signalling of reproductive status by kings and queens.

21-6 SLEEP DEPRIVATION IMPAIRS PRECISION OF WAGGLE DANCE SIGNALING IN HONEY BEES

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Sleep is essential for basic survival and insufficient sleep leads to a variety of dysfunctions. In humans, one of the most profound consequences of sleep deprivation is imprecise communication. Communication in non-human animals may suffer analogous degradation of precision. However, society-specific functions of sleep have rarely been explored and no function of sleep has been ascribed to a eusocial organism in the context of its society. Our study investigates a possible social function of sleep by testing the effect of sleep deprivation on the precision of signaling among European honey bees (*Apis mellifera*). We hypothesized that honey bees perform basic colony functions more efficiently with sleep and predicted that depriving honey bees of sleep would decrease the precision of their dance's direction and distance information. Because forager honey bees sleep primarily at night, we magnetically disturbed a select subset of foragers in the hive for 12 h during one night, and compared results of sleep deprivation with results following a daytime disturbance period, which served as a control for stress. We adhered magnetic steel to treatment bees and nonmagnetic copper to control bees. Bees with magnetic tags performed waggle dances with less directional precision (i.e., standard deviation of dance zenith angles was greater) than their nonmagnetic copper-tagged siblings following a night of sleep deprivation, as compared with the day following daytime disturbance ($z = 2.36$, 1-tailed $P = 0.0499$, $n = 545$ observations of 17 bees, using a mixed-effects model programmed in R). There was no effect of sleep deprivation on precision of distance. The deterioration in sleep-deprived bees' ability to communicate is expected to harm the foraging efficiency of fellow bees. In studying the effects of sleep deprivation on honey bee communication, we begin to see the functional significance of sleep to societies.

21-7 Bumblebee behavior: how do past and present foragers at the patch affect individual decisions?

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For the bumblebees' local foraging behavior and the pollination event, the local insect composition may be as important as the local floral composition. However, much more effort has been employed in studying the effects of local composition of plants than of local composition of insects on the foraging patterns of pollinators. By using field observations from a Norwegian meadow, we studied here, for the first time, how the local density of previous and simultaneous foraging bumblebees influenced the local foraging behaviour of the three most common pollinators of *Centaurea jacea*: *Bombus pascuorum*, *Bombus lucorum* / *B. terrestris* and *Bombus lapidarius*. In general, pollination activity by single individuals increased with the density of other bumblebees in the patch, as shown by the positive relationships between the number of simultaneous foragers and the number of arrivals to patches, the number of inflorescences contacted per visit, and the duration of visits to inflorescences. The effects that previous foragers had on the behaviour of particular bumblebees were, however, species-specific and variable in their directionality. Thus, we found negative relationships between foraging activity and the previous presence of more similar functional bumblebee species, while positive relationships otherwise. The effect of previous and simultaneous foragers increased in some cases at low Centaurea densities. Our results show that the local abundance of previous and simultaneous conspecific and heterospecific foragers affect the foraging behaviour of particular bumblebee individuals in a complex manner. More studies like ours will shed light to the effect of local bumblebee densities on the behavior of different bumblebee species, and the underlying mechanisms.

21-8 The cues have it; nest-based, cue-mediated recruitment to carbohydrate resources in a swarm-founding social wasp

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This study explores whether or not foragers of the Neotropical swarm-founding wasp *Polybia occidentalis* use nest-based recruitment to direct colony-mates to carbohydrate resources. Recruitment allows social insect colonies to rapidly exploit ephemeral resources, an ability especially advantageous to species such as *P. occidentalis*, which store nectar in their nests. Although recruitment is often defined as being strictly signal-mediated, it can also occur via cue-mediated information transfer. Previous studies indicated that *P. occidentalis* employs a type of cue-mediated recruitment; the presence of conspecifics at a site attracts foragers. This recruitment is resource-based, and as such, is a blunt recruitment tool, which does not exclude non-colony-mates. We therefore investigated whether *P. occidentalis* also employs a form of nest-based recruitment. A scented sucrose solution was applied directly to the nest. This mimicked a scented carbohydrate resource brought back by employed foragers, but, as foragers were not allowed to return to the nest with the resource, without the possibility of recruitment behavior. Foragers were offered two dishes—one containing the test scent and the other an alternate scent. Foragers chose the test scent more often, signifying that its presence in the nest induces naïve foragers to search for it off-nest. *P. occidentalis*, therefore, employs a form of nest-based recruitment to carbohydrate resources that is mediated by a cue, the presence of a scented resource in the nest.

21-9 PROMISCUOUS HONEY BEE QUEENS GENERATE COLONIES WITH A CRITICAL MINORITY OF WAGGLE-DANCING FORAGERS

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Honey bees have a sophisticated communication system that employs the well-known waggle dance to recruit workers to food resources that have been discovered by their forager nest mates. Recent studies have revealed that a high level of within-colony genetic diversity --conferred to a honey bee colony when its polyandrous queen produces multiple patrilines of workers --enhances the production of waggle-dance signals and the corresponding activity of the forager work force. However, how the presence of multiple patrilines in a single colony generates these increases remains a mystery. To explore this phenomenon, we assayed the foraging and dancing performances of workers in multiple-patriline and single-patriline colonies as they visited the same food resource to discover how within-colony genetic diversity gives rise to a more productive foraging effort. *Per capita*, foragers in multiple-patriline colonies visited the food resource more often and advertised it with more waggle dances than foragers in single-patriline colonies. Within each multiple-patriline colony, a minority of patrilines emerged whose workers produced the majority of their colony's foraging and dancing activity. Although the workers from these patrilines were more likely to become engaged as foragers and dancers, once engaged, their per-capita level of activity was similar to that of workers from patrilines that were less likely to contribute to their colony's pool of foragers and dancers. Our results demonstrate that extreme polyandry by honey bee queens does not enhance the foraging effort and production of waggle-dance signals through the introduction of low dance-threshold, high-activity workers into a colony's population. Rather, genetic diversity is critical for injecting into a colony's work force social facilitators who are more likely to become engaged in foraging-related actives, so boosting the production of dance signals and a colony's responsiveness to profitable food resources.

21-10 ASSOCIATIONS BETWEEN DIFFERENT ANT SPECIES: INTERSPECIFIC NESTMATE RECOGNITION AND POTENTIAL BENEFITS

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Aggression between ant colonies is ubiquitous. However, there are exceptions to this rule - in particular, ants of the genera *Crematogaster* and *Camponotus* often occur together across the world. The closest of these associations is the parabiosis, where they share a common nest. Why they tolerate each other, however, is largely unknown. We therefore studied interspecific nestmate recognition in a parabiotic association from the rainforest of Borneo, as well as the potential benefits for each partner. In particular, we compared their cuticular hydrocarbons, which ants use to discriminate nestmates from non-nestmates, to other, associated or non-associated species.The two parabiotic species were surprisingly tolerant towards each other and did not discriminate between colonies of the partner species. *Crematogaster* produced cuticular compounds of a hereto novel substance class. In bioassays, these compounds significantly reduced aggressiveness of its partner *Camponotus*. Moreover, both species had notably longer hydrocarbons than congeneric, non-associated ants. Due to their lower volatility, long-chain hydrocarbons are harder to perceive than shorter ones. We therefore suggest that they provide fewer recognition cues, and thereby hamper interspecific nestmate recognition. Similarly long hydrocarbons were detected in parabiotic ants from the Neotropics, but not in a looser *Camponotus-Crematogaster* association from the Mediterranean, and may hence be typical of parabioses. In the Bornean parabiosis, both species profited from the association. *Camponotus* followed *Crematogaster* pheromone trails and thereby benefited from *Crematogaster's* foraging activity. *Crematogaster*, in turn, seemed to profit from nest defense by *Camponotus*. However, *Camponotus* never occurred without *Crematogaster*, while non-parabiotic *Crematogaster* nests were common. Thus, *Camponotus* seemed to be more dependent on *Crematogaster* than vice versa.

21-11 RESPONSE TO FERTILITY INFORMATION IS NOT FIXED AND CHANGES WITH COLONY GROWTH IN THE ANT *CAMPONOTUS FLORIDANUS*

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Fertility signals are among the most important signals in a social insect colony because of their role in regulating worker reproduction. Fertility signals induce voluntary worker sterility by indicating the presence of a fertile queen and allow the enforcement of worker sterility by providing information necessary for worker policing. However, the fertility signal of a queen is not constant across colony development; incipient queens lack the fertility signals of mature queens. Does the response of workers to fertility information change as the colony grows? In the ant *Camponotus floridanus*, we find the response to fertility information is not fixed and, in fact, changes radically during colony growth. Workers from mature colonies destroy eggs laid by individuals of low fertility (e.g., workers and incipient queens), whereas workers from incipient colonies tolerate all eggs regardless of the fertility of the egg-layer. If the response to fertility signals is not hard-wired, what factors are responsible for modifying the response to fertility information? We hypothesize that experience with fertility signals influences workers' responses. We present data indicating experience can affect the response to fertility signals in the context of egg policing in the ant *C. floridanus*.

21-12 SPECIES-SPECIFIC VIBRATIONAL BEHAVIORS AGAINST PHOTOSTIMULATION IN TERMITES

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Termites attack woody structures with a large number of their nestmates, sensing environmental stimuli vibration of foundation, plant chemicals, sun-light, air-blow, etc. They integrate the information of food, nests, or enemies, and form a well-established society by communicating with nestmates using various types of behaviors. Some of them shake themselves back and forth / right and left, or bump their head / abdomen against foundation in response to the stimuli. In this study, we focused on two types of vibration behaviors, i.e. tremulation (especially back and forth movement) and tapping behaviors (head bumping), in 4 species of termites (*Coptotermes formosanus*, *Reticulitermes speratus*, *Incisitermes minor* and *Zootermopsis nevadensis*) just after strong photostimulation using an LED light source. These behaviors were recorded by a high-speed video camera, and the recorded movies were analyzed with TEMA 2Dsoftware (Photoron Co., Ltd.) to compare the differences of the behaviors among species. Sounds during tapping were also recorded using a condenser microphone with an amplifier (B & K, Co.,Ltd.) and analyzed with Avisoft-SAS Lab Pro software (Avisoft Bioacoustics Co., Ltd.).Tremulation and tapping behaviors were found to be species-specific. *C. formosanus*, *R.speratus* and *Z. nevadensis* showed tremulation at similar frequencies of 15-20 Hz, but with different temporal patterns among the three species. Displacement amplitudes of tapping tended to be larger than those of tremulation in these speces. Exceptionally, *I. minor* did not show tapping behaviors, but tremulation with frequency of 1.0 - 1.5Hz, which was clearly slower than the other species. In addition, *Z. nevadensis* produced audible sounds with a fundamental frequency of 1 kHz with coincidence of the tapping behaviors.

21-13 RECRUITMENT STRATEGIES AND COLONY SIZE IN ANTS

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Ants use a great variety of recruitment methods to forage for food or find new nests, including tandem running, group recruitment and scent trails. It has been known for some time that there is a loose correlation across many taxa between species-specific mature colony size and recruitment method. Very small colonies tend to use solitary foraging; small to medium sized colonies use tandem running or group recruitment whereas larger colonies use pheromone recruitment trails. Until now, explanations for this correlation have focused on the ants' ecology, such as food resource distribution. However, many species have colonies with work forces that grow from a single queen over several orders of magnitude, and little is known about how a colony's organization, including recruitment methods, may change during growth. After all, recruitment involves interactions between ants, and hence the size of the colony itself may influence which recruitment method is used - even if the ants' behavioural repertoire remains unchanged. Here we show using mathematical models that the observed correlation can also be explained by recognizing that failure rates in recruitment depend differently on colony size in various recruitment strategies. Our models focus on the build up of recruiter numbers inside colonies and are not based on optimality arguments, such as maximizing food yield. We predict that ant colonies of a certain size should use only one recruitment method (and always the same one) rather than a mix of two or more. These results highlight the importance of the organization of recruitment and how it is affected by colony size. Hence these results should also expand our understanding of ant ecology.

21-14 RAID ORGANISATION AND DIVISION OF LABOUR IN SLAVE-MAKING ANTS

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The North American slave-making ant *Protomognathus americanus* is an obligate slavemaker, meaning that its colonies completely rely on enslaved host workers for all colony tasks such as foraging and brood care. To satisfy the colony's demand of slaves, some slavemaker workers - the scouts - leave their nest in summer and search for host nests in the vicinity. Successful scouts recruit nestmates and subsequently raid these host nests for slave brood, but they can also attack the nest without assistance.We performed laboratory experiments to gain insights into processes regulating this complex raiding behaviour. We observed more raids when the slave to slavemaker ratio inside the colony was low, indicating a higher need for slaves. Slavemaker workers could determine the colony's need for additional slaves by their nutritional status, as they are exclusively fed by slaves. An experiment with different feeding regimes revealed that in colonies with a lower food provisioning rate, an increased proportion of slavemaker workers search for host nests. This result supports the hypothesis that reduced food supply leads to increased host nest searching. Behavioural observations of unmanipulated colonies during raids showed that some workers were invariably involved in raiding activities, whereas others stayed inside the nest. Cuticular hydrocarbon analyses demonstrated differences between active and inactive workers, and subsequent determination of morphology and fertility parameters added to our understanding of division of labour in *P. americanus*.

21-15 DECODING WAGGLE DANCES TO DETERMINE FORAGING PATTERNS OF HONEY BEES THROUGHOUT THE YEAR

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Loss of habitat as a result of human activities (such as modern farming) is a well known problem for many species; this is also the case for the honey bee (*Apis mellifera*). Lack of foraging habitat may be a significant factor in the 75% decline in the number of colonies over the last century. The `Sussex Plan for Honey Bee Health and Wellbeing' aims to address this problem in part by determining where and when the Sussex landscape has limitations on foraging for bees and other pollinating insects. To exploit resources that are patchy both spatially and temporally honey bees use their unique, symbolic dance language to convey information on the location and quality of resources to other foragers in the hive. We have decoded over 2,000 of these dances from 3 individual hives, allowing us to assess the quality of the surrounding landscape from a colony's perspective. Where previous studies have taken a “snapshot” view of foraging patterns, here we present data for foraging patterns throughout the year (during the months the bees are foraging) in both urban and rural environments. This allows us to examine which areas are lacking in forage during various times of the year. So far, we have found that bees must travel much further during the summer months (August mean 3950m) to find resources than in spring (March mean 710m).

21-16 THE SMELL OF COMPETITION - DOES COLONY SCENT INFLUENCE WORKER REPRODUCTION IN *BOMBUS TERRESTRIS*?

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In the primitively eusocial bumblebee *Bombus terrestris* workers are capable of laying eggs in the presence of a fertile queen. Although the queen advertises her reproductive function by means of a fertility signal and/or a queen pheromone throughout colony development, dominant workers start to compete for reproduction at the end of the annual colonies. Thus, they are able to enhance their individual fitness. Former studies suggested that there should be signals from the nest environment that provide reliable information on nest size, number of fertile workers and caste of brood, enabling individual workers to time their reproduction. We investigated whether nest structures made from wax contain the respective information. To address this hypothesis we collected and chemically analyzed volatiles in the wax of honey pots, egg cups, and brood cells at different stages of colony development. We identified aldehydes, alkanes, alkenes, and fatty acid esters with chain lengths from C19 to C35 in all wax types sampled. In previous studies the same substances were also found on the cuticle surface and in various glands of bumblebee workers. The analyzed nest structures differed in the quantitative composition of these compounds. Moreover, we found pronounced differences in the composition of the wax bouquets before and during the competition phase. We suggest that, as an information source accessible to all colony members at all times, the wax bouquet may influence worker reproduction in mirroring the colony condition.We thank the Carl Zeiss Foundation for financial support.

21-17 STRUGGLE FOR ROYALTY: QUEENS OF *ROPALIDIA MARGINATA* EMPLOY BOTH PHEROMONES AND AGGRESSION

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Ropalidia marginata is a primitively eusocial wasp with colonies consisting of a fertile queen and several functionally sterile workers. The queen is periodically replaced and one of the workers takes over as the new queen. There is growing evidence that despite her remarkable docility, the queen maintains her reproductive monopoly by means of a pheromone derived from her Dufour's gland which she applies on the nest surface using a behaviour we have termed rub abdomen. We therefore argued that the queen should be overthrown if she is prevented from applying her pheromone to the nest. To test this prediction we collected and introduced the queen and all the workers into a fresh cage without the nest, so as to remove the substrate for pheromone application. Contrary to our expectation, queens maintained their status in at least 4 out of 6 experiments; in one experiment the queen was clearly overthrown and another she was not overthrown but neither did she continue to lay eggs. It appears that the queens maintained their status (and laid eggs in newly constructed nest) in 4 experiments, by continuing to rub their abdomens (and presumably apply pheromone) to the walls of the cage even in the absence of the nest. We expect that such attempts to apply pheromone to the cage would be relatively inefficient as the surface area would be very large. In agreement with this we found that although these queens maintained their status, they were aggressively challenged by the workers and they in turn reciprocated with aggression toward their workers. Such aggressive interactions either directed by the queen to the workers or vice versa, are almost never seen in natural colonies and were also not recorded in 12 control experiments where the nest also was introduced into the cage, with or without brood. Our results show that the pheromone helps queens of *R. marginata* to maintain their status but that if necessary they can also supplement the pheromone with physical aggression.

21-18 SIGNALS AND CUES USED FOR ORIENTATION AND COMMUNICATION BY MELIPONINE BEES DURING DEFENSE AND AGGRESSION

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Meliponines (`stingless bees') represent wonderful research objects to study the defensive mechanisms and the biology of societies possessing a flying worker caste. Among the worldwide more than 400 species one can find a great variety of defensive strategies. I studied the behaviour and chemical ecology of sympatrically occurring neotropical species to identify the signals and cues important for the intra- and intercolonial communicative interactions both on intra- and interspecific level. After a focus on two relatively well known species (*Scaptotriona* aff. *depilis* [often still called "*postica*"] and *Trigona spinipes*), I will compare my own observations with prior observations from the literature. This will enable a better identification of some of the most relevant signals and cues used by meliponines during aggressive and defensive actions. Finally, I will present data showing the importance of simple optical cues used by the bees to locate and orient towards potential nest intruders.

21-19 MULTI-COMPONENT TRAIL PHEROMONE IN TERMITES (ISOPTERA)

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In termites, trail pheromones generally consist of a single compound, but very recently multi-component trail pheromones have been identified in *Prorhinotermes simplex* (Rhinotermitidae, Rhinotermitinae), in *Amitermes evuncifer* (Termitidae, Termitinae), in *Armitermes euamignathus* (Termitidae, Syntermitinae), and in many species of Nasutitermitinae (Termitidae). In some species, the multi-component nature of the pheromone could be detected only after GC-EAD. In most of these species, the trail pheromone is made of (3Z,6Z,8E)-dodeca-3,6,8-trien-1-ol and neocembrene. The proportions of the compounds vary according to species, but neocembrene is always much more abundant than (3Z,6Z,8E)-dodeca-3,6,8-trien-1-ol. Both compounds act in synergy. Other components, such as (11E)-trinervita-1(14),2,11-triene, and (3Z,6Z)-dodeca-3,6-dien-1-ol were also identified in trail pheromones, but their function remains unknown until now.Pheromone blends allow for an increased behavioural sophistication. Regarding termite trail pheromones, they could allow an increased biological activity and longevity. They might also be involved in species-specificity of trails.The similar chemical structure of the trail pheromone of the basal termite *Prorhinotermes*, and the more evolutionarily derived termites *Amitermes* and *Nasutitermes* highlight the highly conservative nature of the chemical communication in termites.

21-20 PHEROMONE DISRUPTION OF ANT TRAILING BEHAVIOR IN TWO SPECIES

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Ants are some of the most aggressive and invasive species in the world. With toxic bait systems affecting non-target species, thus not ideal in sensitive ecosystems, new tactics to control ant species are being tested. The fire ant *Solenopsis invicta* uses recruitment pheromones to organize the retrieval of food resources back to the colony. *Z,E-alpha*-farnesene is responsible for the orientation of workers along trails. We disrupted worker trail orientation after presenting of an oversupply (30 or 300 µg) of this compound. Established trails were recorded by overhead webcam and ant positions digitized, before and after presentation of the treatment. The coefficient of determination or trail integrity statistic, r^2 was then calculated. Ants initially showed high linear trail integrity (r^2 = 0.75). Within seconds of pheromone presentation, the ants showed little or no further evidence of trail-following behavior (r^2 = 0.16-0.49).Our fire ant work follows on from the success of Argentine ant *Linepithema humile* trail following disruption. A breakdown of trail following integrity has been achieved using *(Z)-9*-hexadecenal a major component of the trail pheromone. *Linepithema humile* individuals had high trail following integrity r^2 = 0.947 along natural trails before disruption was applied. Integrity dropped (r^2 = 0.164) after a point source of pheromone was placed next to the trail. In Hawai'i Volcano National Park, the disruption of *L. humile* trail formation and a reduction in the number of ants at bait cards lasted for 14 days in the field using a sprayable (multiple point source) microencapsulated trail pheromone formulation. Movies showing the trail disruption phenomenon and the use of machine vision for ant digitization will be demonstrated. Further research is needed to establish the long-term effects and control potential of trail disruption for ants. Negative effects on resource collection, colony size and nest migration are expected.

21-21 INHIBITING WORKER REPRODUCTION IN *DINOPONERA QUADRICEPS*: THE EFFECTS OF BEHAVIOR ON REPRODUCTIVE SIGNALS

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Animal societies are characterized by cooperation and conflict. In the large number of social insects in which the worker caste can never mate, thus being restricted to laying unfertilized eggs, conflicts are mostly expressed in the form of male production. Reproductive individuals are selected on the basis of either aggressive interactions or related aspects and the asymmetry that underlies the dominance relationships is thought to be a good indicator of reproductive potential. Reproductive individuals can signal their presence and/or reproductive capacity by means of signals. The cuticular hydrocarbons are correlated with ovarian activity and signal reproductive status in many social insects. In addition, there are endocrine factors involved directly to the reproductive conditions, such as juvenile hormones. In this study the hypothesis that colonial odors prevent workers from reproduction in *D. quadriceps* was verified. For that, we tested whether the presence of the gamergate in a same divided colony would inhibit the worker reproduction. We also accessed the physiological and behavioral signals of reproduction in the colonies. Our results confirmed that physically direct dominance is important to regulate the reproduction in ponerine ants. The direct contact between the reproductive female and workers was more important than supposedly volatile pheromones. We also found a negative correlation of juvenile hormones and reproduction.

21-22 IDENTIFICATION OF TERMITE QUEEN PHEROMONE REGULATING CASTE DIFFERENTIATION

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Reproductive division of labor based on caste is one of the major characters in eusocial insects. Factors determining caste differentiation are poorly understood but are long believed to involve chemical signals, or pheromones, produced by functional reproductives that inhibit other colony members from differentiating into the reproductive caste. In eusocial insects, queen pheromone is well known about the inhibition of reproductivity of workers. Compounds of queen pheromones have been identified only in a few eusocial Hymenoptera such as ant and honeybees. In termites, which evolved eusociality independently of Hymenoptera, the existence of queen pheromone inhibiting supplementary queen differentiation has been suggested for long time. In spite of researchers' trials over the last half century, none of the termite queen pheromones have been chemically identified. Here we report the first identification of a social insect queen pheromone that inhibits caste differentiation. We demonstrated that queen volatiles suppress new queen differentiation and identified two chemical compounds of the queen pheromone. Moreover, we found that eggs also emit the same volatiles as the queen pheromone. An artificial pheromone blend consisting of these compounds had the full inhibitory power of a live queen. Our results demonstrate that the volatiles from queens and eggs consist of identical compounds and function as a signal of queens' fertility and thus inhibit the differentiation of new queens.

Oral Presentations

Semiochemicals in insect societies: the effects of genes and environment and their interaction

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THE PLASTICITY OF THE CHEMICAL SIGNATURE IN ANTS AND THEIR GUEST: FROM PARASITISM TO MYRMECOPHILY

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Nestmate recognition is a fundamental trait in eusociality to ensure that altruistic behaviours are directed towards related individuals. Discrimination of non nestmates is determined by cuticular hydrocarbons that form a gestalt colony odour. Nevertheless, intruders from alien colonies or different species are frequently observed in ant colonies. I will present a review of this phenomenon ranging from intraspecific robbing to slavery, parasitism and guests and how these intruders can be tolerated within the host colony. Callow workers have small quantities of cuticular hydrocarbons and therefore are tolerated in any colony. This quantity increases in a few days to attain the mature level and adjust to the colony odour. Parasites and guests can synthesize their own hydrocarbons and / or obtain them from the host (chemical mimicry) and accordingly can have a large range of hosts, while others are strictly limited to one. Slave-makers ants *Polyergus* can be reared by various *Formica* species, indicating a large plasticity, but cannot enslave ants of another genus. They develop the host odour but keep some differences with the slaves. This is also observed in *Rossomyrmex* enslaving *Proformica*. Social parasite ants mimic their host odour, for example *Myrmica karavajevi*, the parasite of *M. scabrinodis*. Myrmecophiles are guests of ants; they are tolerated as they have the colony odour of the host. *Sternocoelis* beetles live only in *Aphaenogaster senilis* colonies, as they are able to synthesize the host's hydrocarbons. Coevolution has probably shaped and limited the extent of phenotypic plasticity to select hydrocarbon biosynthesis limited to a close profile of the host. Inside the host colony, contacts permit the integration into the gestalt of the colony. As guests and parasites keep some identity, learning is necessary to permit the mutual tolerance.

BIOGENESIS OF INSECT HYDROCARBONS USED IN CHEMICAL COMMUNICATION

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The recognition of the critical roles that hydrocarbons serve as sex pheromones, kairomones, species and gender recognition cues, nestmate recognition, dominance and fertility cues, chemical mimicry, primer pheromones and task specific cues has resulted in an explosion of new information in the past several decades, especially among the social insects. The presence of complex mixtures of *n*-, methyl branched and unsaturated hydrocarbon components provide a large number of possible components for chemical communication. While in no case do we completely understand the biosynthetic process that regulate the formation of complex mixtures of hydrocarbons present on a given insect, we now appreciate some of the regulatory points in hydrocarbon production. Our knowledge of hydrocarbon formation comes primarily from work on non-social insects, especially flies and cockroaches, and from work in termites. Chain length specificity of the hydrocarbon products appears to reside in the chain length specificity of the microsomal acyl-CoA elongation steps and the acyl-CoA reduction step. The methyl-branch groups arise from the insertion of propionate as the methylmalonyl-CoA derivative in place of malonyl-CoA at specific point during chain elongation. It appears that a microsomal fatty acid synthase is involved forming the fatty acyl precursors to hydrocarbons, and it inserts the methyl-branches early in chain synthesis rather than towards the end of the process. The very long chain acyl-CoAs are reduced to aldehydes that are then converted to hydrocarbon and CO_2 by a cytochrome P450 that functions as an oxidative decarbonylase. Hydrocarbons are produced in the oenocytes, transported by lipophorin in the hemolymph, and cross epidermal cells and the cuticle by a poorly understood mechanism. The selective transport of hydrocarbon components has been shown to play a role in the final cuticular profile of hydrocarbons in some cases.

CASTE DETERMINATION IN *MELIPONA* STINGLESS BEES IS BASED ON GENETIC PREDISPOSITION AND GERANIOL FROM LARVAL PROVISION

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Stingless bees of the genus *Melipona* are unique among eusocial bees because queens and workers develop in brood cells of equal size and feed on similar amounts of food. Thus, trophic caste determination, as in honey bees and all other species of stingless bees, appears unlikely. The actual mechanism that triggers queen development in *Melipona* remained unresolved to this day. We now identified geraniol, the main compound in labial gland secretions of nurse workers, as an exogenous caste determination factor in *M. beecheii*. When 10µg of this terpene were added to the larval food in brood cells, 25% of the females developed into queens, which is a significantly higher proportion as compared to untreated brood cells (9% queens; χ^2 statistics on absolute numbers, $p < 0.001$). This finding corroborates the two-locus, two-allele model of genetic caste determination in *Melipona* proposed by Kerr more than 50 years ago, in which only females that are heterozygous at both loci are capable of developing into queens (25% on average). Caste fate in *Melipona* apparently is controlled both genetically and trophically: Female larvae that are genetically predisposed towards being queens only follow this developmental pathway if they received sufficient amounts of a caste determining compound. In *M. beecheii* this compound is geraniol, which represents the first caste determination substance identified from the larval provision of a social insect. Furthermore, the identification of a nutritional factor triggering queen development clearly refutes a theoretical model, in which *Melipona* larvae are able to personally decide to become queens.

BIASED EXPRESSION OF FATTY ACID ELONGASE GENES AND FERTILITY SIGNALING IN AN ANT.

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Reproductive division of labor among one or a few dominant fertile individuals and many functionally sterile nestmates is the hallmark of the eusocial insects. Reproduction in these societies is assumed to be regulated through fertility signals, though how these signals are produced remains poorly understood. Here, we investigate the association between putative fertility signaling and the expression hydrocarbon synthesis enzymes in the ponerine ant *Harpegnathos saltator*. This ant species is characterized by a low queen worker dimorphism. When queens die, some workers take over reproduction (gamergates) while shifting their cuticular hydrocarbon profile to include compounds with elongated chain length. This characteristic profile acts as a fertility signal that informs sterile workers about the presence and reproductive status of gamergates. To elaborate upon this mechanism, we characterize fatty acid elongase proteins and measure their expression in different tissue types in gamergates and infertile workers. Six fatty acid elongases are upregulated in the gaster of gamergates, indicating that fatty acid elongation is accelerated in gamergates, which correlates with reproduction and behavioral differentiation. Many of these enzymes are also involved in basic fatty acid metabolism and are highly conserved among many insect species. These new results strongly support the hypothesis of fertility signaling through cuticular hydrocarbon profiles and further elucidate the molecular mechanisms that regulate reproduction in eusocial insects.

KIN-INFORMATIVE RECOGNITION CUES IN LEAF-CUTTING ANTS

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Although social groups are characterized by cooperation, they are also often the scene of conflict. Unless group members are clones, their reproductive interests will differ and individuals may benefit by exploiting the cooperative efforts of other group members. Selfish behaviour is therefore predicted to arise in all non-clonal systems. However, it has been shown to be suppressed to a great extend in one of the classic examples of cooperation, social insect societies. Selfishness could result in costs at the colony level and lead to the breakdown of the social organisation. Theory suggests that cues permitting workers to recognise their close relatives and act nepotistically are therefore selected against. Here, we investigate the cuticular hydrocarbon profiles and relatedness of workers from four colonies of the leaf-cutting ant *Acromyrmex octospinosus*. We show that the profiles of these ant workers are informative enough to allow for recognition of patrilines with a very high accuracy and thus may permit within-colony nepotism to occur. The results fundamentally change our understanding of the social insect paradigm and suggest that within-colony conflicts may be more widespread in genetically diverse societies than previously thought or are restrained by alternative mechanisms.

EVOLUTION OF CUTICULAR HYDROCARBON PROFILES - FROM SOLITARY TO SOCIAL HYMENOPTERA

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Cuticular hydrocarbons are ubiquitous in insects. Their primary function is hypothesized to be a barrier against water loss. Additional multiple functions concerning intra- and interspecific communication have been evolved with the most elaborated complexity in social insects. Currently we know the composition and the function of these chemical profiles in many insect species. However, the evolutionary mechanisms shaping the specific profiles or leading to the evolution of specific substance classes often remain unknown. Aside from intraspecific communication and prevention of water loss that may constrain profile diversity other selection pressures such as escape from parasitoids may favour greater profile diversity. To this end, I conducted comparative studies between various groups of solitary and social Hymenoptera to investigate the evolutionary trajectories towards specific cuticular profiles. I will give an overview in this talk on mechanisms and factors which are responsible for altering cuticular hydrocarbon profiles in ants, bees and wasps.

SOURCE AND MAINTENANCE OF CUTICULAR HYDROCARBONS IN THE ANT *FORMICA EXSECTA*

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Within social insect species colony signatures are required so that altruistic behaviour can be appropriately directed. It is widely accepted that within ant species nest-mate discrimination is down to a chemical signature determined by the cuticular hydrocarbons present. The ant species *Formica exsecta* is recognised as having one of the simplest chemical profiles consisting of various (Z9)-alkenes and alkanes, present in colony specific ratios. In this study we investigated the synthesis, transportation and secretion of the cuticular hydrocarbons in the ant species *F. exsecta*. Preliminary studies involved feeding small groups of ants *ad libitum* a diet containing: amino acids, acetates or acids, labelled with either carbon-13 or deuterium atoms. At different time intervals the cuticular hydrocarbons were extracted with hexane and these extracts were analysed using gas chromatography coupled to mass spectrometry. Different substrates appeared to produce greater abundance of isotope peaks after the same amount of time, indicating that the conversion of the labelled substrate into hydrocarbons was not consistent for all substrates. Additional experiments allowed us to track the time taken for labelled substrates to be incorporated into the hydrocarbon profile, whilst the incorporation of labelled oleic acid revealed information regarding the use of precursors in the manufacture of these hydrocarbons. In addition we looked at whether there was any relationship between the type of colony profile and the environmental factors surrounding the colony such as; temperature, humidity and light levels. These results, as well as the importance of the research in terms of the benefits and potential impact that it may have in future research within social insects and chemical ecology in general, will be discussed.

KIN DISCRIMINATORS IN THE SWEAT BEE *LASIOGLOSSUM MALACHURUM*: THE RELIABILITY OF CUTICULAR AND DUFOUR'S GLAND ODOURS

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Social sweat bees (Halictidae) have been excellent systems for studying kin recognition since the seminal study of Greenberg (1979) on *Lasioglossum zephyrum*. We explored the possibility of odour-based kin recognition in the primitively eusocial *Lasioglossum malachurum*, a species in which queens partially cede reproduction to unrelated (alien) workers but not to own workers i.e. *L. malachurum* appears to discriminate among nestmates on the basis of kinship. In particular, we investigated the reliability of worker odour cues to act as indicators of nest membership and kinship. We used GC and GC-MS to determine the odour bouquet of workers (cuticular extracts and Dufour's gland extracts), GC-EAD to determine which odour components could be perceived by worker antennae, and microsatellite DNA markers to estimate genetic relatedness. Cuticular and Dufour's gland compounds varied significantly among colonies, providing a basis for nestmate discrimination. A significant, though weak, negative correlation between chemical distance and genetic relatedness ($r = -0.055$, $p = 0.0001$) suggests a genetic component to variation in cuticular bouquet, but odour cues were not informative enough to discriminate between different degrees of relatedness within nests. This pattern of variation was identical for Dufour's gland bouquets. Both the presence of unrelated "alien" individuals within nests and the fact that aliens are not chemically different from their nestmates suggest that the discrimination system of *L. malachurum* is prone to acceptance errors. Compounds produced by colony members are likely combined to generate a colony chemical signature (gestalt odour). The correlation between odour cues and nest membership is greater for perceived compounds than for non-perceived compounds, suggesting that variability in perceived compounds is a result of selection for nestmate recognition despite possible counter-selection to reduce costs derived from an excess of nepotistic behaviour.

INTER- AND INTRA-COLONIAL DIFFERENCES IN NON-POLAR COMPOUNDS IN THE CLONAL ANT *CERAPACHYS BIROI*

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Cuticular and glandular chemicals are the most important sources of information used in insect communication. Although chemical signalling has been widely investigated in social Hymenoptera, there are no studies on the chemical ecology of clonal ants. *Cerapachys biroi* is one of only two ant species showing obligatory thelythokous parthenogenesis; it has an army ant-like colony cycle, in which stationary periods (statary phases) alternate with foraging periods. *Cerapachys biroi* also shows a peculiar division of reproduction: "queen-like" individuals lay eggs throughout their lifetime, and workers lay eggs only during the initial colony cycles. These queen-like individuals spend all their time in the nest taking care of the brood, whereas workers switch their behaviour and turn into foragers after becoming functionally sterile. The aim of this study was to assess if there are chemical differences between different classes of individuals (e.g. young workers *vs* old workers) and different colonies, given that the genetic variability in this species is expected to be limited by the clonal structure. We collected individuals of different age and caste from eight colonies, and we analyzed their chemistry using GC-MS. We found that cuticular hydrocarbons, and non-polar compounds in general, differ at both inter- and intra-colonial levels. These differences are correlated with the individual's age, task, reproductive role and colony.

Poster Presentations

Semiochemicals in insect societies: the effects of genes and environment and their interaction

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22-1 SENSING COLONY SIZE ON THE BASIS OF PHYSICAL CONTACTS

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In social insects various traits of the colony and individuals change with colony growth. This phenomenon implies that each individual can sense own colony size by some means but the mechanism is still largely unknown. Because individual ant is difficulty to count directly the number of individuals in a colony, it is predicted there is an indirect index reflecting the change of colony size. The queen signal is crucial information for the maintenance of group cohesion because under the presence of queen signal, workers refrain from reproducing and engages in altruistic behaviors. In *Daicamma sp.* from Japan, the queen signal is transmitted to workers only through direct physical contacts between a gamergate and workers. A gamergate frequently walks around the nest to spread the queen signal efficiently. Although this way is considered to be effective in the case of small societies, even in small society, the efficiency of regulation via direct contact can vary with colony size. Then, we examined following two hypotheses, 1) the index of colony size for workers is the contact interval to a gamergate: the contact interval lengthens with colony size and reproductive behaviors of workers become active in larger colonies, 2) the index of colony size for a gamergate is the contact interval to reproductive workers: the number of reproductive workers increase with colony size and the patrol behavior become active in larger colonies. As predicted, the reproductive behaviors of workers and the patrol behavior of gamergates were changed with colony size and these behaviors were influenced by the contact interval to a gamergate and reproductive workers, respectively. These results showed in *Diacamma sp.* the change of physical contact interval was used as the index of colony size. Also each sensing mechanism of colony size for workers and gamergates are mutually interacted and form a feedback cycle.

22-2 INTRA-NEST HYDROCARBON DYNAMICS AND WORKER REPRODUCTIVE DECISIONS IN THE ANNUAL BUMBLE BEE *BOMBUS TERRESTRIS*

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One of the most interesting features of insect societies resides in the great diversity of individual reproductive strategies. In the annual bumble bee, *Bombus terrestris*, workers have two reproductive options. They can reproduce inside their own nest at the end of the colony cycle, where intense worker-worker and queen-worker conflicts occur, or they can drift to other nests and reproduce. Comparing those two strategies allows to investigate the underlying proximal mechanisms, as well as to study individual behaviour in two social contexts leading to distinct reproductive conflict structures. Behavioural experiments strongly suggest that chemical cues are involved in both intra- and inter-colonial reproduction. However, those cues have until now been poorly investigated. Thus, in order to clarify which kind of information colony members could use to take their reproductive decisions, our study aimed at finely investigating the different signals present in the nest. The development of colonies headed by wild-caught queens was daily monitored and the queens, workers, wax and worker- and queen-laid eggs profiles were regularly analysed all through the colonial cycle using SPME, gas chromatography and mass spectrometry. The chemical pattern of each group and their evolution were studied and compared at the intra- and inter-colonial level. We discuss the importance of the various chemical cues with regards to the two reproductive strategies, and the way they correlate with the beginning of worker reproduction and the behavioural interactions observed in the nest.

22-3 MAINTAINING THE GESTALT: A POTENTIAL ROLE OF NEST MATERIAL IN NESTMATE RECOGNITION

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Nestmate recognition is of paramount importance in social insects, since it enables individuals to protect their colonies against intruders and parasites. Recognition is thought to depend on the degree of matching between a label (odour profile of a given individual) and a template (neural representation of the colony odour profile). In ants, there is strong evidence that long-chain cuticular hydrocarbons (CHC) are used as recognition cues (signature odours). These cuticular hydrocarbon blends can consist of dozens of different chemical compounds, and both environmental and genetic factors can contribute to this label. In this fieldstudy, we tested whether nest material can potentially act as a holder of ant-produced odours that influences the label and/or template in the colony. We conducted aggression tests involving *Camponotus aethiops* workers that had been exposed to soil from either their own or a different colony. The exposed ant was used as either the focal or target ant in aggression tests. Aggression was markedly lower when the target ant had been exposed to nest material from the attacking ant's colony, relative to the control. However, exposing the focal ant to foreign nest material did not significantly affect its aggression level towards this foreign colony. Treatment with foreign colony soil produced changes in ants' CHC profiles that were detectable by GC-MS, although the changes were subtle. Our results demonstrate that nest material has a role in nestmate recognition, being able to change the label of individual ants, and that the recognition system of ants is extremely fine tuned, able to recognise small differences in odour profile.

22-4 A PROTEOMIC APPROACH TO THE STUDY OF SOLUBLE PROTEINS INVOLVED IN HONEYBEE OLFACTION.

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Chemical communication in the honeybee is a sophisticated system, making use of several pheromones regulating interactions and exchange of information between castes and individuals. Odorant Binding Proteins (OBP) and Chemosensory Proteins (CSP) are soluble proteins highly expressed in insect chemosensilla where they interact with pheromones and odorants and lead to Odorant Receptor (OR) activation (Pelosi *et al*. 2006). Insect genomes contain a remarkable number of genes encoding OBPs (57 in the malaria vector *Anopheles gambiae*, 21 in the honeybee), and a lower number of genes encoding CSPs (7 in *An. gambiae*, 6 in the honeybee). The expression of such proteins is not limited to olfactory organs, with some being ubiquitous and others being expressed in pheromonal glands, where they are possibly involved in pheromone release. Unraveling the role of OBPs and CSPs requires both detailed studies of their expression in different physiological studies as well as studies of their affinity for known pheromones and general odorants. Until recently expression patterns of such genes in insects have been almost exclusively investigated through genomic analysis. We used a proteomic approach to study expression of OBPs and CSPs in antennae and mandibular glands of honeybee workers and queens. Protein extracts were separated through 2D gel electrophoresis and proteins were identified by High Performance Liquid Chromatography coupled to a LTQ Orbitrap Mass Spectrometer. We found that several OBPs were expressed in antennae, while a lower number was expressed in the mandibular glands. Moreover considerable differences were found between OBP expression in mandibular glands of individuals of different ages and castes. Affinity of selected OBPs with honeybee pheromones was studied in binding competitive assays.

22-5 IDENTIFICATION OF AN ANT QUEEN PHEROMONE REGULATING WORKER STERILITY

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Social insect societies range from paragons of cooperation to uneasy alliances in which conflict is rife. The interplay between altruism and self-interest is especially apparent in the evolution of worker sterility. In many species, queens are thought to produce primer pheromones that modulate worker sterility; these may either be honest signals of quality to which it benefits workers to respond, or manipulative agents of control. Although queen pheromones underpin both the proximate and ultimate causes of reproductive division of labour, progress has been hampered by the fact that they have only been conclusively identified in the honeybee. By synthesising candidate pheromones, we experimentally demonstrate that the queen surface hydrocarbon 3-MeC31 reduces worker ovarian development and aggression in the ant *Lasius niger*. Production of 3-MeC31 is lower in immune-challenged queens, showing that it is condition-dependent and potentially costly. The chemical is also correlated with queen fertility and ovarian activation, and is potentially used by workers to identify and eliminate unproductive queens in multi-queen colonies. The pheromone appears to be an honest signal of the queen's value as a reproductive, suggesting that workers altruistically remain sterile as long as their queen is healthy and fertile.

22-6 UNCOUPLING FERTILITY FROM FERTILITY-ASSOCIATED PHEROMONES IN WORKERS HONEYBEES (*APIS MELLIFERA*)

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Fertility-associated pheromones, chemical signals delineating ovarian development, were favourably selected in the course of evolution because it is in the best interest of both the signallers (in recruiting help from other colony members) and the receivers (in assisting them to reach an informed decision of how to maximize fitness). Such signals therefore should constitute honest, deception-proof indicators of ovarian development, suggesting, theoretically, that the processes of ovarian development and signal production are irreversibly coupled. Here we demonstrate that these processes can be uncoupled by treating queenless (QL) honeybee callow workers with a juvenile hormone (JH) methoprene, JH analog. While methoprene effectively inhibited ovarian development, it neither inhibited Dufour's fertility signal nor the mandibular glands' dominance signal. In fact, there was even a slight augmentation of both in the methoprene-treated bees. Thus, although fertility and fertility signals are tightly associated, they can be uncoupled by experimental manipulation. Based on these results we hypothesize that ovarian development and fertility-associated signal production are triggered by a common event/signal (e.g. queen pheromone disappearance) but comprise different regulatory systems. However, the results may have confounded because of the pleitropic effects of JH in many insects. We therefore repeated this experiment using RNA interference (RNAi)-mediated silencing of the vitellogenin gene function. Injection of vitellogenin double stranded RNA (dsRNA) blocks ovary development seemingly as well as affect JH titre in workers, and will shed further light on the regulatory process governing reproduction and signal production.

22-7 WE ARE FAMILY - ARGENTINE ANTS LIVING IN HARMONY!

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Introduced populations of the Argentine ant, *Linepithema humile*, are characterised by a social structure known as unicoloniality, where intraspecific aggression between nests is absent and there are no territorial boundaries, resulting in the formation of large and spatially expansive supercolonies. Ants within these supercolonies show very low levels of aggression towards more distant colony members, however, ants from different supercolonies are mutually aggressive. We studied the behavioural relationships of Argentine ant populations in the Western Cape, South Africa, using aggression bioassays and chemical analysis of cuticular hydrocarbons (CHCs). Both the aggression bioassay and chemical data revealed that the Argentine ant populations can be divided into two behaviourally and chemically distinct supercolonies. Although intraspecific aggression was rare among nests ranging over many kilometres, it was evident between the two supercolonies of Argentine ants in the Western Cape with ants able to discriminate among conspecific non-nestmates. Ant CHC profiles varied within and between populations, possibly due to a wide range of environmental influences, however these differences did not influence the levels of aggression between colony-pairs. The presence of these two distinct supercolonies is suggestive of at least two introduction events of this ant within the Western Cape. Moreover, the pattern of colonisation observed in this study, with the two colonies interspersed, is in agreement with global patterns of Argentine ant invasions.

22-8 DEFEAT OF THE CLONES: RAPID SIGNAL DEVELOPMENT IN *APIS MELLIFERA CAPENSIS* DOES NOT GIVE THE UPPER HAND IN INVASIONS

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A. m. capensis workers can successfully establish themselves as social parasites, of their own and other subspecies. This means workers must enter host colonies undetected, however the acceptance rates of the clonal population of *A.m. capensis* workers which are established north of the hybrid zone in South Africa is exceptionally low. We asked if clone workers produced a mandibular gland pheromone (MGP) that made them conspicuous to the host colony workers and also whether they were genetically predisposed to producing queen-like MGP and consequently outcompeting native Cape workers. We aged clone and native workers in a controlled environment and followed their MGP signal development over time. The clone workers produced (E)-9-hydroxy-2-decenoic acid (9HDA) from 12hrs while the native workers only started producing this compound after 24hrs. Not only were they quicker in synthesising 9HDA but they also produced variable quantities of (E)-9-keto-2-decenoic acid from 24hrs on. Clearly, these clone Cape workers out-compete their native sisters in the onset of producing queen compounds. This rapid synthesis of queen compounds by these clone workers significantly lowered their acceptance by host workers (Spearman $\rho = -0.34$, $p < 0.0001$). However, once past the host colony defence, does this rapid signal development culminate in pseudoqueen establishment by outcompeting native workers? A single clone worker was placed together with a group of native workers for 10 days after which time their MGP were analysed. Clone workers produced a queen-like MGP but did not always succeed in outcompeting native workers who produced a more queen-like signal in 71% of the trials. Although clone workers have a propensity to develop their MGP rapidly, this is not advantageous in gaining access to host colonies, nor does this early head start result in pheromone dominance. Clone workers are therefore no more advanced on the queen-worker pheromone continuum than native workers.

22-9 The Cape honeybee (*Apis mellifera capensis*); a social parasite by predisposition?

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Worker reproduction in honeybees is mainly regulated by pheromones produced by the brood and the queen. The queen pheromones influencing worker reproduction have been located in the mandibular glands and in non-laying workers this gland's profile is dominated by fatty acids that are incorporated into the food given to the brood and to nestmates via trophallaxis. After queen loss and onset of reproductive activity, workers can synthesize different fatty acids, which are normally produced by queens and that contribute to their reproductive status and success. *Apis mellifera capensis* workers can rapidly produce queen-like mandibular profiles, which could represent a major factor in their ability to behave as facultative intraspecific social parasites. Indeed, *A. m. capensis* workers can dominate reproduction and outcompete the host queens in colonies of other subspecies. Even in the presence of their own queen, the mandibular gland profile of *A. m. capensis* workers contains the precursor of the major compound of the queen pheromone. This unique trait amongst honeybee workers supports that *A. m. capensis* workers are primed for reproduction and that this phenomenon represents a pheromonal predisposition to social parasitism.

22-10 Chemical comparison between contents of cephalic salivary glands and cuticular profile of *Scaptotrigona postica* workers

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The contents of the cephalic salivary glands (CSG) of *Scaptotrigona postica* workers have been analysed at various stages after adult emergence and compared with the cuticular hydrocarbons at these stages.The CSG of the newly emerged workers are almost empty. They contain tiny amounts of 2-tridecanone, 2-tridecanol and ethyl oleate. Older workers functioning as nurses inside the nest have more secretion, consisting of various esters (tetradecenyl acetate and hexadecenyl acetate), together with those found in the newly emerged workers. The gands of forager workers contain much more secretion and a more complex mxture of unsaturated alcohols and acetates, and 2-ketones and and corresponding alcohols.It has been suggested that the CSG make some contribuition to the mixture of hydrocarbons that waterproof the cuticle of stingless bees. Since wings of worker bees are likely to be the body part least contaminated by plant and hive waxes, these were also analysed. The wings of newly emerged workers contain chief n-tricosane, followed by heptacosane and nonacosene. Nurse workers contain chiefly heptacosane, nonacosene and nonacosane. Forager workers contain a similar mixture of heptacosene, heptacosane and nonacosene. Conclusions:1. The CSG of this stingless bee only became fully supplied with secretion in forager workers. 2. The CSG contain a complex mixture of oxygenated organic compounds of intermediate volatility. 3. The CSG do not contribute hydrocarbons to the cuticular profile. 4. The composition of the cuticular hydrocarbons also change with age of worker. Further work is proceeding the identification of the substances in the CSG, and their preparation for behavioral experiments.

22-11 An oriental orchid seems to be mimicking the mandibular gland components of *Apis cerana*

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An oriental orchid *Cymbidium floribundum* is known to aggregate the *Apis cerana japonica* (*Acj*) and not to attract *Apis mellifera* (*Am*). We have devised an assay system using *Acj* taken from a hive and released into two-choice arenas and have estimated the aggregation rate to an attractant effectively. This system can be used to distinguish clearly between an attracting orchid and a non-attracting one. To investigate the mechanism of the aggregation we prepared the extracts of the petal, sepal and labella of the flowers separately and found that the acid fraction of the petal and the sepal extracts attracted *Acj*. The main component of the fraction was 3-hydroxyoctanoic acid (3-HOAA) Keeling *et al.* reported Indonesian *A. cerana* and *Apis nigrocincta* mandibular glands of mated queens and workers contain 3-HOAA. It is known that the pheromone secreted from queen mandibular gland has activities such as drone attraction and induction of retinue behavior of workers. The gas chromatography - mass spectrometry for methanol extracts of heads of *Acj* workers and *Am* showed that they contained 10-hydroxydecanoic acid (10-HDAA) and (E)-10-hydroxydec-2-enoic acid (10-HDA) known as royal jelly components. In addition, *Acj* had 3-HOAA as the major component. Using our assay system the extract of *Acj* was found to attract *Acj* specifically. We tested the attracting activity of 3-HOAA (synthesized racemate) mixed with other mandibular gland components; (E)-9-oxodec-2-enoic acid, (E)-9-hydroxydec-2-enoic acid, 10-HDAA, and 10-HDA. Several different mixture patterns of these components showed clear attractiveness to *Acj* workers. Also the activity was observed with two other related subspecies, *A. cerana cerana* and *A. cerana indica*. We concluded that 3-HOAA mixed with the other mandibular gland components function as aggregation pheromones for *A. cerana*. It is thought that *C. floribundum* is mimicking the mandibular gland components of *A. cerana*.

22-12 Blending of heritable recognition cues among ant nestmates to create a gestalt odour prevents within-colony nepotism

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The evolution of sociality is facilitated by the recognition of close kin, but if kin recognition is too accurate, nepotistic behavior within societies can dissolve social cohesion. In social insects, cuticular hydrocarbons act as nestmate recognition cues and are usually mixed among colony members to create a colony Gestalt odor. Although earlier studies have established that hydrocarbon profiles are influenced by heritable factors, transfer among nestmates, and additional environmental factors, no studies have quantified these relative contributions for separate compounds. Here, we use the ant *Formica rufibarbis* in a cross-fostering design to test the degree to which hydrocarbons are heritably synthesized by young workers and transferred by their foster workers. Bioassays show that nestmate recognition has a significant heritable component. Multivariate quantitative analyses based on 38 hydrocarbons reveal that a subset of branched alkanes are heritably synthesized, but that these are also extensively transferred among nestmates so that they appear unsuitable for within-colony kin discrimination. In contrast, especially linear alkanes are less heritable and little transferred, which may prove suitable cues for the assessment of genetic diversity within colonies. These results indicate that heritable compounds are suitable for establishing a genetic Gestalt for efficient nestmate recognition, but that recognition cues within colonies are insufficiently distinct to allow nepotistic kin discrimination.

Oral Presentations

Nestmate and other kin-recognition systems: from ecology and behaviour to molecular and neurophysiological techniques

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ADAPTIVE SHIFTS IN THE ACCEPTANCE THRESHOLDS OF HONEY BEE GUARDS

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Reeve (Reeve H. K. 1989. The evolution of conspecific acceptance thresholds. American Naturalist 133: 407-435) presented a general model of acceptance thresholds. When environmental conditions vary, discriminating individuals can increase their fitness or inclusive fitness by adopting variable rather than fixed acceptance thresholds. In nest defence in social insects, the model predicts that entrance guards should adopt less permissive conspecific acceptance thresholds when the frequency of intrusions by non-nestmates and the cost of these intrusions increase. Honey bees, *Apis mellifera*, are an excellent species for testing these predictions. Workers frequently rob honey from other colonies, and robbing can kill the victim colony by depleting the honey reserves it needs to survive. Robbing intensity varies greatly both seasonally and over shorter periods, thereby providing environmental variation. Finally, the number of guards and fights, and the acceptance or rejection of individual nestmates or non-nestmates, can all be observed and quantified at hive entrances. Experimental results provide strong support for the model. As predicted, guard acceptance thresholds are less permissive when the frequency of robbing by conspecifics is higher. When nectar conditions changed from dearth to plenty over several weeks the threshold became progressively more permissive finally becoming “accept all”. But the threshold can also change rapidly. When the number of intruders increased rapidly guards became less permissive within minutes. Guards are faced with trading off two errors, accepting non-nestmates and rejecting nestmates. These two errors are negatively correlated when the intruders are conspecifics but not when the intruders are allospecific *Vespula vulgaris*, which also robs honey bee colonies. This shows that acceptance threshold variation is also driven by an underlying informational constraint in ability to discriminate between nestmate and non-nestmate conspecifics.

A COMPARATIVE PHYSIOLOGICAL STUDY ON NESTMATE RECOGNITION SYSTEMS BETWEEN MONO- AND POLYGYNE ANT SPECIES

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Territorial boundaries between conspecific social insect colonies are maintained through nestmate recognition systems. Previously, in a monogyne ant, *C. japonicus*, it was shown that the colony specific cuticular hydrocarbon (CHC) blend is a chemical cue for nestmate versus non-nestmate discrimination, and that the particular antennal CHC sensilla responded to non-nestmate CHCs but did not to nestmate CHCs. Thus, the CHC sensilla of *C. japonicus* function as a well-developed differential CHC pattern detector, which is based on a sensory desensitization for the nestmate CHC blend. In contrast, in supercolony-forming polygyne ant species, which have developed an extraordinary social organization style known as unicoloniality, individuals mix freely among physically separated nests. The underlying mechanism is considered to involve remarkable reduction of intraspecific aggressiveness, while maintaining interspecific competitiveness. *Formica yessensis* is a polygyne ant species that constructs the largest supercolonies, comprising numerous nests, ever found in Japan. We compared the composition of CHCs of workers from different localities of a supercolony, as well as the chemosensory discrimination efficiency of the CHC sensilla. Workers from colonies within the supercolony had more similar profiles compared to workers from colonies outside the supercolony. Total response of the active CHC sensilla increased stepwise, suggesting that discrimination of conspecific workers at the peripheral system is limited, in particular among members of the same supercolony, but is fully expressed for allospecific workers. Thus, we illustrate that the full and the limited nestmate versus non-nestmate discrimination potential of the CHC sensilla, which relies on the intraspecific similarity of the CHC pattern, is one of the important factors for the characteristic aggressiveness and/or tolerance in *Camponotus japonicus* and *Formica yessensis*.

FRIENDS AND FOES FROM AN ANT BRAIN'S POINT OF VIEW - FUNCTIONAL IMAGING OF COLONY ODORS IN *CAMPONOTUS FLORIDANUS*

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Ants discriminate colony members (nestmates) from members of different colonies (non-nestmates) by their colony odor, which consists of colony-specific hydrocarbons on the cuticle (label). During the recognition process, the label is compared to a neuronal template (label-template matching) and a mismatch will lead to aggression. The neuronal basis of label-template matching is unknown. In search for the neuronal correlate of the label, we retrogradely stained output neurons of the primary olfactory center of the ant brain, the antennal lobe (AL), and presented label-treated dummies. We measured neuronal activity in the upper half of the AL in response to both nestmate and non-nestmate label using calcium imaging with a CCD camera. Our results show that workers can perceive the label of their own colony. A template implemented simply as a sensory filter in the antenna, where olfactory receptor neurons are adapted to the ever-present nestmate label and only non-nestmate label is detected, is hence unlikely to be the main mechanism of nestmate recognition. Alternatively, sensory information might be specifically modified along the olfactory pathway to allow discrimination, with specific modifications that result in a template. We compare neuronal activity patterns elicited by nestmate and different non-nestmate labels using multi-variant statistics in search for specific modifications of neuronal representations. In order to map the neuronal activity in the whole AL, we use calcium imaging with a 2-photon microscope and analyze the responses of glomeruli located in the lower part of the AL. Differences in the neuronal responses to the label in different parts of the AL might indicate that different mechanisms are realized in parallel to achieve efficient and reliable nestmate recognition.Funding: DFG SSB554/A6 & GSLS Würzburg

NESTMATE RECOGNITION AND TOLERANCE THRESHOLDS IN THE FACULTATIVE POLYGYNOUS ANT *PACHYCONDYLA VERENAE*

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The fact that many ant species vary their level of aggressiveness according to the status of their neighbours suggests some mechanism of intercolony recognition. However, non-nestmate recognition mechanisms can greatly vary according to the social system and ecological conditions of each species. In the ponerine ant *Pachycondyla verenae* workers forage solitary at distances of up to 30 meters. At these distances, foraging areas of distinct nests can overlap and allocolonial ants may often interact. This species presents territorial behaviour, but only the vicinity of the nest is highly defended. Here, we studied the intraspecific level of aggression in a dense population where nests could be found less than 3 meters apart. We investigated the effect of nest distance on aggressive behaviour on a fine scale and whether and how chemical and genetic divergence among nests are correlated to it. We also investigated if acoustical signals play a role during intraspecific interactions in this species. Dyadic encounters were conducted between individuals from colonies distributed over different distance ranges, which corresponded to sympatric neighbours, sympatric non-neighbours, and allopatric colonies. The variability in the composition of the cuticular hydrocarbons between the colonies used in the behavioural tests was investigated by GC-MS SPME analysis as well as the population structure using species-specific microsatellite markers. We discuss the relative importance of these different factors in the set up of different tolerance thresholds in *P. verenae*. Supported by the Programme AlBan, the European Union Programme of High Level Scholarships for Latin America scholarship n° E06D101212BR, and the French Entomological Society (Grant Germaine Cousin).

NOT ONE ODOUR BUT TWO: A NEW MODEL FOR NESTMATE RECOGNITION

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Recognition systems play a key role in a range of biological processes, including mate choice, immune defence and altruistic behaviour. Social insects provide an excellent model for studying recognition systems because workers need to discriminate between nestmates and non-nestmates, enabling them to direct altruistic behaviour towards closer kin and to repel potential invaders. However, the level of aggression directed towards conspecific intruders can vary enormously, even among workers within the same colony. This is usually attributed to differences in the aggression thresholds of individuals or to workers having different roles within the colony. Recent evidence from the weaver ant *Oecophylla smaragdina* suggests that this does not tell the whole story. Here I propose a new model for nestmate recognition based on a vector template derived from both the individual's innate odour and the shared colony odour. This model accounts for the recent findings concerning weaver ants, and also explains why the level of aggression expressed by a colony decreases as the diversity within the colony increases, even when odour is well-mixed, as recently noted in *Formica exsecta*. The model makes additional predictions that are easily tested, and represents a significant advance in our conceptualisation of recognition systems.

ADAPTIVE COGNITIVE SPECIALIZATIONS FOR CONSPECIFIC FACE PROCESSING IN *POLISTES* PAPERWASPS

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Whether cognitive evolution is process general or domain specific has been the subject of intense debate. The general process view argues that evolution shapes the basic rules of learning and memory. Alternatively, domain specificity argues that selection shapes cognitive abilities depending on the particular tasks that are important for a given species. Specialized face recognition in humans has provided some of the best evidence for specialized domain specific cognitive abilities in any taxa. Like humans, the paper wasp *Polistes fuscatus* uses variable facial patterns to recognize individuals; so testing for the occurrence of specialized face processing in wasps will provide an important comparison with previous vertebrate research. Importantly, *P. metricus*, a close relative of *P. fuscatus*, lacks individual recognition allowing us to test whether cognitive specializations for face processing are restricted to species with individual recognition. To examine domain-specificity we trained wasps on various kinds of images. Our results show that *P. fuscatus* learn conspecific faces more rapidly and accurately than simple patterns or abnormally configured faces. In contrast, *P. metricus* fails to learn faces. In fact, *P. fuscatus* learns both heterospecific and conspecific face images more rapidly than *P.metricus*. This finding is especially surprising given that hymenopteran vision is thought to be well adapted to high contrast images, such as simple black and white patterns. Despite the complexity of the facial images, *P. fuscatus* appears to be specialized for learning faces. Domain-specific visual processing in an insect suggests that specialized cognition may be a common feature of neural systems in general rather than particular to vertebrates. Additionally, the contrast in domain specificity associated with individual recognition suggests that cognitive specialization is evolutionarily labile and adaptive in *P.fuscatus*.

Poster Presentations

Nestmate and other kin-recognition systems: from ecology and behaviour to molecular and neurophysiological techniques

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23-1 REGULATION OF REPRODUCTION IN THE PRIMITIVELY EUSOCIAL WASP *ROPALIDIA MARGINATA*: ON THE TRAIL OF THE QUEEN PHEROMONE

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As expected for a primitively eusocial wasp, queens and workers are not morphologically differentiated in *Ropalidia marginata*. As unexpected for a primitively eusocial wasp however, *R. marginata* queens are remarkably meek and docile. Upon removal of the queen, one of the workers becomes extremely aggressive but immediately drops her aggression if the queen is returned. If the queen is not returned, this hyper-aggressive individual will develop her ovaries, lose almost all her aggression, and become the next queen of the colony. Hence we call her the potential queen (PQ). Because of the non-aggressive nature of the queen and because the PQ loses her aggression by the time she starts laying eggs, we consider the hypothesis that regulation of worker reproduction in *R. marginata* is mediated by pheromones rather than by physical aggression. Using the immediate loss of aggression by the PQ upon return of the queen, we develop a bioassay to demonstrate that the queen's Dufour's gland is at least one of the sources of the queen pheromone. Extracts of the queen's Dufour's gland (but not that of the worker's Dufour's gland) mimic the queen in making the PQ drop her aggression. We are also able to correctly classify queens and workers by a discriminant function analysis based on the chemical compositions of their respective Dufour's glands.

23-2 CUTICULAR HYDROCARBONS OR PEPTIDES: WHICH ONE IS RESPONSIBLE FOR NESTMATE RECOGNITION IN *POLISTES DOMINOLUS*?

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A colony of social insects is like a fortress. Access is allowed only to the members of the colony. The mixture of hydrocarbons of the cuticle, whose primary function is to prevent dehydration, was widely reported to be involved in nestmate recognition in all social insects. Recent studies have shown the occurrence of a peptidic fraction on the cuticle of the paper wasp of the genus *Polistes* that may be involved in the communication system. Present work is aimed at separating the two cuticular fractions, peptides/polar *versus* hydrocarbons/apolar, to verify whether only the latter component is responsible for nestmate recognition as reported in the literature. Several colonies of *Polistes dominulus* were collected in the field and brought to the laboratory. Conspecific wasps, not belonging to the same colonies, were also collected to be used as non-nestmates in discriminatory tests. These spare individuals were killed by freezing and washed through several steps in a mixture of polar and apolar solvents in order to obtain two distinct cuticular fractions: hydrocarbons and peptides. These fractions were checked for purity through Gas Chromatography coupled with Mass Spectrometry and MALDI-TOF mass spectrometry, respectively. Binary choice tests were performed on each colony by presenting the two isolated fractions, previously extracted from a non-nestmate conspecific in order to evaluate the behavioural response. Our results confirmed that it is the hydrocarbon fraction, and not the peptidic one, the chemical mediator prompting nestmate recognition in paper wasps.

23-3 *KLADOTHRIPS* VS. *KOPTOTHRIPS*: COMPARING THE PERIPHERAL CHEMOSENSORY SYSTEMS OF A EUSOCIAL THRIPS AND ITS PARASITE

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Chemoreception is essential for insects, e.g. both olfaction and contact chemoreception ('taste') are involved in host plant selection as well as in nestmate recognition, enabling the integrity of insect societies. *Kladothrips intermedius* is a eusocial *Acacia* gall-inducing thrips, presenting 2 castes: dispersers, the reproductive one, and soldiers, mainly defensive. *Koptothrips dyskritus* is a kleptoparasite invading *Kl. intermedius* galls, a relevant resource for these species representing both food and shelter in the harsh environment of the Australian outback. Soldiers evolved in at least 7 species of the genus *Kladothrips* probably in response to the selective pressure by their lethal parasites (4 species belonging to the genus *Koptothrips*). We analysed through SEM and TEM the morphology and ultrastructure of their flagellar chemosensilla in order to offer an initial step to further investigations into their chemical ecology: host plant choice, anal droplet function, nestmate recognition and potential camouflage. Sensilla represent the peripheral nervous system in insects and they host different sensory neurons. Chemosensilla host ORNs (Olfactory Receptor Neurons) or GRNs (Gustatory Receptor Neurons). The 2 species, in spite of their weak relatedness and their main morphological differences, show similar antennae. They are both moniliform and 8-segmented, but the last 2 flagellomers are almost fused together in the eusocial thrips. The antennae of soldiers look like a shrunk version of those of dispersers. Even the sensillar array is very similar, presenting olfactory SW trichoid sensilla as well as DW coeloconic ones and contact chemoreceptive chaetic sensilla. The latter are sexually dimorphic in *Kl. intermedius*. The parasite presents only 2 additional olfactory sensilla dorsally. The ultrastructural analysis revealed a higher number of ORNs in dispersers than in soldiers. Electrophysiological recordings corroborate the olfactory function of some sensilla.

23-4 GENOME AND COMMUNICATION - THE GENETIC ARCHITECTURE OF COMMUNICATION IN ANT SOCIETIES

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The recent availability of multiple ant genomes provides an unprecedented opportunity to better understand the genetic basis of communication in insect societies, both from the perspective of the sender and the recipient. Here we report our work on a series of candidate genes involved in communication, which we annotated in the newly sequenced Red harvester ant (*Pogonomyrmex barbatus*) genome. In particular, the organization of insect societies is critically dependent on its members' capability to distinguish between nestmates and non-nestmates. In ants, this discrimination is based on the detection of multicomponent cues encoded by cuticular hydrocarbon (CHC) profiles. Due to their chemical diversity, methylalkanes and alkenes are thought to be particularly effective discriminatory components of these profiles. We began to investigate genes involved in the production of alkenes as part of the CHC pattern in ants. In *Drosophila*, the gene desaturase 1 plays a pivotal role for the production and variation of cuticular alkenes. Drawing upon genomic and transcriptomic data, we identified a cluster of homologous desaturase genes in *P. barbatus*. To explore the importance of cuticular alkenes for communication in this species, we used RNAi technology to decrease desaturase gene transcription levels and thus experimentally change worker CHC patterns.

23-5 Worker castes of leaf-cutting ants show differences in aggressive behavior

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Nestmate recognition is crucial for the stability of social insect colonies because it allows individual workers to distinguish between nestmates and non-nestmates. This ability to discriminate ensures that costly altruistic behaviors are directed towards colony members (which are usually related individuals), while at the same time intruders will be attacked and expelled from the colony. The objective of this study was to investigate the possible variation in the aggressive behaviour of workers of the leaf-cutting ant *Acromyrmex echinatior*. Colonies of this species contain morphologically different workers, which can be grouped into three distinct castes: large, medium and small workers. We conducted a series of aggression tests in which workers from each of the three different castes were presented to either nestmates or non-nestmates. The results show a clear difference in the level of aggression among the worker castes. Large and medium workers were highly aggressive towards non-nestmates; aggression level was particularly high for large workers. However, small workers were generally less aggressive and showed lower discrimination between nestmates and non-nestmates. These experiments demonstrate that there is a difference in the perception or action component of the recognition system in different castes. This may be due to the task specialization between the different worker castes: the small workers perform mainly intra-nidal tasks and therefore do not need to be as effective in nestmate discrimination as large and medium workers that are responsible also for nest protection. Our study creates the basis for further investigations of behavioural syndromes within insect societies.

23-6 My house, my rules: Context-dependent acceptance of non-nestmate conspecifics in a primitively eusocial paper wasp

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Animals that live in social groups often employ recognition cues to differentiate nestmates from non-nestmates that can pose a threat to their social system. Therefore, a central problem is how to optimize the balance between acceptance errors (accepting non-nestmates) and rejection errors (rejecting colony nestmates). The Optimal Acceptance threshold model predicts that discrimination is context-dependent and discrimination errors will depend on the fitness cost of rejecting nestmates and accepting non-nestmates. Here I show that in colonies of the paper wasp *Mischocyttarus mexicanus*, acceptance of non-nestmates in a foreign colony was context dependent. Colony nestmates were less aggressive and non-nestmates were more frequently accepted in early colonies. In contrast, the acceptance threshold shifts towards nestmates becoming more restrictive in the acceptance of non-nestmates as the colony transitioned from newly founded to the imminent emergence of offspring as adult workers. A higher percentage of young non-nestmates were accepted in comparison to older non-nestmates, particularly in early colonies. Conversely, there was a higher frequency of young non-nestmate rejection in late colonies with a high investment in offspring suggesting a higher fitness cost of accepting non-nestmates that may pose a usurpation threat to the colony. These shifts in the acceptance threshold posit that discrimination of non-nestmates is related to their cost of being accepted depending on the investment in offspring, providing evidence for the effect of the specific social context on non-nestmate acceptance.

23-7 NESTMATE RECOGNITION IN THE ANT *DINOPONERA QUADRICEPS*: TASK-RELATED EFFICIENCY AND CHEMICAL CUES

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Recognition of mates and foes is one of the more remarkable mechanisms of social groups. In social insects, this extends to the nest mates, parasites, predators and others. Chemical substances communicate colony affiliation for both adults and brood; thus, in theory, all colony members should be able to accept fellow nest mates and reject aliens. In a series of experiments we investigated the ability of *Dinoponera quadriceps* workers to discriminate nest mates and non-nest mates (brood and adults). The results showed that brood recognition depends on the functional role of evaluator (cognitive ability), while the adult recognition is cue-bearer dependent. We verified that cuticular hydrocarbons present on brood and adults provide the cues of discrimination.

23-8 CUTICULAR HYDROCARBONS ON HARVESTER ANT (*POGONOMYRMEX BARBATUS*) MIDDENS GUIDE FORAGERS TO THE NEST

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Colony-specific cuticular hydrocarbons (CHC) are used by social insects in nestmate recognition. Here we show that CHCs are used to facilitate the return of foragers to the nest. Harvester ants use colony-specific CHCs to mark the pebbles placed on the nest mound. CHCs are found in a concentration gradient, growing stronger near the nest entrance in the center of a 1-2 m diameter nest mound. Foraging behavior was disrupted when the gradient of CHCs was altered experimentally. When midden material was replaced with artificial pebbles lacking the colony-specific CHCs, the rate of returning foragers decreased significantly, and returning foragers spent more time locating the nest entrance. Current research examines how the CHC gradient on the nest mound is produced and maintained.

Oral Presentations

New insights into social evolution: Molecular and genomics approaches to comparative neuroethology

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CHEMOSENSORY SOCIOGENETICS: HOW INSECTS SENSE THEIR SOCIAL ENVIRONMENT

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All animals use a sophisticated array of sensory apparati to monitor their biotic and abiotic environments. Recent advances in the genetic, and genomic analyses of sensory physiology yielded a significant progress in our understanding of the molecular and cellular basis of diverse sensory transduction pathways. In spite of these advances, our understanding of how the social environment is sensed and coded by the nervous system is still in its infancy. Here I will focus on recent progress in understanding the role of cells and genes in the gustatory and olfactory systems in mediating social interaction in insect models, and their possible implications for general understanding of behaviors in social insects.

HONEYBEE ODOR CODING AND LEARNING COSTS – NEURAL NETWORKS AND CIRCADIAN INTELLIGENCE

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Bees collect nectar and pollen, and learn to associate color and odor with these rewards. This appears as an adaptive behavior that ensures efficient foraging by the hive. But how are these memories stored in the nervous system? We investigated the effect of associative learning on early sensory processing, by combining classical conditioning with in vivo calcium imaging of secondary olfactory neurons, the projection neurons in the honeybee antennal lobe. We found associative changes of odor representations 2 to 7 hours after appetitive odor conditioning. These changes affected both the global projection neuron response strength and the spatial pattern of activated neurons. Our data suggest that odor learning affects the intra-glomerular network at the level of olfactory receptor neuron-to-projection neuron synapses and inhibitory local neuron-to-receptor neuron synapses. The observed changes are consistent with the idea that odor learning optimizes odor representations and facilitates the detection and discrimination of learned odors. Several studies have shown that learning is metabolically expensive. We therefore asked whether the learning capacity of bees is constant, or modulated with respect to pollen and nectar occurrence. A meta-analysis of flowering angiosperms indicated that pollen and nectar are more widely available in the morning hours than later in the day. Using a proboscis extension paradigm, we tested the discriminative learning ability of bees at different times during the day. We found that bees learn best in the morning. This performance peak is likely to be genetically entrained, given that it was independent of even prolonged exposure to timed food sources at different times of the day. Furthermore, we observe that consistently there is a percentage of bees that are "bad learners".These observations indicate that evolution carefully titers investment into learning and memory, and that olfactory memory directly affects olfactory processing.

MICROARRAY ANALYSIS PROVIDES UNPRECEDENTED INSIGHT INTO THE PHYSIOLOGY OF REPRODUCTIVE AND NON-REPRODUCTIVE HONEYBEES

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The success of a honeybee colony depends largely on the reproductive altruism of the worker bees, which usually remain sterile, and instead help their mother queen to reproduce. As yet, little is known about the genomic basis of this spectacular form of altruism. In this study, we compared gene expression patterns among 16 laying and 16 non-laying 18-day old honeybee workers using a third generation microarray and using whole-body RNA extracts. Our results demonstrate that there were massive differences in the gene expression between these two sets of workers, with a total of 1284 genes being differentially expressed (BH corrected p-value<0.05), of which 871 being up-regulated in fertile workers and 413 being up-regulated in sterile workers. GO enrichment analysis demonstrated that ca. one quarter of all the GO-terms enriched in egg-laying workers were linked to oogenesis, mitosis or meiosis, whereas the GO-terms enriched in non-laying workers were associated with wing muscle contraction, metabolism and flight behavior. Interestingly, these results therefore suggest that non-laying workers were foraging, whereas laying workers of the same age were not, in accord with theoretical predictions that reproductive workers should tend to carry out less work and less risky tasks inside the colony. In addition, we also discovered several genes of interest, such as *Apis mellifera* odorant receptor 156 which was nearly 2-fold up-regulated in sterile workers and farnesyl pyrophosphate synthase (involved in juvenile hormone synthesis), odorant binding proteins 9 and 7 and chemosensory protein 5, which were up-regulated in reproductive workers. Several of these genes of interest are situated within the region of previously identified QTLs linked to differences in worker reproductive capacity in honeybees. Overall, our results provide unprecedented insight into the detailed physiology of non-reproductive honeybee workers.

INVOLVEMENT OF DNA METHYLATION IN MEMORY PROCESSING IN THE HONEY BEE (*APIS MELLIFERA*)

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Epigenetic mechanisms such as histone acetylation and DNA methylation have recently been implicated in learning and long-term memory in vertebrates. Epigenetic system components are also found in many invertebrate genomes, for example the honeybee has complete and functional DNA methylation enzymology similar to that of vertebrates. We examined whether DNA methylation has a role in learning and memory in the honeybee, as it does in vertebrates. We focussed on the role of DNA methylation in extinction memory, which to date has not been assessed in any system despite known dissociations in the molecular mechanisms underpinning acquisition and extinction memories. Treatment with the DNA methyltransferase (DNMT) inhibitor zebularine significantly reduced extinction retention or altered within-session extinction in either direction, depending on when DNMT function was inhibited. Extinction was also found to be more DNMT-dependent than acquisition. Our findings add to the understanding of epigenetic processes in learning and memory, extending known roles of DNA methylation to olfactory associative memories and extinction memory, as well as implicating for the first time a function for DNA methylation in memory in invertebrates.

APPOINTMENT KEEPING AND THE CIRCADIAN ANTICIPATORY STATE: MICROARRAY ANALYSIS OF TIME-TRAINED FORAGER HONEY BEES

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Honey bees feed on flowers, many of which produce nectar and pollen during certain restricted, but consistent, time windows in the day. Previous research has shown that bees have the ability to form distinct spatiotemporal memories that allow them to return repeatedly to different food sources at specific times of day. Because foragers tend to specialize on one or just a few of the several food sources that their colony might be exploiting at any given time, different foragers in a colony often will be active at different times of day. This study was designed to explore whether different spatiotemporal foraging patterns are associated with distinct brain gene expression profiles. Different groups of foragers from the same colony were trained to collect sucrose solution from an artificial feeder at one of two different times of day, one in the morning and the other in the late afternoon; morning and afternoon feeders were located in different places. Both groups of foragers were collected twice, 30 minutes prior to each of the two training periods. Each collection thus yielded one group of bees that was showing stereotypical food anticipatory behavior and one group of bees that was inactive. Microarray analysis revealed that hundreds of genes were differentially expressed in the brain based on either time of day or activity state. A subset of these genes showed unique patterns of expression in the two groups of foragers. As expected, genes involved in regulating circadian rhythms are differentially expressed in association with different spatiotemporal memories, but surprisingly, some of the clock genes, such as *period* and *cyrptochrome*, showed changes in expression due to activity state rather than time of day. Overall, these results indicate that distinct spatiotemporal foraging memories in honey bees are associated with distinct neurogenomic states that involve genes that regulate temporal activity and genes that are prime the brain for feeding behavior.

MOLECULAR DYNAMICS AND SOCIAL REGULATION OF CONTEXT-DEPENDENT PLASTICITY IN THE CIRCADIAN CLOCKWORK OF THE HONEY BEE

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Honey bees switch between activities with and without circadian rhythms according to their social task. Forager bees have strong circadian rhythms, whereas nurse bees typically care for the brood around the clock with no circadian rhythms in behavior or clock gene expression. In the current study we further found that young nurses tended brood with no circadian rhythms in behavior or clock gene expression, even under a light-dark illumination regime or when placed with brood, but no queen, in a small cage outside the hive. By contrast, we found that nurse-age bees that were restricted to a broodless comb inside or outside the hive showed robust behavioral and molecular circadian rhythms. These findings suggest that direct interaction with the brood modulates the circadian system of honey bees. Nurse bees that were removed from the hive into in individual cages in the laboratory, and were orally treated with a brood extract showed attenuated circadian rhythms in locomotor activity. This finding is consistent with the premise that the brood influence on worker activity is mediated by contact pheromones. Circadian rhythmicity is context-dependent because nurses showed circadian rhythms in locomotor activity shortly after removal from the hive, and in clock gene expression after ~16 hrs. The dynamics of rhythm development support a model positing that at least some pacemakers continue to oscillate and be entrained by the environment in nurses that are active around the clock. These cells set the phase to the clock network when the nurse is removed from the hive. These findings suggest that despite its robustness, the circadian system exhibits profound plasticity enabling adjustment to rapid changes in the social environment.

Poster Presentations

New insights into social evolution: Molecular and genomics approaches to comparative neuroethology

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24-1 Dopamine biosynthesis and oogenesis in the honeybee, *Apis mellifera*: a link between reproductive biology and behaviour?

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Dopamine is important for many aspects of honeybee biology, it is involved in cognition, locomotion and also regulates aspects of development. Dopamine also influences reproduction via the levels of juvenile hormone and 20-hydroxyecdysone, the key gonadotropic hormones in adult insects. Intriguingly a recent study in the crustacean *Macrobrachium rosenbergii* has shown that dopamine is produced locally in the ovary, and this has led to the suggestion that dopamine may also be directly involved in reproduction; particularly in ovarian development and differentiation of oocytes. However, this has not been systematically studied in insects, and the function of dopamine in the ovary is unknown. Understanding how oogenesis is controlled in honeybee queens and repressed in workers is key to understanding how sociality evolved in this species. Dopamine biosynthesis may constitute part of this regulation as dopamine links the brain and reproductive axis therefore localised dopamine production in the ovary has the potential to influence both biology and behaviour. Here we examine the role of dopamine and dopamine biosynthesis in the honeybee ovary. Using quantitative RT-PCR we have examined the mRNA levels of the two major dopamine biosynthetic enzymes (tyrosine hydroxylase and dopa-decarboxylase). We find that mRNA levels for these biosynthetic enzymes correlate with active oogenesis. We have also used in situ hybridisation to examine the distribution of mRNA in the ovary, and immunohistochemistry to detect dopamine in the ovary. The results from these analyses imply that the dopamine may have a role in reproduction in the honeybee but does not seem to specifically influence oocyte differentiation. While systemic dopamine levels are known to influence reproduction in the honeybee, this is the first suggestion that localised dopamine production in the ovary may also affect reproduction.

24-2 Brain scaling in ants: from total brain size to individual neuropil

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Ants have great size diversity and are one of the most dominant insect groups in the world. Within the leaf-cutting ants of the neotropics, the genus *Atta* exhibits a large degree of physical polymorphism, with workers ranging in size from only a few millimeters in length to over two centimeters. We used this size diversity in *Atta colombica* to study the brain to body size ratio within a single species, thus eliminating phylogenetic considerations when making comparisons across species. We also examined how different neuropil in the brain scale to overall brain and body size. Although the overall brain size increases with body size the ratio is not linear. We discuss how physical constraints on brain size may influence brain structure and behavior, how overall body size may influence this ratio and how it influences division of labor. We compare our data to theoretical predictions of how brain size scales to body size.

Oral Presentations

My brain made me do it: Neurological basis of behavioral repertoire changes in social insects

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MY MOTHER MADE ME DO IT: HONEY BEE QUEENS REGULATE THE BEHAVIOUR OF WORKERS TO SECURE THEIR OWN SURVIVAL

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Exposing young worker bees to queen mandibular pheromone (QMP) has profound effects on dopamine signalling in the brain. QMP lowers brain dopamine levels, selectively alters the levels of dopamine receptor gene expression and modifies the responses of brain tissues to this amine (Beggs *et al.* 2007 *PNAS* 104, 2460-2464). At a behavioral level these changes appear to have three major consequences: aversive learning in young worker bees is blocked (Vergoz *et al.* 2007 *Science* 314, 384-386), their activity levels are suppressed (Beggs *et al.* 2007) and they are more likely to show attraction to the queen (Vergoz *et al.* 2009 *PNAS* 106: 20930-35). One of the major components of QMP, homovanillyl alcohol (HVA) bears a striking structural resemblance to the biogenic amine dopamine and has recently been found to activate the honey bee D2-like dopamine receptor, *AmDOP3* (Beggs & Mercer 2009 *Current Biology* 19, 1206-1209). Analysis of HVA's effects is providing valuable insights into dopamine's functions in the brain while highlighting the complexity of neural circuits that guide the behaviour of this highly social insect.

MECHANISMS OF AGE-DEPENDENT DIVISION OF LABOR IN THE SOCIAL APHID *TUBERAPHIS STYRACI*

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Social aphids parthenogenetically produce altruistic nymphal individuals termed soldiers that perform colony defense, housekeeping, and gall repair for their colony mates. In highly social aphids, soldiers are morphologically differentiated from reproductive nymphs of the same instar and unable to grow, constituting a sterile caste. *Tuberaphis styraci* is a highly social aphid with a sterile soldier caste in the second instar. When the soldiers are young, they perform relatively safe tasks inside the nest such as gall cleaning by removing waste products. As the soldiers get older, their tasks change more dangerous activities outside of the nest such as colony defense. To revealing the mechanism of polyethism, understanding of age-dependent division of labor in the social aphid is useful. However, its mechanism is not understood.To understand the mechanisms of age-dependent division of labor in the aphid social system, we focused on cGMP-dependent protein kinase (PKG) pathway because recent studies indicate that PKG pathway is involved in the division of labor in honeybee and ants. We conducted a feeding experiment to assess the role of PKG pathway in the transition from non-aggressive to aggressive in soldier behavioral development. We administered various concentrations of the cGMP-analog for young soldiers (5~10 day old), and after three hours tested their aggressive response with a fine brush. Young soldiers which fed higher concentrations of the cGMP-analog were significantly more aggressive than those which fed lower concentrations of the cGMP-analog or distillated water only. This study suggests that PKG pathway is involved in the expression of defensive behavior in age-dependent division of labor in social aphids.

OPTICAL IMAGING OF ODOUR-EVOKED ACTIVITY IN THE LATERAL HORN OF THE HONEYBEE *APIS MELLIFERA*

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Through different processing steps, olfactory systems create evolving internal representations that differently represent odors' chemical characteristics and/or biological value. In insects, odors are detected by sensory neurons on the antennae, which project to a primary processing centre, the antennal lobe. Then projection neurons convey odor information to higher brain centers, the mushroom bodies and the lateral horn. Previous work has intensely detailed the representation of odor stimuli in the antennal lobe and in the mushroom bodies, but that in the lateral horn has remained mostly unaddressed, although this structure may be a pre-motor center for stereotyped and/or learned olfactory behavior. We studied odor representation in the lateral horn of the honeybee, a social insect using both floral odors for foraging and pheromones for communication within the hive. We used calcium imaging based on two staining methods emphasizing respectively general activity or the responses of projection neurons. All tested odors, including floral volatiles and social pheromones, induced clear calcium responses in the lateral horn. Specific recordings of projection neurons showed a transformation of odor representation, as the lateral horn arbors an odor-specific map, with the same intensity, but different odor-similarity relationships as in the antennal lobe. Lastly, two alarm pheromones produced by different glands and with clearly different chemical structures give rise to the most highly overlapping representations in the lateral horn. The lateral horn thus contains an odor-specific map that may be biased for representing odors with a pheromonal function.

INTRA- AND INTERSPECIFIC NEUROANATOMICAL VARIATION AMONG BEHAVIORALLY DIFFERENTIATED WORKERS IN THE ANT GENUS *PHEIDOLE*

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The relative contribution of individual behavior and its neural basis to decentralized processes of group action in ants is poorly understood. What is the role of underlying neuroanatomical variation among workers? Neuroethological studies of social hymenopterans have shown that the mushroom bodies, paired neuropils implicated in learning, memory, and behavioral flexibility, increase in volume as workers develop new behavioral competencies with age, suggesting variation in neuroanatomy may be important in generating division of labor. Our analysis of a large neuroanatomical dataset from the hyperdiverse ant genus *Pheidole* indicates that the behaviorally differentiated worker subcastes and age cohorts of three *Pheidole* species are identifiable by distinct, size-independent neural phenotypes. The relative sizes of functional brain subregions and their ontogenetic allometries vary intra- and interspecifically, indicating *Pheidole* worker neuroanatomy is developmentally and evolutionarily labile. Relatively large mushroom bodies are found in worker groups with broader behavioral repertoires, providing novel evidence that this neuropil is functionally linked to worker behavioral diversity. Our results are consistent with the hypothesis that repertoire differences among worker groups are in part a result of developmental processes leading to distinct species-, subcaste-, and age-specific worker neural phenotypes, which bias worker task performance patterns.

BIOGENIC AMINES AND AGGRESSIVE BEHAVIOUR OF THE RED WOOD ANT (*FORMICA POLYCTENA*)

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The role of the biogenic amines serotonin (5-HT), dopamine (DA) and octopamine (OA) in the mediation of various forms of aggressive and predatory behaviour has already been documented in ants from various phyla tested in various contexts. We revisited the question of the involvement of biogenic amines in the mediation of ant aggressive behaviour by investigating the effects of abdominal injections of dopamine (DA), serotonin (5-HT), octopamine (OA) and tyramine (TA) on the behaviour of foragers of the red wood ant (*Formica polyctena*) tested in 4 types of dyadic aggression tests consisting of a 10 min encounter with a nestmate, an alien conspecific, a worker of another ant species (*Formica fusca*), and a small larva of the house cricket (*Acheta domesticus*). We also investigated the effect of chronic oral administration of OA on aggressive behaviour of foragers of *F. polyctena* from queenless and queenright colony fragments during dyadic encounters with nestmate or non-nestmate conspecifics and with larvae of *A. domesticus*. DA treatment had a stimulatory effect on some forms of aggressive behaviour shown by *F. polyctena* during encounters with allospecifics. The occurrence of worker death caused by exposure to formic acid was highest in the ants treated with 5-HT which suggests strongly that the most aggressive ants were more frequent in that group. Surprisingly, OA had no effect on any form of aggressive behaviour of the tested ants, irrespectively of the mode of its administration. Significant behavioural effects of biogenic amine treatments were discovered only if the behaviour of the tested ants was quantified and analysed in a very detailed way. They were not observed if the outcome of each aggression test was quantified as a single score. This provides a striking illustration that the conclusions drawn from the same set of experimental data may depend in a crucial way on the exact method of data quantification.

FORAGING TASK SPECIALIZATION AND SENSORY ALLOMETRY IN WILD, HYBRIDIZED AND ARTIFICIALLY SELECTED HONEYBEES

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One of the best-supported models explaining self-organized task specialization in social insects is the response threshold model, which predicts specialization due to inter-individual variability in sensitivity to task associated stimuli. However, the proximate factors underlying the differences in individual sensitivity remain elusive. In the honeybee *Apis mellifera*, the tendency to collect nectar, pollen or water correlates with differences in sensory sensitivity. What factors underlie sensory sensitivity in honeybees and how do they lead to inter-individual variation? Answering this question is important for understanding causal relationships and for testing the generality of the model across species.In solitary species, sensory sensitivity correlates with size variation of the sensory organs. Assuming a similar association in honeybees, we examined whether sensory allometry correlated with foraging task specialization in Africanized and European honeybees. We focused on the number and size of olfactory sensilla, the length of the antenna and the head size. We also examined the association between sensory allometry and quality and quantity of resources exploited: nectar foragers, known to exhibit low sensory sensitivity, have fewer and smaller sensilla than pollen and water foragers. Also, sensory allometry positively correlated with pollen load and negatively with nectar concentration. We further examined sensory allometry in honeybee strains artificially selected for their tendency to store more (high strain) or less (low strain) surplus pollen. As with other stimuli, high and low strains differ in olfactory sensitivity. We found that ‘high-strain’ workers have fewer but larger olfactory sensilla, longer antenna and bigger heads than ‘low-strain’ bees. Our results suggest an association between sensory sensitivity and sensory allometry in honeybees, which appear to be affected by artificial selection of the tendency to store surplus pollen.

Poster Presentations

My brain made me do it: Neurological basis of behavioral repertoire changes in social insects

25

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25-1 NEURAL CODING IN THE DUAL OLFACTORY PATHWAY OF THE HONEYBEE *APIS MELLIFERA*

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Sensory systems use parallel processing to extract and process different features of environmental stimuli. Parallel processing has been studied in the auditory, visual and somatosensory systems, but research in the olfactory modality has shown little progress. An invertebrate model like the honeybee is well-suited for such research, as it provides relative neuronal simplicity with good experimental access to the brain and robust behavioural paradigms. Strikingly, the honey bee brain contains a dual olfactory system, with a clear dichotomy from the periphery to higher-order centres subtended by two main neuronal tracts (median and lateral Antenno-Cerebral Tract, m and l-ACT). The function of this dual system is utterly unknown, and attributes like odour quality and odour quantity may be separately encoded in these subsystems. We have thus started a thorough functional study of olfactory coding in both subsystems, using *in vivo* calcium imaging to reveal neuronal activity. As one of the subsystems (m-ACT) has never been imaged before, a novel imaging preparation was developed and responses to a panel of aliphatic odorants at different concentrations were compared in both subsystems. Our data show a global redondance of olfactory coding in both subsystems, but unravel some specificities of each system for encoding the chemical group and carbon chain length of odour molecules. Our current experiments aim to understand neural processing at higher levels of both pathways.

25-2 SUCROSE ACCEPTANCE THRESHOLD AND FEEDING BEHAVIOUR IN NECTIVOROUS ANTS: STARVATION AND SEROTONIN EFFECTS.

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Feeding is a complex behaviour and its regulation involves the integration of a wide range of external and internal factors. Such is also the case for the ant *Camponotus mus*, which modifies its feeding behaviour depending on the nectar quality but also on its internal state, determined by the colony's sugar requirements. Physiologically, the biogenic amine serotonin (5-HT) is involved in numerous processes related with feeding behaviour. We examined the effects of serotonin on feeding related behaviour and its relationship with starvation in *C. mus* ants. Feeding behaviour and electrical activity of the sucking pump were recorded in ants that received different concentrations of orally administered serotonin. This was compared with the effects produced by starvation. In addition, we developed a novel protocol to analyse the sucrose acceptance threshold in ants. Our results have shown that 5-HT promoted a decrease in sucrose feeding in a dose-dependent manner. Intake rate of 5-HT-treated ants was lower than that of control ants, similar to the decrease observed for non starved ants. Our recordings lead us to discern that intake rate reduction by serotonin was mainly due to a decrease in the volume of solution ingested per pump contraction while intake rate reduction by satiation was due to a decrease in pumping frequency. Sucrose acceptance threshold changed with the level of carbohydrate starvation: the higher the starvation level, the lower the solution concentration that elicited a response. On the other hand, oral-administered 5-HT did not modify sucrose acceptance threshold under our experimental conditions. Our results show that both 5-HT and satiation promote a depressant effect on sucking pump activity, however, they act by different mechanisms. These findings are discussed in the frame of feeding control and the role of biogenic amines in insect behaviour.

25-3 Evolution of elaborate mushroom bodies in the Hymenoptera

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The mushroom bodies are learning and memory and sensory integration centers in the insect brain. In the aculeate Hymenoptera, these higher brain centers are extremely large relative to the rest of the brain and possess elaborately convoluted sensory input regions called calyces. In most aculeates the calyces also contain a novel subcompartment called the collar that receives visual input from the optic lobes. Since this unique mushroom body morphology (termed "elaborate" mushroom bodies) appears to be characteristic of the aculeate Hymenoptera, it has been proposed to be associated with the cognitive demands of sociality as exhibited by several lineages in this group. However, reconstructions of the evolutionary history of elaborate mushroom bodies indicate that they first arose at the base of the Euhymenoptera (Orussidae + Apocrita), 50 MY prior to the evolution of sociality in the aculeates. The base of the Euhymenoptera marks the first appearance of species having a parasitoid lifestyle, suggesting that the initial acquisition of elaborate mushroom bodies, particularly the novel inputs from the optic lobes, may have been driven by the cognitive demands of spatial and other types of visual learning associated with host location. As is the case for many evolutionary novelties, the transition from small, simpler mushroom bodies in the most basal hymenopteran lineages to large and elaborate mushroom bodies is not abrupt, and species close to the base of the Euhymenoptera possess mushroom bodies with different combinations of characters. Comparisons of mushroom body morphology with the behavioral and sensory ecologies of species throughout the Hymenoptera will further clarify the forces driving the evolution of elaborate mushroom bodies, and provide additional insight into the functions of these higher brain centers.

25-4 Neuronal modifications of octopaminergic and tyraminergic systems in termite soldiers

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Termites are the oldest eusocial insects, with a division of labor that has evolved independently from social hymenopterans. Soldiers, the defensive caste specific to the lineage, are essential for colonies, because termite colonies are always threatened by various predators. Soldiers are differentiated from workers (or pseudergates or larva) in the way they behave against predatory invasion. Workers tend to escape rapidly into their nest, whereas soldiers typically aggressively attack enemies. This behavioral transition appears during the soldier differentiation, and thus can be regarded as age polyethism. However, the neurological underpinnings of behavioral differentiation of soldier are completely unknown. Octopamine (OA) is known as a neuroactive substance that adjusts aggression levels in various insects. We hypothesized that high aggressiveness of termite soldiers involves the OA action. Therefore, we first compared the soma sizes and distributions of OA-immunoreactive neurons in the brain and suboesophageal ganglion (SOG) between soldiers and pseudergates in the damp-wood termite *Hodotermopsis sjostedti*. The results indicate that some somata of dorsal unpaired median (DUM) neurons in the SOG were significantly larger in soldiers than in pseudergates. Intracellular staining of these neurons revealed that some of them projected into mandibular nerves and tritocerebrum. Considering that mandibular muscles control biting and that the tritocerebrum integrates some information including mechanoreception, these projection areas are thought to relate to defensive behavior. Next, we quantified biogenic amines in the brain and SOG. Although the OA levels in both brain and SOG showed no significant differences, the levels of tyramine (TA), the precursor of OA, was significantly higher in soldiers than in pseudergates. These results suggest that TA or OA in enlarged DUM neurons may be involved in the high aggressiveness in soldiers.

25-5 DIFFERENTIAL BRAIN MORPHOGENESIS AND TRANSCRIPTION PROFILE OF GENES RELATED TO NEUROGENESIS IN CASTES OF *A. MELLIFERA*

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The differential feeding regime experienced by the queen and the worker larvae of the honeybee *Apis mellifera* shapes a complex endocrine response cascade that ultimately sets up differences in brain morphologies. Herein we report on aspects of brain morphogenesis during larval development of both castes and the expression profiles of eight neurogenesis-associated genes: tetraspanin 5D (*tsp5d*), dachshund (*dac*), failed axon connection (*fac*), cryptocephal (*crc*), ataxin-2 (*atx-2*), ephrin receptor (*EphR*), short stop (*shot*) and kruppel homolog-1 (*kr-h1*). Our morphological results revealed a delay in worker brain development when compared to queens. The quantitative transcript analyses in brains of third to fifth instar larvae revealed three expression patterns: one represented by *tsp5D*, with a continuous decrease in expression; a second, represented by *dac*, *fax*, *crc* and *kr-h1*, all of which showed increasing transcript, and the third, represented by *atx-2*, *EphR* and *shot*, is a group of genes with an expression peak in fourth instar larvae. *Shot* whose expression is required for axon extension and cell proliferation in *Drosophila*, was found to be more expressed in fourth instar queen larvae compared to worker larvae. *kr-h1*, whose protein product has been reported to be involved in neuronal remodeling and morphology, was more expressed in fifth-instar queen larvae. These results contribute to explaining the observed faster brain development in queens and the existence of a larger area of neuroblasts in brains of fifth instar queen larvae when compared to those of workers, suggesting *shot* and *kr-h1* as players in the gene expression cascade induced by differential feeding in the honeybee *Apis mellifera*. Financial support: CNPq 473748/2008-8; FAPESP 2005/03926-5; 2009/00810-7.

25-6 AGE-RELATED CHANGES IN HONEY BEE RESPONSES TO *APIGUARD*, A THYMOL-BASED TREATMENT USED TO CONTROL *VARROA* MITE

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The parasitic mite *Varroa destructor* is responsible for heavy losses in honey bee colonies and represents a major threat for the beekeeping industry. Several treatments have been developed to control this parasite. The most common solutions involve the use of synthetic chemicals. However, mite populations are developing resistance to the most commonly used miticides. Essential oils such as thymol offer an attractive alternative. However, thymol-based treatments such as the gel formulation *Apiguard* are reported to have adverse effects on honey bee colonies. We investigated the effects of *Apiguard* on honey bee behaviour using laboratory-based behavioural assays. Initially, bees were placed in a behavioural arena with *Apiguard* at the centre. The bees' location relative to the thymol source and the number of antennal contacts with the *Apiguard* were recorded. The bees' responses to *Apiguard* appeared to be age dependant, with forager bees displaying strong avoidance of the *Apiguard* gel. Further observations indicated that avoidance behaviour was mediated by the antennae, and seemed to be triggered by taste rather than smell. Subsequent assays were carried out using a Y-maze designed to test the influence of *Apiguard* treatment in the hive on bees' responses to thymol. Exposing bees to *Apiguard* in the hive altered their subsequent reactions towards thymol. Only forager bees from non-treated hives avoided *Apiguard* when tested in the Y-maze, suggesting that bees may become habituated to the treatment. These observations suggest that *Apiguard* exposure can have both behavioural and physiological impacts on the honey bee. A better understanding of the age-related changes in responses to *Apiguard* is required to ensure effective spread of the gel throughout the hive, and to maximize the effectiveness of *Apiguard* as an anti-*Varroa* treatment.

25-7 PHEROMONAL REGULATION OF BEHAVIOURAL PLASTICITY IN THE HONEYBEE

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In honeybee colonies the female worker caste expresses a pronounced polyethism. Summer bees perform a rich behavioural repertoire ranging from various indoor duties (e.g. feeding, building) to foraging nectar, pollen and water outside the hive. Switching between these behaviours depends on age and goes along with plastic changes in the synaptic architecture of the mushroom-body calyces. However, division of labour in a honeybee colony is not purely age-dependant. In fact, the transition from nurse bees to foragers may additionally be modulated by chemical communication signals enabling the colony to respond in a flexible manner to environmental changes by shifting the work force between indoor and outdoor duties. One of these chemical cues is ethyloleate (EO) which has been described to delay the onset age of foraging (Leoncini *et al.* 2004 *PNAS*), EO is found at high concentrations only in foraging bees, thus making it a colony wide accessible signal for work force distribution. Therefore, the concentration of EO can be regarded as a primer pheromone that accelerates or delays the onset of foraging in a concentration dependant manner. As it was unclear where and how EO is received we investigate in this study whether EO is received as an olfactory cue. A first indication for this hypothesis was delivered by electroantennography (EAG) recordings of whole antennae using EO as a stimulus. Furthermore, we currently apply Ca2+-Imaging using selective staining of projection neurons with calcium sensitive dyes to investigate neuronal representation and processing of EO in antennal lobe (AL) glomeruli. Preliminary results indicate that EO is processed in glomeruli of the T1 cluster, and potentially the T3 cluster. These results provide evidence that EO is at least partly received as an olfactory cue suggesting that it may mediate plastic changes in brain during the transition of nurse bees to foragers via the olfactory pathway. Supported by Human Frontier Science Program

25-8 ACUTE DISRUPTION OF THE NMDA RECEPTOR SUBUNIT NR1 IN THE HONEYBEE BRAIN SELECTIVELY IMPAIRS MEMORY FORMATION

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Memory formation is a continuous process composed of multiple phases that can develop independently from each other. These phases depend on signaling pathways initiated upon the activation of receptors in different brain regions. The NMDA receptor acts as a sensor of coincident activity between neural inputs, and as such its activation during learning is thought to be crucial for various forms of memory. In this study we inhibited the expression of the NR1 subunit of the NMDA receptor in the honeybee brain using RNAi. We show that the disruption of the subunit expression in the mushroom body region of the honeybee brain during and shortly after appetitive learning selectively impaired memory. Although the formation of mid-term memory and early-long-term memory was impaired, late-long-term memory was left intact. This indicates that late-long-term memory formation differs in its dependence on NMDA receptors activity from earlier memory phases.

25-9 *FOS*-LIKE IMMUNOREACTIVITY EXPRESSION IN DIFFERENT SUBTYPES OF KENYON CELLS

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The *fos*-like immunoreactivity in *Apis mellifera* brain was first studied by Fonta *et al*. (1995), but in this research they used a different system for the bee development, with nynphal stages, while honeybees have larvae, pupae and adult development stages. In the bee brain, mushroom bodies are structures responsible for the process of information received from other brain regions. They are formed by intrinsic neurons, the kenyon cells, that are divided in three populations: inner and outer compacts and non compacts cells. These cells show a differential expression for some genes, like immediate early genes. This way, the aim of this work was to evaluate the *fos*-like expression in the kenyon cells of *Apis melliferea* workers. With this in mind, *Apis mellifera* workers newly-emerged and foragers were collected and sacrificed for removal of the brain. The brains were fixed and routinely processed for immunohistochemical techniques with fluorescence. The *fos*-like immunoreactivity was present in the inner and outer compact cells, but could not be seen in the non compact cells. However, in forager bees, the immunoreactitivty was stronger than newly-emerged. This results can be explained because foragers are under a different kinds of environmental stimulus, which can start the differential gene expression. Inner and outer compact cells can have different function from non compact cells in brain. Another important result is that glial cells show *fos*-like immunoreactivity, which does not happen in vertebrates. It can indicate an important role in bee brain physiology.

25-10 EFFECT OF SUBLETHAL DOSE OF FIPRONIL IN THE NEURAL ACTIVITY OF MUSHROOM BODIES FROM NEWLY-EMERGED WORKER *APIS MELLIFERA*

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Fipronil acts in the nervous system of honeybees by inhibiting γ-aminobutyric acid (GABA) and can affect the gustation, the olfactory learning and the motor activity, which are essential functions for successful foraging and pollinization. The indiscriminate use of pesticides, exposes the pollinators to severe stress, leading to economic losses due the constant decrease of honeybees around agricultural fields of the world. Thus, this study aimed to evaluate the sublethal effect of fipronil under laboratory conditions on the neural activity of newly-emerged workers of *Apis mellifera*, which were obtained from the Department of Biology/UNESP/Rio Claro, keep at 25±2 °C, RH of 60±10 %. Daily, 10 µL of the syrup (sucrose + H_2O) with 0.1 ng of fipronil were given for each honeybee and at the 3rd, 5th, and 8th days after the exposure, few specimens were collected, dissected and the brain fixed and prepared by histochemical methods to examine the neural activity of the mushroom bodies (Wong-Riley 1979; Armengaud *et al.* 2000). Our findings revealed that fipronil affects the mushroom bodies that are responsible in the learning process of honeybees, by changes in the cell metabolism of neural activities, inducing increase in the respiratory activity and enzymatic activity of mitochondria.

25-11 THE EFFECT OF OCTOPAMINE TREATMENT ON RESPONSES OF THE RED WOOD ANTS (FORMICA POLYCTENA) TO INSECT PREY IS CONTEXT DEPEN

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The biogenic amine octopamine (OA) is implicated in the control of many behavioural and physiological processes in social insects. In particular, administration of OA may partly counterbalance diverse behavioural effects of various types of social deprivation. We compare the results of three experiments investigating the effects of OA treatment on responses to potential insect prey in workers of the red wood ant *Formica polyctena*. In the first experiment groups of about 25 foragers were subjected to chronic oral OA treatment (2 weeks) at an increasing dose of 2, 5 and 10 mg/ml of carbohydrate food and then tested by exposing them to a dead adult housefly (Musca domestica) in the foraging areas of their nests during 45 min or up to the moment of prey retrieval to one of the nest chambers. OA treatment significantly enhanced the occurrence of seizing of the prey, its transport, and its retrieval. The effects of OA treatment on responses of ants to prey were, however, less strong and less durable than previously studied effects of manipulations of the social context (increase of the group size). In the remaining two experiments, we recorded the behaviour of foragers of *F. polyctena* during a 10 min encounter with a small live house cricket larva (*Acheta domesticus*) taking place inside a set of two connected test tubes. OA was administered either in a form of an abdominal injection (0.5 µl of 0.08 mol/l OA) carried out 1 h before the test (Experiment 2) or in a form of chronic oral treatment (5 mg/ml of 50% sucrose solution provided during 2 weeks; Experiment 3). In both these cases, irrespectively of the mode of treatment, OA did not influence in a significant way the responses of the ants to cricket larvae. OA injections only exerted a stimulatory effect on the number of episodes of locomotory activity of the tested ants. The effect of octopamine treatment on responses of the red wood ants (*Formica polyctena*) to potential insect prey is thus strongly context dependent.

25-12 DEVELOPMENTAL STAGE- AND TASK-DEPENDENT CHANGE IN THE EXPRESSION OF A NON-CODING RNA, *NB-1*, IN THE WORKER HONEYBEE BRAIN

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The female honeybees differentiate into two castes, workers and queens. In addition, workers shift their tasks with an age-dependent manner: younger workers (nurse bees) are engaged in nursing their brood, while older ones (foragers) forage for nectar and pollen. Although juvenile hormone regulates worker task transition, the molecular and neural mechanisms underlying the worker task transition remain largely unknown. With an aim to analyze the molecular mechanisms, we previously identified a novel non-coding RNA, termed *Nurse bee brain-selective gene-1* (*Nb-1*), and suggested its possible role in the worker task transition based on its gene expression patterns (Tadano *et al.* 2009 *Insect Molecular Biology* 18, 715-726). Here, we analyzed developmental stage-dependent expression of *Nb-1*.The *Nb-1*-expression is higher in nurse bee brain than in forager brain. Northern blot analysis revealed that the *Nb-1*-expression in the brain is already visible in early pupal stage and decreases gradually through the pupal stages. In addition, *in situ* hybridization revealed that the *Nb-1*-expression spreads more diversely in the brain in the pupal stages than in the adult stages. Although the *Nb-1*-expression is restricted to octopamine-positive neurons, which are supposed to be related to worker task transition in adult worker brains (Tadano *et al.* 2009), it was enriched in the proliferating cells (neuroblasts and ganglion mother cells) in the developing pupal brains, especially those in the mushroom bodies, a higher center in the insect brain. In addition, we found that the *Nb-1*-transcripts were always localized in the cytoplasm in the proliferating cells, whereas they were present in both nuclei and cytoplasm in the other cells, suggesting the possible role of cytoplasmic *Nb-1*-transcripts in cell proliferation. Our findings suggest that *Nb-1* might have dual functions in the worker brain, which are related to pupal brain development and worker task transition, respectively.

25-13 THE 5-HT1A RECEPTOR OF THE HONEYBEE AND INVOLVEMENT OF SEROTONIN IN THE REGULATION OF PHOTOTACTIC BEHAVIOR

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The biogenic amine serotonin (5-hydroxytryptamine, 5-HT) plays a key role in modulating various physiological and behavioral processes in both protostomes and deuterostomes. The multifaceted actions of serotonin are mediated by binding to integral membrane receptors, most of which belong to the superfamily of G-protein-coupled receptors (GPCRs).By screening the honeybee (*Apis mellifera*) genome, we found several candidate genes encoding for putative 5-HT receptors. One of these receptors (Am5-HT1A) shares high similarity with members of the 5-HT1 receptor class. The activation of this receptor leads to an inhibition of the production of the second messenger cAMP. Furthermore, the pharmacological profile of Am5-HT1 was determined, enabling us to manipulate signal transduction via Am5-HT1A *in vivo*. A receptor specific antibody was generated in order to investigate the spatial distribution of the receptor protein. Strong anti-Am5-HT1A immunoreactivity was observed in the ocellar nerve, in the three optic ganglia (viz., lamina, medulla, and lobula), and in the alpha- and beta-lobes, the pedunculi, the lip and the basal ring neuropil of the mushroom bodies.Using *in vivo*-pharmacology and behavioral testing, we could demonstrate that Am5-HT1A receptor ligands had a strong impact on phototactic behavior of individual honeybees. Thus, the data presented here mark the first comprehensive study - from gene to behavior - of a 5-HT receptor in the honeybee, paving the way to the eventual elucidation of additional roles of this receptor subtype in the physiology and behavior of this social insect.

25-14 THE ROLE OF DOPAMINE RECEPTORS IN THE REGULATION OF WORKER STERILITY IN THE HONEY BEE *APIS MELLIFERA*

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When a honey bee colony becomes queenless, workers respond to the absence of queen pheromones by activating their ovaries and laying eggs that if reared develop into functional males. The aim of this study is to examine the role of dopamine receptors in the regulation of ovary activation. Even though dopamine modulates diverse physiological pathways involved in learning and memory and locomotor activity, there are now multiple lines of evidence suggesting that dopamine also plays a key role in the regulation of worker sterility in the honey bee worker. There is a strong correlation between ovary activation and high dopamine levels in the brain. However, it is not clear if the action of dopamine on ovarian tissue is direct, indirect, or a combination of both. In order for dopamine to have a direct effect at the ovary activation, dopamine receptors must be present in this tissue. Our preliminary results suggest that, as in vertebrates, honey bee ovaries express specific dopamine receptors and that their expression level depends on caste (worker *vs* queen). This result suggests a direct gonadotropic effect of dopamine in this non-neuronal tissue.

25-15 THE BIOLOGICAL CLOCK OF *MELIPONA QUADRIFASCIATA* (APIDAE; MELIPONINI): AN ANATOMICAL STUDY OF THE FORAGER'S BRAIN

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Foraging of *Melipona quadrifasciata* is a rhythmic process that implies visits to the field during the day and rest inside the hive in the night. Biological rhythms like these are found in other insect groups as well and are generated by the circadian system localized in the central nervous system and in the retrocerebral complex. This system consists of a web of multiple oscillators - neurons and glial cells - which do not assemble together to establish a discrete structure. Circadian oscillators receive inputs from the visual system and diffuse the generated rhythm to other parts of the body through nerves and hormones. The aims of this study were to detect and describe components of the circadian system in the brain and retrocerebral complex of *M. quadrifasciata* foragers, using classical histological and immune techniques.The optic lobes neuropils: *lamina*, *medulla* and *lobula* are distinct and evident. The protocerebrum is the largest portion in the frontal-dorsal region of the head. Two neuropilar groups are present: *corpora pedunculata*, the large, bilateral "mushroom bodies"; and the central complex in the median line. Spherical nucleous and granular soma are some features of cells lying under the calyces of the *corpora pedunculata*. These cells (neurosecretory cells) cluster together in two protocerebral regions: *pars intercerebralis* and *pars lateralis*. The presence of neurosecretion was evinced by the fuchsin-paraldehyde technique, mainly in some cells of *pars intercerebralis* and also at *corpora cardiaca* and *corpora allata*, in the retrocerebral complex. Tridimensional reconstructions helped to locate specific areas in the nervous system. In both the central nervous system and retrocerebral complex, species specific traces can be recognized in morphological features and spatial localization of certain structures. The use of markers for corazonin and pigment dispersing factor allowed the identification of putative components of the circadian system of stingless bees.

Oral Presentations

Trajectories towards sociality across arthropod taxa

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DECONSTRUCTING SOCIAL EVOLUTION: SOCIAL TRAJECTORIES AND THE EVOLUTION OF COLONY FOUNDING STRATEGIES

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The evolution of social life involves changes in a suite of traits, including patterns of dispersal, reproduction and cooperation. Mapping these decisions in a single framework, a "social trajectory," can help clarify social strategies available to individuals, selective pressures acting on social structure, and evolutionary routes to social life. In this talk, I will discuss insights generated by a trajectory approach, illustrated with experiments we have conducted on evolution of queen social behavior in the ant *Messor pergandei*. The temporal relationship between evolved social traits, or "decisions," at different points in the life-cycle connects seemingly independent transitions in critically important ways. Early decisions determine the social context in which later decisions are expressed, and can thus be both adaptive responses and selective agents in their own right. A related effect is that sociality creates novel downstream decision points that did not previously exist, so species moving from a solitary to a social lifestyle bring their solitary behavioral repertoire with them into a social context. Seemingly derived social traits, such as alloparental care or division of labor, can emerge spontaneously in groups when individuals respond with ancestral behaviors to the novel environment. This is critical for key hypotheses to explain the evolution of sociality, as it suggests that kin-selected or direct benefits of these behaviors were present and may have been important at sociality's origin rather than requiring a second evolutionary step. Finally, the social trajectory highlights an often-neglected historical aspect: that the same social structure can be arrived at via multiple evolutionary routes. The costs and benefits of any social transition depend critically on the direction of change: correctly reconstructing this history can be challenging, but is essential if we want to identify general principles underlying social evolution.

EVOLUTION OF SOCIALITY IN SPIDERS

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Spiders are predatory and cannibalistic, and most are solitary. Nevertheless, group living has arisen independently in several spider families in two fundamentally different forms. Colonial spiders have individual territories within a shared living space, forage individually, compete for prey, and do not cooperate in raising young. Cooperative breeding (social) spiders construct communal webs, and feed and raise young communally. Colonial species have some juvenile dispersal: they are assumed to out-breed and to have a 1:1 primary sex ratio. Juvenile dispersal is lost in the social species; they inbreed regularly and have a female-biased colony sex ratio. I discuss the demographic consequences of these two modes of group living and their different breeding systems. In both colonial and social spiders, per-capita prey consumption and reproductive success peak at intermediate group sizes. As the colony grows, physical constraints on capture web size and competition for prey reduce average prey consumption and individual productivity. In colonial spiders, juvenile dispersal results in long-lived colonies with stable population sizes at or near the optimum for foraging and reproduction. In social spiders, the female-biased sex ratio and cooperative breeding result in rapid colony growth. Lacking juvenile dispersal, colony size quickly exceeds carrying capacity. Colony collapse due to competition for food may be averted by fission, whereby the group separates to form sub-nests. Fission reduces competition for food without slowing overall colony growth. Thus, nest fission could maintain stable colony sizes. Nevertheless, social spider colonies exhibit boom and bust dynamics and typically survive only a few generations. A possible explanation for this pattern is rapid loss of colony viability due to disease and parasites.

MATERNITY-RELATED PLASTICITY IN CIRCADIAN RHYTHMS OF BUMBLE BEE (*BOMBUS TERRESTRIS*) QUEENS

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One of the key transitions in the evolution of insects societies is the expression of brood care behaviour in non-reproductive workers. It has previously been suggested that sibling brood care evolved from maternal behaviour. Brood care behaviour in workers is associated with around-the-clock activity with no circadian rhythms. We tested whether bumble bee (*Bombus terrestris*) queens that care alone for their first batches of offspring, are also capable of around-the-clock activity. We monitored locomotor activity of queens at various life cycle stages, and of queens for which we manipulated the presence of brood. We found that gynes typically emerged with no circadian rhythms, but later in life showed robust rhythms. Mating and diapause did not affect the expression of circadian rhythms, but colony-founding queens with brood showed no, or only attenuated circadian rhythms. By contrast, queens for which we removed the brood or that lost it for other reasons, switched to activity with strong circadian rhythms. This remarkable plasticity in queens is consistent with the hypothesis that task related plasticity in the circadian system of workers evolved from maternity-related plasticity in the circadian clock.

SURPRISINGLY SOCIAL: EUSOCIALITY IN DIGGER WASPS?

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Eusociality is defined by reproductive division of labour, overlapping generations and cooperative care of young. Lower levels of sociality may lack one of those qualities. Prominent examples for eusociality occur in Hymenoptera. While ants are all eusocial, bees and wasps consist of both social and solitary species. Digger wasps (Crabronidae and Sphecidae), on the other hand, normally show a solitary lifestyle. *Cerceris*, the largest crabronid genus with more than 900 species which occur on all continents, comprises a few exceptional species that show supposedly communal nesting. One such example is *Cerceris rubida*. The nests of this Mediterranean species are shared by up to 8 females. According to the "communality" hypothesis, nest-sharing in this species was assumed to be the result of random nest choice combined with low intraspecific aggression. An alternative hypothesis is that colonies are matrifilial systems and *C. rubida* is eusocial. In this study we show that nests of *C. rubida* are founded by single females, and they become social when their daughters emerged and remain with the mother in their nest. In support of this, nest foundresses differ from the following generations in size, patterns of body pigmentation and cuticular hydrocarbon profile. Additionally, non-foundress females of *C. rubida* within a nest exhibit a more similar cuticular profile representing a degree of kinship compared with females from foreign nests. Even founding females show this similarity plus additional compounds. Behavioural assays with hydrocarbon extracts on filter paper show the ability of females to discriminate between nestmates and non-nestmates. Thus, at least four evidences (overlap of generation, phenotypical "castes", nestmate recognition, and potential "queen" signal) suggest complex sociality in *C. rubida*. Our findings suggest a possible new independent origin of eusociality in the Hymenoptera.

How individual trajectories may contribute to the rise of a worker caste in termites

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Eusociality, achieved by the differentiation of a sterile soldier caste, evolved once at the origin of termites. Few elements remain in present-day termites to track the origin of this caste, which is best envisioned by analogy with other social insects possessing a defender caste and living in food-rich and confined environments, such as social aphids and thrips. However, several termite lineages crossed the eusociality threshold a second time, when a second altruistic caste (workers) arose. Many intermediate conditions exist between immatures of small-colony drywood termites, which do little work, remain developmentally totipotent and usually proceed selfishly to the alate or neotenic stage, and true workers of specialized Termitidae, which differentiate early as altruistic helpers and are completely sterile. Here, I will emphasize the intermediate situation, occurring in some wood-dwelling termites with larger colonies: an actual working caste - a functional category of individuals performing helper tasks - is present, but is composed of immatures retaining developmental flexibility. I will discuss how such a working caste can result from the juxtaposition of trajectories of individual immatures, faced with a choice between selfish and altruistic opportunities. Recent results on caste patterns in the Rhinotermitidae and Serritermitidae will be presented in this context. For instance in *Glossotermes*, there is a single pre-alate nymphal (wing-padded) instar preceded by totipotent working immatures, whose actual chances of reproduction are however reduced by a heavily biased sex ratio. In other taxa, such as *Prorhinotermes inopinatus* or the kalotermitid *Neotermes papua*, mutilations of the wing rudiments by nestmates likewise constrain the future development of working immatures. The various ways a working caste develops in those large-colony wood dwellers illustrate possible steps towards the evolution of permanent workers such as those of the Termitidae.

Direct and indirect fitness benefits of philopatry and cooperative care in *Xyleborina* (Curculionidae: Scolytinae)

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Reproduction of ambrosia beetles is characterized by parental care and colonial breeding, and eusociality has been described for one species. Social behaviour has not been studied in this group, however, despite its outstanding suitability to serve as model system for the study of genetic and environmental factors in the evolution of cooperation and advanced sociality. We studied the parental and alloparental behaviour of two species of the haplodiploid *Xyleborina* and estimated fitness effects to distinguish between the potential importance of individual and kin selection. We show experimentally that philopatric females gain direct fitness benefits from pre-emergence feeding in their natal gallery, which leads to a higher reproductive output after dispersal and foundation of an own nest. Philopatric females may also breed within their natal gallery. Indirect fitness benefits of alloparental care seem to be important as well: all colony members cooperate in gallery maintenance, brood care and fungus farming, with various task specializations among individuals of different status and age. This polyethism is reminiscent of the behaviour of the socially most highly-developed hymenoptera and represents an advanced level of sociality in Coleoptera.

Poster Presentations

Trajectories towards sociality across arthropod taxa

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26-1 INTRINSIC AND EXTRINSIC FACTORS IN SOCIAL EVOLUTION AND THE GEOGRAPHICAL DISTRIBUTION OF SPIDER SOCIALITY

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The geographical distribution of species with different social systems should provide clues as to the factors responsible for social evolution. Social spiders are notable for having a distinctly tropical distribution. In the genus *Anelosimus*, which contains the largest number of social species of any spider genera, this latitudinal pattern is replicated altitudinally: social species are restricted to wet low to mid-elevation tropical areas, while subsocial species predominate at higher elevations and latitudes. We postulate that this pattern results from an interaction between the dense three-dimensional webs characteristic of social spiders with two separate environmental gradients. That social species are restricted to the lower elevation wet tropics may reflect a gradient in insect size, which we show decreases with elevation and latitude. Large insects compensate for a decline in the number of prey caught per capita with increasing colony size that results from a declining surface area to volume ratio of the prey capture snares. Where large insects are abundant the spiders make up for this decline by cooperatively capturing larger insects in larger colonies. The result is a biomass per capita that is maximal at intermediate colony sizes. Absence of subsocial species in the lowland rainforest, on the other hand, may reflect gradients on the intensity of precipitation and abundance of potential ant predators, which we show increase with proximity to the rainforest. Through transplant and rain exclusion experiments we show that dense 3D webs may be unsustainable for solitary living spiders in environments where intense rains cause their frequent destruction. Dense 3D webs may also provide better protection against predators, especially when large. Overall, these findings illustrate how broad scale patterns of sociality, and social evolution itself, is the result of an interaction between intrinsic features of organisms and the environments in which they live.

26-2 DYNAMICS OF AGGREGATION IN WOODLICE: INDIVIDUAL PREFERENCES AND COLLECTIVE CHOICE

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Woodlice are intensively studied to understand the adaptation to land-life of crustaceans. In this respect, individuals prefer dark and damp sites. Moreover, it is well known that aggregation participates to this adaptation by reducing the water loss. However, even if the benefits of such behavior have long been known, little evidence reveal its dynamics. In this study, the aggregation of 40 *Porcellio scaber* was followed. First of all, experiments were carried out in homogeneous environments (in circular arena and under 3 different light intensities). Secondly, experiments were carried out in heterogeneous environments where woodlice could choose between two shelters with different brightness. The study confirms the individual preferences of woodlice since 95.6% of aggregates were observed under dark shelters. Our results describe for the first time the dynamics of aggregation: whatever the experimental condition, most of woodlice were aggregated in less than 5 minutes possibly in several aggregates. Subsequently, during the experiments, the proportion of aggregated woodlice remained high but were relocating in only one big aggregate. Main differences were observed in high brightness where aggregates seemed less stable. Our results also show the implication of a strong social component in this aggregation process. Indeed, 88% of experiments carried out in heterogeneous environments showed the selection of only one of both shelters. This kind of result can only be explained by the social interaction between congeners. Finally, aggregation in woodlice results from a trade-off between individual preferences of woodlice (that means to be in obscurity) and strong inter-attraction in this species (that induces to be near congeners). We discuss the implication of such inter-attraction in the behavioral ecology of woodlice since, it can induce grouping of woodlice in non optimal decision by selecting unfavorable bright places.

26-3 SOLDIERS ARE NOT CREATED EQUALLY

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Gall induction by thrips evolved once on Australian *Acacia*, and within the subsequent radiation emerged a defensive caste (soldiers). The soldiers typically have reduced wings, antennae, and melanisation of the cuticle. However, it is the enlarged forelimbs that enable the soldiers to more effectively defend the colony from invertebrate invasions. The soldier caste is made up of males and females, and between species this caste differs in number of soldiers and the sex ratio. Previous work has not considered relative fighting ability between the sexes, nor has the variation in morphology within a sex been shown to be related to fighting ability. We begin this investigation with the species *Kladothrips intermedius*. This species has larger female soldiers that possess more robust forelimbs compared to males. Furthermore, males have longer wings than females, which might suggest that males are less committed in their morphology to a fighting role. We tested two hypotheses: (1) Are females more effective than males at fighting invaders? (2) Is wing length negatively correlated with fighting ability? The main outcomes of our investigations are that females are not more effective fighters, but they appear to be slightly more willing to engage in fighting behaviour. And, there is evidence that increased wing length decreases willingness to fight for males. While female wing length is not related to willingness to fight, but shorter wings are associated with an increased ability to kill an invader. Thus, the individuals within this caste are not equally committed to the defensive role.

26-4 ON THE THRESHOLD OF EUSOCIALITY: THE REPRODUCTIVE STATUS OF THE INDIVIDUALS IN PASSALID BEETLE COLONIES FROM THAILAND.

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The Passalids (Passalidae: Coleoptera) include some of the most highly social of all beetles, and indeed routinely satisfy two of the three traditional criteria of eusociality: overlap of generations and cooperative brood care. All species are social and most live in galleries in rotting wood. Offspring usually remain with their parents until adult; the parents jointly provide food (faecal material) for the young; and the larvae and adults (both mature and teneral) cooperate in the construction and repair of the pupal cases of siblings. However, it is not clear whether there is any marked reproductive division of labour amongst the adults within a colony. We investigated this in *Aceraius telferi* in Northern Thailand. We carried out a detailed census of twenty-eight colonies, containing between two and thirteen adults. We dissected all the adults and assessed their reproductive maturity, apparent age (through the degree of body cuticular wear and colour), and the mating status of the females. There was no clear evidence of high reproductive skew within the colonies: there could be more than one reproductively active individual in the larger colonies. There appears to be a high level of inbreeding, since almost all the females, including the most teneral, had been mated. These results will be discussed in the context of the trajectories towards sociality within the beetles as a whole.

26-5 PHYSIOLOGICAL EFFECTS AND FITNESS CONSEQUENCES OF MATERNAL MANIPULATION IN A TROPICAL SWEAT BEE (*MEGALOPTA GENALIS*)

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Although developmental maternal effects are a potentially important source of phenotypic variation, they can be difficult to distinguish from other environmental factors. This is an important distinction within the context of social evolution, because maternal manipulation may act as a selective force on worker behavior. We investigated the influence of maternal effects on social caste in a mass-provisioning facultatively eusocial sweat bee, *Megalopta genalis*. Although all female *M. genalis* eclose without obvious impediments to initiating their own nests and reproducing independently, many females forego dispersal to remain in their natal nests as subordinate workers in small social groups with a strong division of labor. We tested the hypothesis that maternal provisioning behavior has been a primary path of selection on worker behavior. We found evidence that females actively manipulate the quality and quantity of pollen masses provided to their daughters, independent of ecological constraints. We then measured caste differences in physiological correlates of nutrition, including body size, ovary development, and levels of circulating vitellogenin. Measuring these parameters in females raised independently of social interactions allowed us to determine the amount of variation in these caste-related traits that was the result of differences in maternal provisioning behavior. Finally, we investigated the fitness consequences of maternal effects by quantifying the direct and indirect costs and benefits of social behavior in workers, queens, and solitary females, using molecular parentage analysis and detailed behavioral observations. This combined approach tests for a selective pathway through which maternal effects may have acted on the evolution of worker behavior.

26-6 SOCIAL EVOLUTION IN TERMITES

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Termites are the oldest and second largest group of social insects yet they have received much less attention than social Hymenoptera. All termites are eusocial but the degree of social organisation largely differs among taxa: Phylogenetically basal wood-dwelling species, which live in a single piece of wood that serves at the same time as food and shelter, have totipotent worker individuals that can explore all caste options. They can develop into soldiers, and two types of reproductives: primary reproductives that leave the nest as winged sexuals to found a new nest elsewhere and neotenic reproductives that inherit the natal breeding position when the current reproductives die or become unhealthy. By contrast, in those termite species that sooner or later have to forage for food (foraging species) developmental flexibility becomes gradually reduced. These differences in ecology and development result in different pay-offs that individuals can receive from direct versus indirect fitness gains. The workers of wood-dwellers are less altruistic and largely represent immature individuals that finally become sexuals. This contrasts with foraging species where workers do intensive brood care. I will give an overview of termites' social evolution by presenting data on the degree of brood care and inheritance opportunities in different families.

26-7 SOCIAL POLYMORPHISM IN THE AUSTRALIAN SMALL CARPENTER BEE, *CERATINA* (NEOCERATINA) *AUSTRALENSIS* (HYMENOPTERA: APIDAE)

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The bee tribe Ceratinini provides important insights into the early stages of sociality. The facultatively social bee, *Ceratina australensis*, exhibits all the prerequisites for successful group living yet also presents ecological and behavioural characteristics that seemingly disfavour frequent colony formation. It provides a unique system to naturally observe behavioural differences and fitness consequences of solitary versus social behaviour in sympatry. Here we weigh intrinsic properties (genetic relatedness, brood productivity) against extrinsic environmental constraints (resource limitation, parasite pressure) to elucidate the conditions favouring the emergence of social life. Maternal behaviour and mutual tolerance provide the behavioural repertoire for social interaction. The bivoltine reproductive cycle and nest reuse are key life history traits contributing to multi-female nesting. However, dispersal habits and brood parasitism may inhibit more frequent social colony formation. Social colonies consist of two adult females, one that both forages and lays eggs and one that remains at the nest as a passive guard. Social nesting provides no overt advantage over solitary nesting, although brood survival tends to be greater in social colonies. Genetic analysis indicates that nestmates in social colonies are sisters, suggesting that kinship is important for sociality. Indirect fitness benefits and female longevity provide sufficient staying incentives for females to remain at the nest as hopeful reproductives, foregoing foraging and reproduction until they inherit the nest.

26-8 THE EVOLUTION OF ALLOPARENTAL CARE IN SOCIAL COMB-FOOTED SPIDERS

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The potential for sociality to arise in any given taxa depends on a suite of ecological and phylogenetic factors. Phylogenetic factors include the amount of selectable variation in social behaviour (inertia) and the co-optative potential of pre-existing traits (preadaptation). In this study, I explore the significance of a preadaption for alloparental care in the multiple parallel evolution of sociality in tropical comb-footed spiders (Theridiidae: *Anelosimus spp.*). Focusing on the care of egg-sacs, I demonstrate that two species of social comb-footed spiders perform alloparental care. I then establish a positive correlation between the degree of alloparental care provided and various metrics of sociality. I follow by showing that the subsocial sister species of both social species readily perform alloparental care given the opportunity. I argue that alloparental care is an obligate corollary of parental care and that no specific behavioural evolution beyond the evolution of grouping behaviour was necessary for its appearance in social species. I discuss that, in the case of social spiders, the co-optative potential of indiscriminate parental care may have thus assisted in the shift from solitary to social living.

26-9 MALE AND FEMALE MEDIATED GENE FLOW IN THE SOCIAL SPIDER *STEGODYPHUS DUMICOLA*

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Population structure and genetic relatedness among colony mates of social spiders have been investigated using allozymes, mtDNA, and DNA fingerprinting. All of these approaches have indicated high levels of relatedness among colony mates and strong population structuring, particularly among colonies. This population structure is a function of the method of colony initiation - typically by dispersing, mated females - and dispersal among established colonies. There is little evidence for movement of immatures among colonies, and most inter-colony dispersal is thought to be carried out by mated females and possibly by adult males in search of mates. However, the amount of male and female dispersal among colonies is very difficult to assess in the field. In this study we investigated population structure of the cooperatively social spider *Stegodyphus dumicola* (Eresidae), focusing on the effects of gene flow mediated by male versus female dispersal. Our questions were: (1) Is there detectable pre-mating/mating dispersal among colonies, (2) is this dispersal carried out by males, females or both, and (3) what are the typical dispersal distances of males and females? We sampled adult males and females from 54 colonies at two locations in South Africa, collecting just after the main period of mating in order to capture any males that might have immigrated into the colonies. We employed DNA-fingerprinting (using TE-AFLPs) to estimate similarity among sampled individuals, and carried out separate analyses of molecular variance (AMOVA) and spatial autocorrelation analyses for males and females. As in earlier studies, we detected strong differentiation among colonies. Results of autocorrelation analyses will be reported.

26-10 TERMITE SOLDIERS ARE RESPONSIBLE FOR THE REGULATION OF PRESOLDIER MOLT BY RAPID JH-DECREASING EFFECTS IN THE COLONY

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The regulation of caste differentiation is essential to insect sociality. Termite soldiers are sterile, and cannot eat by themselves as specialized defensive features. Nevertheless, almost all termite species have a soldier caste, and the soldier ratio per colony is maintained at a low level, indicating that the regulation of soldier differentiation is very important to maintain termite sociality. Soldier differentiation can be induced artificially by juvenile hormone (JH) application to workers. Thus, the key factor *underlying the regulation of soldier differentiation* is JH, but proximate mechanisms are still unclear. To elucidate additional data on the mechanisms mediated by JH, various dish assays were performed using the rhinotermitid termite *Reticulitermes speratus*. First, we investigated the effects of soldiers on morphogenetic changes during the molt into presoldiers induced by JH III application to workers. The rates of presoldiers induced in treatments with soldiers were lower than in treatments with no soldiers. Moreover, induced presoldiers in treatments with soldiers had shorter mandibles and incomplete frontal glands in their heads. To identify the physiological effects on workers prior to molt into presoldiers, JH of workers in soldier presence and absence was quantified by LC-MS 0, 5, 10 and 15 days after JH application. As a result, JH titers (endogenous + applied JH III) of workers with soldiers were significantly lower than without soldiers 5 days after treatment. Finally, we manipulated the duration of soldier existence after JH application. Presoldier differentiation was inhibited by only 4-day interaction with soldiers, but the mandibular elongation of presoldiers was influenced by 8-day interaction with soldiers. Our results suggest that soldiers inhibit presoldier production and affect soldier-specific morphogenesis by JH-decreasing effects, which are probably rapidly transmitted to other colony members.

Oral Presentations

Immunity and sociogenomics of host-parasite interactions

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STRATEGIES OF IMMUNE DEFENCES IN SOCIAL INSECT SOCIETIES

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Social insect societies are constantly threatened by a variety of microbial pathogens and parasites. Immunity is one cornerstone of defence and it can be used in various ways. Defences are also costly in principle, and these costs might affect the colony in various ways. Furthermore, intensive brood care as is typical for social insects has opened up additional immune defence strategies to protect offspring. Finally, the question will be asked how selection on parasites might affect their evolution.

EVOLUTION AND SPECIFICITY IN INSECT IMMUNITY

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The modern study of immunity in insects relies heavily on paradigms taken from work on *Drosophila*. I will give a brief overview of our current knowledge about humoral and cellular immunity in this species, and discuss to what extent that knowledge can be extrapolated to other insects. I will argue that whereas signaling pathways are in general relatively well conserved between different insect orders, this may not always be true for the specificities of pathogen recognition factors or the effector mechanisms.

RESIN COLLECTION AS COLONY-LEVEL IMMUNE DEFENSE IN HONEY BEES

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Individuals within a densely populated society, such as a honey bee colony, defend themselves against parasites through evolved defenses expressed at the colony-level due to collective behaviors of individuals. This study aimed to further our knowledge about the role of resins, complex plant secretions with diverse antimicrobial properties, as a form of colony-level defense, or social immunity. The harvesting and incorporation of antimicrobial compounds in nest architecture by honey bees is a relatively unexplored behavior. Our previous study indicated that individual bees in resin-enriched environments were able to invest less on immune function, likely due to overall reduced in-hive bacterial loads. This finding was based on differences in expression of two immune-related genes (hymenoptaecin and *AmEater*), with 7d old bees from resin-enriched colonies having significantly lower expression as compared to bees from control colonies.Subsequent studies examined how in-hive resins affect immune-gene expression of bees and the rate of resin foraging in healthy and pathogen-challenged colonies. Individuals from resin-enriched colonies had reduced investment in immune function compared to bees from resin-poor colonies after challenge with the fungal pathogen *Ascosphaera apis*. However, bees from resin-enriched colonies did show an up-regulation in immune expression after pathogen challenge, demonstrating that resin does not inhibit immune function. The self-medication hypothesis was tested to determine if bees increase the rate of resin collection after pathogen challenge; results will be presented. These are the first known studies examining the effect of in-hive resins on honey bee immunity at baseline levels or during a pathogen challenge and to investigate self-medication behaviors by honey bees. More generally, this is a rare example of an environmental compound that can modulate immune function.

MOLECULAR BASIS OF SELF-SACRIFICING GALL REPAIR BY SOLDIER APHIDS IN THE SOCIAL APHID, *NIPPONAPHIS MONZENI*

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Fortress repair was reported from a gall-forming aphid, *Nipponaphis monzeni*, in which monomorphic first-instar soldier nymphs repair their gall in a self-sacrificing manner (Kurosu *et al.*, 2003). *N. monzeni* forms completely closed galls on the tree *Distylium racemosum*. Since the wall of growing galls is still soft in early spring, gall-feeders such as lepidopteran larvae often invade the gall by tunnelling through the wall. When a hole was bored in the gall wall, the soldier nymphs of *N. monzeni* immediately gathered around the hole, discharged a large amount of body fluid from their cornicles on the damaged area, and mixed the fluid with their legs. The discharged fluid soon became viscous and solidified, whereby the hole was filled up completely. In an attempt to understand the molecular basis of gall repair, especially of the solidification, we analyzed proteinacous components of the body fluid. We found that the body fluid consisted of six major proteinacous components, one of which was phenol oxidase, a key enzyme involved in melanization and hemolymph clotting in insects. Molecular and enzymatic analyses revealed that the expression levels of the phenol oxidase in gall-repairing soldiers was much higher than those in the non-repairing individuals. Other components in the fluid were novel proteins, that contained highly repetitive sequence motifs and showed no sequence similarity to protein sequences deposited in the databases. It seems likely that these proteins are cross-linked due to the action of highly reactive intermediate substances in the melanin pathway, represented by quinones, that results in solidification of the body fluid. From these results, we suggest that the aphid's innate immune and wound-healing mechanisms have been recruited to the social task, gall repair, in the lineage leading to *N. monzeni*.

EVOLUTION OF IMMUNITY-RELATED GENES IN MYRMECIINE ANTS

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Pathogenic microorganisms impose strong selection pressures on animal immune systems. Immunity-related genes include the most rapidly evolving genes in insect genomes. In social insects pathogens can be easily transmitted among nest mates. Thus they are very well suited for studying the evolution of immunity-related genes. Here, we study the molecular evolution of immunity-related genes in Australian hopper and bulldog ants (genus *Myrmecia*), and in the "dinosaur" ant *Nothomyrmecia macrops*. We cloned and sequenced several immunity-related genes whose products include pathogen recognition, signal transduction, and effector molecules. We discovered positive selection in some but not all immunity-related genes studied in myrmeciine ants applying codon-based comparative analyses. Moreover, we produced a molecular phylogeny facilitating insights into the evolution of phenotypic characters in myrmeciine ants.

SOCIAL IMMUNITY AND THE EXPRESSION OF IMMUNE-RELEVANT GENES IN THE EASTERN SUBTERRANEAN TERMITE

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For social insects, there are two levels of immune defence. Individual immunity consists of a conserved innate response involving the expression of antimicrobials upon infection. Social immunity, by contrast, is a derived phenomenon specific to social animals whereby individuals cooperate to reduce each other's pathogen load. Although examples of social immunity are known from a few well-studied taxa, the extent to which this phenomenon occurs in termites is unknown. Moreover, it is not known the potential impact that socially-enabled defences might have on individual-level immunity. In this study we manipulate two social variables that are expected to affect the number and nature of social interactions, and measure the ability of individuals within groups to resist infection. From laboratory experiments we report that both group size and caste composition affect individual survivorship, despite an individually uniform pathogen load. This pattern suggests that contact rate and type are important for controlling contagion in termite societies, and further suggests that social immunity is affected by caste-based divisions in labour. Secondly, from a novel cDNA library that is enriched for immune genes, we report the first comparative test of termite immune gene diversity against other social and non-social genomes. Like the honey bee, the Eastern subterranean termite appears to harbour relatively few immune genes when compared to *Drosophila* and *Anopheles*. Social living and social immunity may therefore be generally associated with loss of immune loci in insects.

Poster Presentations

Immunity and sociogenomics of host-parasite interactions

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27-1 DIFFERENTIAL HOST DEFENSE AGAINST MULTIPLE PARASITES OF DISTINCT IMPACT

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Host-parasite interactions are an ideal system for the study of coevolutionary processes. Although infections with multiple parasite species are presumably common in nature, most studies focus on the interactions of a single host and a single parasite. To the best of our knowledge, we present here the first study on the dependency of parasite virulence and host resistance in a multiple parasite system. We evaluated whether the strength of host defense depends on the potential fitness cost of parasites in a system of two Southeast Asian army ant hosts and five parasitic staphylinid beetle species. The potential fitness costs of the parasites were evaluated by their predation behavior on host larvae in isolation experiments. The host defense was assessed by the ants' aggressiveness towards parasitic beetle species in behavioral studies. We found clear differences among the beetle species in both host-parasite interactions. Particular beetle species attacked and killed the host larvae, while others did not. Importantly, the ants' aggressiveness was significantly elevated against predatory beetle species, while non-predatory beetle species received nearly no aggression. As a consequence of this defense behavior, only the less costly parasites are able to achieve a high level of integration in the ant society. We conclude that the selection pressure on the host to evolve counter-defenses is higher against costly parasites and thus a hierarchical host defense strategy has evolved that depends on the parasites' impact.

27-2 UNDERSTANDING THE COST-BENEFIT BALANCE OF IMMUNE ACTIVATION IN NATURE: A FIELD EXPERIMENT WITH BUMBLEBEES

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An immune challenge in an individual can result in both positive and negative effects. While costs of immune system activation are well documented under controlled laboratory conditions, beneficial effects linked to immune priming that affords increased protection on future parasite exposure occur. These beneficial effects are epitomised by the success of vacinnation strategies in humans and domesticated animals. Costs and benefits of this kind have been shown to exist in bumblebees, *Bombus terrestris*, but the influence of immune boosting episodes on colonies of this social insect under natural settings is still unknown. In order to investigate this we conducted a field experiment in which colony members were periodically immune boosted with a benign bacterial based immune elicitor. As costs of immune activation in laboratory maintained bumblebees materialise under food stress we also included food supply (natural and supplemented) as a factor in the experiment. The results give an insight into the influence of immune boosting on colony performance in the field, and allow conclusions to be drawn on the balance between the costs and benefits of immune activation under natural conditions.

27-3 Beeworm: Gene expression in a Host-Parasite Interaction

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Host immunity presents a formidable obstacle for parasite infection. To overcome such complex defences, parasites have evolved elaborate mechanisms, including immunosuppresssion or evasion. One such parasite, *Sphaerularia bombi*, an entomopathogenic nematode, infects overwintering bumble bee (*Bombus* spp.) queens. Infection induces behavioural and physiological alterations in the host with the main pathological effect of parasitism being host castration. At present, there is no genetic data for the mechanisms involved in parasitism. Therefore, we plan on investigating the host-parasite interaction of *S. bombi* and its bumble bee host, *Bombus terrestris*, at the gene expression level. As a first step towards understanding this interaction, we have constructed referent transcriptomes for both host and parasite. Here we describe a catalogue of expressed sequence tags (ESTs) from different developmental and life stages of *B. terrestris*. Complementary DNA (cDNA) from *B. terrestris* larva, pupa and adults was sequenced using 454 sequencing. Sequencing generated 1,609,302 ESTs. Overlapping ESTs were assembled into unique contigs and then annotated preliminarily with BLAST2GO. Based on gene ontology (GO) terms, candidate genes involved in immune and developmental processes were identified. A number of immune pathways were represented in the set, including the Toll, Imd, JNK and prophenoloxidase pathways. In addition, developmental genes, which are known to be targeted by *S. bombi during infection*, such as vitellogenin and juvenile hormone, were present. The ESTs were compared across developmental life stages to determine whether or not a difference in immune-related and developmental gene expression was evident. Our preliminary analysis indicated variation in the overall EST profile across the different developmental stages. Our EST catalogue will provide an important resource for gene discovery and annotation in future *B. terrestris* genomic studies.

27-4 Genomic analysis of the effects of *Nosema apis* infection on honey bee (*Apis mellifera*) workers

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Pathogens represent a major threat to pollinator populations. Globally, honey bees are declining, and loss of their pollination services would have profoundly negative consequences for both agricultural and natural landscapes. *Nosema apis*, a microsporidian pathogen of honey bees, causes commercial losses due to reduced hive productivity. *N. apis* spores are spread via the fecal/oral route and infections are initiated in the midgut. Infected bees suffer from diarrhea, mature at behaviorally accelerated rates and have shortened life spans. Using microarrays, we characterized gene expression in worker bees with *N. apis* infections to better understand the molecular pathways underlying the physiological and behavioral symptoms associated with infection. We compared midgut gene expression in healthy and infected bees at 1 and 2 days post-infection and fat body gene expression at 2 and 7 days post-infection. We hypothesize that genes regulating metabolism, behavioral maturation and immunity will differ between control and infected bees. In addition, we expect to characterize, for the first time, genes associated with the local midgut immune response to *N. apis*. This study will contribute to our basic understanding of bee immunology and lay the groundwork for future studies of the impact of *Nosema apis* and an additional pathogenic microsporidian, *Nosema ceranae*, at the molecular, physiological, behavioral and colony levels.

27-5 NAVIGATING STRATEGIES OF TRAIL-FOLLOWING IN TERMITES

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Termites have evolved effective antifungal mechanisms to combat pathogenic fungi, including antibiotic glandular secretions, grooming, removing diseased individuals, and innate and acquired immune responses. The evolved immunity overcomes environmental challenges from microbes and decrease infection susceptibility and transmission. Here, we examine group facilitation of inhibition and infection resistance against the fungal pathogen *Paecilomyces variotii* (Eurotiales) in the mound-building termite *Globitermes sulphureuse*. Introductions of termite groups onto nest materials showed an effect of termite demography on fungal pathogenicity, where termite group size negatively correlated with fungal population and termite mortality. We verified this by introducing termite groups to the same dose of fungal culture. Treatment with larger groups had lower mortality and faster rates of fungal population reduction. Fungal consumption and dermal contamination were determined from the green-colored fungal spores in the gut and upon the integument. Surviving workers confined in groups and subsequently exposed to the same pathogen exhibited significantly higher survival compared to isolated workers, which had higher survival rate than naïve workers, suggesting the development of fungus-induced immunity. These results demonstrate that group living may facilitate infection resistance thus benefit colony survival despite the increased risks of pathogen transmission that can accompany sociality.

27-6 IMMUNE DEFENSE COSTS, STORAGE OF PROTEINS AND REPRODUCTIVE STATUS IN *APIS MELLIFERA* QUEENLESS WORKERS

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Insects are able of initiating an efficient innate immune response to kill invading pathogens and parasites. Recently, we demonstrated that bacterial challenge in honey bees cause a drastic down-regulation of storage proteins. As synthesis and secretion of these proteins are dependent on a proteinaceous diet, and are intimately related with reproduction in honey bees, we investigated the influence of immune and nutritional factors on (1) the levels of storage proteins in hemolymph, (2) the reproductive status of queenless workers and (3) the expression of ovarian genes. Different diets provided a range of proteinaceous resources for storage protein synthesis in confined bees. Following a period of alimentation, they were infected with *Serratia marcescens*. As expected, the levels of vitellogenin, hexamerin 70a, apolipophorin-II/I and apolipophorin-III in hemolymph, and of vitellogenin receptor transcripts in the ovaries were reduced in infected workers. This down-regulation was only evident in bees fed on a proteinaceous diet (beebread), which provided significant synthesis of storage proteins than other diets (syrup or royal jelly). Beebread was the only diet promoting ovary activation in queenless workers, and workers fed on this diet, and also infected, showed a significant reduction in ovary activation compared to non-infected ones. The expression of apolipophorin receptor and vasa (a germline marker) in the ovaries are not regulated by diet, but by the reproductive status. These results demonstrate that immune response impairs protein storage and, by extension, ovary activation in honey bee workers. Financial Support: FAPESP, FAPEMIG, CNPq

27-7 SYMBIONT-MEDIATED PROTECTION AGAINST FUNGAL INFECTION IN THE DAMPWOOD TERMITE, *ZOOTERMOPSIS ANGUSTICOLLIS*

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Termites have a long co-evolutionary history with prokaryotic and eukaryotic gut microbes. Historically, the role of these anaerobic obligate symbionts has been attributed to the nutritional welfare of the host. We hypothesize, however, that the nature of this mutualistic interaction extends beyond the nutritional benefits to the host and propose that termite gut symbionts enhance the host's defenses against pathogens. To test this hypothesis, a series of *in vivo* and *in vitro* experiments were devised using the primitive dampwood termite *Zootermopsis angusticollis* and the entomopathogenic fungus *Metarhizium anisopliae*. In order to examine the role of symbionts in host protection, termites were experimentally defaunated with oxygen and their survival compared with that of control animals following exposure to fungal conidia. In vivo results indicate that normally faunated termites are significantly less susceptible to infection than their defaunated counterparts. In vitro experiments point specifically to the hindgut protozoa as one factor reducing susceptibility to infection. The associated hindgut protozoa synthesize multiple beta 1-3 glucanases which have significant fungistatic effects. Furthermore, the Eastern subterranean termite, *Reticulitermes flavipes* and the African termite *Cryptotermes secundus* also exhibit symbiont-derived beta 1-3 glucanases, suggesting that symbiont-mediated protection could be a widespread phenomenon in the phylogenetically basal (“lower”) Isoptera. We conclude that the protozoa and their synthesized beta 1-3 glucanases appear to play an important role in termite pathogen defense. This research establishes a novel disease defense role for the mutualistic association between termites and their hindgut eukaryotic microbial consortia, providing new insights into the evolution of this mutualism, as well as the evolution of termite sociality and disease resistance.

27-8 MICROBIAL INHIBITION AND IMMUNE RESPONSE SPECIFICITY IN THE ANT *FORMICA EXSECTA*

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The variety and complexity of insect immune responses has only recently been discovered. Not only has this widespread and diverse group of organisms evolved defence mechanisms analogous to those of vertebrates, but it also shows alternative solutions to the challenges posed by pathogens. Insect societies, such as those of ants, bees and wasps, are a special case in the evolution of host-pathogen interactions. They provide a stable environment where the potential hosts are closely related and occur in high densities, conditions which promote the spread of pathogens. It can therefore be expected that social insect colonies are under constant immune challenge. Even so, social insects are successful in terms of diversity, distribution and ecological dominance. The colony's defences range from division of labour to individual immune defences. In addition to physical barriers, the individual immune response consists of cellular and humoral defences. The cellular component involves e.g. phagocytosis and encapsulation, while the humoral defence consists of circulating defence molecules, such as antimicrobial peptides. My study focuses on the inducibility and specificity of the humoral part of the immune defence in the ant *Formica exsecta*. To test this, I challenged ants with four bacterial species, two gram-positive and two gram-negative, by injecting heat-killed bacteria into the ants' abdomens. After injection, hemolymph was extracted and used to test the difference in the growth-inhibiting effects of the antibacterial peptides on the bacterial cultures. The diameters of the inhibition zones that arose on the cultures were used as a proxy for the strength of the immune response. There was no response against the gram-negative species. The response against one of the gram-positive species was constitutive, whereas the response against the other one was induced by the injection itself. No specificity was found and the degree of inbreeding of the mother colony had no effect.

27-9 SICK ANTS ARE BAD DOCTORS: HEALTH STATE OF GROUP MEMBERS AFFECTS SOCIAL IMMUNITY

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Disease resistance in social insects is characterised by the interplay of individual immunity and collective disease defences. The power of these social defences has been vastly documented, and all studies so far found clear survival benefit of exposed individuals when reared together with nestmates than in isolation. Our study in the ant *Cardiocondyla obscurior* is the first to show an exception to this general pattern revealing no significant survival benefit of ants exposed to the entomopathogenic fungus *Metarhizium anisopliae* when reared in groups, in which all nestmates were exposed, over solitary rearing. However, exposed ants survived better and better the higher the fraction of unexposed group members became. When the proportion of healthy nestmates was higher than that of the exposed ants (75% in our study), we found a significant survival benefit as compared to living in groups of only exposed ants. This indicates that the efficiency of social immunity is plastic and depends on the health status of nestmates. We conclude that particularly in the small societies of *Cardiocondyla* ants it may thus be extremely important to act quickly against incoming infections as the power of social immune defences declines rapidly with the spread of infection through the colony.

27-10 HYGIENIC BROOD CARE: IS IT AFFECTED BY EXPERIENCE?

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Pathogens strongly impact the survival of social insect colonies, where the transmission of disease is facilitated by the geographic and genetic proximity between nestmates. Social organisms evolved anti-pathogen defense mechanisms, hygienic tasks, to acquire resistance at the group level. In *Platythyrea punctata* we examine whether the investment into a hygienic task varies as a function of individual experience. This thelytokous parthenogenetic ant offers the opportunity to study the effect of experience on task performance in absence of genetic diversity among workers. We analyze if increasing experience in grooming larvae treated with spores of the entomopathogenic fungus *Metharizium anisopliae* leads to changes in this antiseptic behavior of individual workers. Further, we investigate whether experienced workers are more efficient in task-performance and we compare colony-level performance between experienced and inexperienced colonies.

27-11 HYPERPOLYANDRY AS A DISEASE RESISTANCE ADAPTATION IN HONEY BEES (*APIS MELLIFERA*)

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Genetic diversity contributes to parasite resistance in ants, bumble bees, and honey bees. Honey bee queens (*A. mellifera*) mate multiply and are known to mate with an average of 7-17 drones. Many hypotheses have been posited to explain this extreme polyandry, including improved division of labor within a colony, heightened probability for sperm acquisition, and decreased disease susceptibility via increased genetic diversity at disease resistance loci. The latter hypothesis, termed the `polyandry versus parasitism hypothesis' (Sherman *et al.* 1988), posits polyandry is a defense mechanism against pathogens and parasites. Here, we test this hypothesis using a combination of PCR analysis at 16 microsatellite DNA regions, three immune function measures, and two disease resistance tests amongst 22 unmanipulated honey bee hives from 2006 and 2009. We predicted colonies with high genetic diversity would display high variability in immune function measures. Our preliminary results are mixed: there were no differences in the mean or the variance around the average encapsulation response across colonies, but there were differences in variance around the average within two colonies. A second measure of immune function - fat body mass - showed a stronger relationship with level of polyandry. The average and variance around the average fat body mass differed across colonies with different numbers of patrilines, and also within half of the colonies. Ongoing study of phenoloxidase activity, microbial-killing ability, and morphometrics may elucidate a mechanistic explanation for the findings of previous studies, which support the polyandry versus parasitism hypothesis.

27-12 IMPLICATIONS OF EUSOCIAL LIFESTYLE ON NUMBER OF IMMUNE GENES IN THE HYMENOPTERAN

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The *Apis mellifera* honeybee genome was previously reported to contain approximately one third as many immune genes compared to the fruit fly *Drosophila melanogaster* and the mosquito *Anopheles gambiae*. It was hypothesized that the evolution of sheltered social living in colonies may have lead to this reduced number of immune genes. The availability of three *Nasonia* jewel wasp genomes allows further insight into this idea, because these solitary hymenopteran parasitoids are likely to experience pathogen pressures similar to the solitary ancestors of eusocial hymenopteran lineages. We examined the conservation and diversification of immune gene families, comparing the *Apis* and *Nasonia* genomes which diverged ca. 150 mya and using the *Drosophila* and *Anopheles* genomes that diverged ca. 250 mya as non-hymenopteran outgroups. Our analysis thus provides new information to the evolution of immune genes in the Hymenoptera, and also revisits the implications of eusociality for innate immunity.

Oral Presentations

Parasites in social insects

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PATHOGENS INFECTING THE RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA*: BIOLOGY OF NEWLY DISCOVERED *S. INVICTA* VIRUSES

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Solenopsis invicta is a polymorphic ant species that serves as host to at least 30 parasites and pathogens. Most of these organisms are absent in areas where *S. invicta* has been introduced, like the United States. A brief overview of the parasites and pathogens of *S. invicta* will be presented with emphasis on the biology of three viruses discovered recently from *Solenopsis invicta* using a metagenomics approach. All of the viruses possess a single-stranded RNA genome, but each possesses a unique genome sequence, structure, and architecture. Two of the viruses appear to cause persistent, asymptomatic infections in the ant host, while the third is highly virulent, resulting in significant colony mortality. Heterologous expression is being employed to mass produce the virulent virus for development as a microbial control agent.

GENOMIC APPROACHES TO HONEY BEE HOST-PARASITE INTERACTIONS

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Honey bees, *Apis mellifera*, face considerable threats from parasites and pathogens ranging from viruses to vertebrates. Genomic techniques can be used as prospective tools by identifying novel or unsuspected pathogens or by identifying honey bee proteins whose genes are activated in response to a disease threat. These techniques also provide an increasingly useful tool for experimental studies of host-parasite interactions, since both honey bees and their disease agents can be queried for genetic changes after infection or after other changes in the environment faced by individual bees or their environment. Here we discuss efforts to define genetic traits of bees and their pathogens through large-scale sequencing. These efforts have targeted causes for the enigmatic `Colony Collapse Disorder' in honey bees and we will show evidence for changing abundances of several viruses and a microsporidian parasite, as revealed by 454 and ILLUMINA next-generation sequencing. We also show how a genomic understanding of bee disease agents ranging from bacteria to mites can point to pathways important for their exploitation of bees, and help identify targets for the control of these organisms. Comparative genomic efforts across insects also provide insights into bee responses to disease and the genetic variation of immune systems.

SEASONALITY OF PREDICTIVE PATHOGEN MARKERS FOR HONEY BEE COLONY COLLAPSE

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Over the last years, high winter losses of managed honey bee, *Apis mellifera*, colonies have been reported from the Northern hemisphere but the underlying reasons remain partly understood. Pathogens can cause colony collapse either alone or when they interact synergistically, thereby creating demand for diagnostics at the colony level. So far, comparisons in pathogen loads have been conducted between dying and healthy control colonies, but long term monitoring appears crucial, especially because pathogens causing colony death may have disappeared leaving room for secondary infections of the weakened colonies. Here we aim to identify predictive markers for colony death over the seasons. Samples were taken from 29 queenright *A. m. carnica* colonies of similar strength in summer, fall and winter 2007/2008 (N=100 workers each colony each season). Pathogen loads were estimated using bottom board counts (*Varroa destructor*, Vd) or a Sybr green RT-qPCR assay (*Nosema ceranae* (Nc), Deformed Wing Virus (DWV), Black queen cell virus (BQCV)) and compared between colonies, which were alive or dead in spring 2008. Colonies that died showed significant higher infections compared to surviving ones with Vd in autumn and winter, Nc in summer and winter, DWV in fall, but not for BQCV. Our data show that Vd, Nc and DWV can be predictive markers in different seasons. Moreover, seasonality appears crucial for the predictive power of the specific markers suggesting that future studies should be long term. More importantly, the results point towards testable mechanisms for colony losses.

FIRST YOU SEE IT AND THEN YOU DON'T: *WOLBACHIA* INFECTION IN THE FUNGUS-GROWING ANTS

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The intracellular bacterium *Wolbachia* is ubiquitous in insects, modifying the reproductive capabilities of its host and having direct effects on host survival. It has consequently received a great deal of interest as a potential biological control agent, as well as being an important model of host-symbiont interactions due to its potential role in speciation. Social Hymenoptera represent a particularly intriguing group for the study of *Wolbachia*. Female workers are functionally sterile, making them, as well as males, an evolutionary dead end for this maternally inherited symbiont. The effects of *Wolbachia* can also apply at both the level of the individual and of the colony, making the relationship more intricate than in solitary counterparts. The uniqueness of this relationship is demonstrated by the novel findings, unknown in other insects, that some *Wolbachia* strains have specialised on New World ants and that natural curing of *Wolbachia* can occur. Here we investigate host-*Wolbachia* relationships in the fungus-growing ant tribe Attini. We demonstrate that the relationship between *Wolbachia* and the fungus-growing ants is complex and dynamic. Importantly, the infection prevalence is frequently different between closely related taxa. We suggest that infection curing and horizontal transmission have driven epidemics or selective sweeps of *Wolbachia*.

RELATIONSHIP BETWEEN VIRULENCE AND REPELLENCY OF ISOLATES OF ENTOMOPATHOGENS TO THE TERMITE *MACROTERMES MICHAELSENI*

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Termites encounter a diverse array of potentially useful and harmful fungi in their subterranean habitats. These vary from symbiotic to harmful species with varying levels of virulence. How these hemiedaphic insects survive in habitats with infective fungi is not well understood. Possible mediation of olfactory signals in avoiding contact with entomopathogenic fungi has been explored by a number of workers. In the present study, we initially found that *Macrotermes michaelseni* detected a virulent isolate of *Metarhizium anisopliae* from some distance and avoided direct physical contact. We hypothesized that there may be a relationship between virulence and repellency of different isolates of *M. anisopliae* and *Beauveria bassiana* to the termite. We compared these for selected isolates of the two fungi. Positive correlations between the two parameters for both sets of isolates of the fungi were obtained. The results show an interesting co-evolutionary phenomenon in which the termite's response to either *M. anisopliae* or *B. bassiana* is directly related to potential harm these fungi can inflict on the insect and that the virulent strains are more likely to be recognized from some distance and avoided.

HOST POPULATION SIZE MEDIATES PARASITE TRANSMISSION IN A SOCIAL INSECT

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Parasites showing predominantly horizontal transmission are likely to be dependent on the number of available hosts. According to the trade-off model for the evolution of virulence the net reproductive rate of a parasite is, all other factors being equal, positively correlated to the host density. Within colonies of social insects a high density of individuals is usually the case. However, horizontal transmission might be seen as the transfer between colonies. Hence, the density of colonies and the possibilities to enter a different host become important. Using a model system of evolutionary ecology, the bumble bee (*Bombus* spp.) and their intestinal pathogen *Crithidia bombi* (Trypanosomatidae), the relationship of colony density and the prevalence of these parasites is tested. Moreover, quantification of the infection intensity and an analysis of genotype by genotype interactions might allow to extract the importance of host density. Three species of bumble bees (*B. terrestris*, *B. lapidarius* and *B. pascuorum*) were collected on three different field sites. Bumble bee workers and drones were genotyped for sibship reconstruction and analysed for *C. bombi* (prevalence, infection intensity and genotype). A significant relationship of parasite prevalence and colony density has been found. Additionally, high density populations showed a higher prevalence of multiple infections and more different genotypes contributing to these multiple infections. Infection intensity is more likely related to the sex of the individual with males being stronger infected than workers. The differences in male and female biology, especially their flight distances and foraging ranges, might explain this pattern. These aspects of parasite epidemiology might be considered in terms of nature conservation issues directed towards pollinators as the bumble bee.

PARASITES IN ANTS: FROM REGIONAL TO GLOBAL AND BACK THROUGH TIME

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Ants have diversified to become the dominant animal life in all terrestrial biomes. Their ecological supremacy (particularly in rainforests) means they are prominent targets of parasites. The diversity of taxa, castes and ecological niches occupied by ants implies parasites choose among possible hosts. Here I tackle the important question of host range via fieldwork, collections, paleobiology and the comparative method. My focus is on *Cordyceps* fungi in ants, a well known example that adorns the defining book in our field (Schmid-Hempel, 1998). Through fieldwork and collections (particularly at Harvard, Kew, New York, Michigan and the personal collection of Harry Evans) I document the occurrence of 10 species of *Cordyceps* (now renamed *Ophiocordyceps*) across >20 countries on 5 continents. I combine that with long-term studies at the regional level in Thailand, Brazil and USA to show that these fungi are highly specialized both in terms of which hosts are infected (taxa and castes) and what effect they have. Looking into the past I present a 47mya fossil of this host-parasite interaction from the Eocene deposits of Europe. Coupled with this direct evidence approach I use the comparative method to find patterns of host associations and parasite exploitation strategies. This reveals that behaviorally manipulating parasites are concentrated in certain ant taxa- not all ant brains are the same. Returning to a single host-single parasite system I discuss the parasite-environment interface and how mass death of infected hosts in rainforests structures ecological communities; even those within the leaves of tropical trees.

LABOULBENIA INFECTIONS IN *LASIUS NEGLECTUS*: AN INVADING PATHOGEN OF AN INVASIVE ANT

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Invasive species typically have a benefit over native species communities due to `parasite release' during introduction and the lack of adapted parasites and pathogens in the new habitat. The invasive garden ant, *Lasius neglectus*, is a recently described pest ant currently spreading in Europe and Asia. It has now been found that two of its approximately 100 known populations suffer from infections with *Laboulbenia formicarium*, an ectoparasitic fungus hitherto unknown in continental Europe. While the collective and individual immune defences of social insects are in general highly effective in preventing infections of colonies, Laboulbeniales persist in ant populations and most individuals carry an infection. It has thus been assumed that Laboulbeniales have no negative effect on ants, as they also apparently do not interfere with the ant's normal behaviour.We have investigated this newly formed host-parasite system in more detail, by analysing how infection with this rare specialist fungus affects the ants' survival under a) nutritional stress and b) exposure to the common generalist fungus *Metarhizium anisopliae*, which the ants are likely to be exposed to in their new habitats. We also tested a possible effect of *L. formicarium* infection on the immune status and the behavioural competence of *L. neglectus* ants to defend themselves or their group members against disease. We find that *Laboulbenia* is not as harmless as previously considered and analyse the impact of this stable infection on the invasive potential of *L. neglectus*.

MULTI-PHASE FILTERING AND TRANSMISSION OF A MIXED INFECTION IN BUMBLEBEE COLONIES

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Social insects present ideal systems in which to study the transmission of infectious diseases within dense groups of frequently interacting host individuals. Here we focus on mixed-genotype infections, which are increasingly recognized as a predominant type of infection and a driver of pathogen evolution. We analyze the infection and transmission success of various strains of an intestinal Trypanosome parasite co-inoculated into colonies of its bumblebee host. Colonies in the field typically become infected by a variety of strains, and the differential ability of colonies to 'filter' the strains of a mixed infection is thought to be an important determinant of the disease dynamics. We find substantial variation in the 'filtering' ability of workers from different colonies, ranging from broad resistance to most strains of the parasite, to narrow resistance to only a few strains. High strain diversity in workers (but not infection intensity) was associated with a high colony reproductive output and an increased probability of transmission to the new queens, which carry infections to the next season. Interestingly, the representation of the different strains changed dramatically throughout the colony cycle, so that the representation of the different strains in the infected new queens contrasted sharply with their representation in early workers. In all, the shaping of parasite diversity through 'filtering' appeared as a crucial determinant of disease dynamics, overshadowing absolute numbers of infective cells. The discrepancy between the short-term success of strains (as it is usually measured in such experiments) and their longer-term transmission underlines the need to consider host ecology and heterogeneity when investigating the spread of infectious diseases.

Poster Presentations

Parasites in social insects

28

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28-1 Wood ants surround their brood with tree resin

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Social insects have evolved collective defenses to combat pathogens. One preemptive line of defense is the use of plant compounds with anti-microbial activity. We previously showed that wood ants actively collect tree resin, which increases the survival of brood and workers confronted with fungal and bacterial pathogens (Castella *et al.* 2008 *Anim Behav* 75, 1591-1596; Chapuisat *et al.* 2007 *Proc. R. Soc. B* 274, 2013-2017). Here, we further investigated how the ants used and distributed resin within nests. We tested if the quantity of resin collected by *Formica paralugubris* workers depended on the presence of brood and if the resin was preferentially placed near the brood. We also tested if the collection and distribution of resin depended on an experimental infection with the fungal entomopathogen *Beauveria bassiana*. We found that workers brought back more resin when brood was present in the nest. Moreover, they placed most resin near the brood, rather than distributing it equally in all areas of the nest. On the other hand, workers did not increase their resin collection in the presence of the pathogen, nor did they place resin closer to infected pupae or infected sections of the nest compared to uninfected ones. This study shows that the collection of resin depends on the presence of the brood and is targeted at it. It also confirms a prophylactic rather than therapeutic use of this substance. The collective use of resin might thus be an efficient but non-specific way to protect the brood against parasites.

28-2 Ants' survival and waste management in *Myrmica rubra* nests

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Sociality increases risks of disease transmission as genetically related individuals live in a confined environment. Therefore, social insects developed specific defences against pathogens by showing hygienic behaviours such as the rejection of cadavers and other wastes. We investigated if the undertaking and waste management is actually an efficient way of increasing ants' survival and of limiting pathogens transmission in *Myrmica rubra* nests. First, we observed the survival curve of ant colonies that were prevented from rejecting their dead nestmates outside the nest. Colonies that were forced to keep their dead inside the nest showed increased mortality of both adults and larvae in comparison to control nests, confirming that undertaking is an effective way for improving colony's survival. Then, we compared the rejection dynamics of single waste items (ant cadavers, preys and clay pellet) introduced into ant nests. Clay pellets were quickly rejected within 12 ± 4 minutes, cadavers were dumped outside after 139 ± 28 min and preys were eaten and rejected only after 17.3 ± 0.3 hours. Spatial localisation inside the nest also differs between different items, as most cadavers stayed far from ants' aggregate and larvae while preys were long-eaten close to larvae and inside ants' aggregate. These results show that cadavers and preys are managed differently before becoming contagious, thus preventing effectively pathogens transmission inside the nest.

28-3 Parasite resistance in the bumble bee *Bombus terrestris* at the molecular level

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Host-parasite interactions are characterised by intensive arms race on both sites. Parasites might increase their virulence in order to exploit hosts efficiently which might be counteracted by activation of the hosts immune system. Insect hosts attack parasites by means of their innate immune system. Parasite related factors (e.g. antigenic peptides, carbohydrates and lipids) switch on the innate immune system by up- or down-regulation of immune related pathways (Toll, IDM, JNK and JAK/STAT). These result in an anti-parasite response via melanization, proteasome-dependent degradation, apoptosis, expression of antimicrobial peptides (AMPs) and cytotoxic enzymes. Different components of the hosts immune response might act as short-term clearance or as long-lasting protection. In order to study short term activation of immune system components we used the bumble bee *Bombus terrestris* and the gram-negative bacterium *E. coli* as a model system. The gene expression patterns of eight immune system genes representing all known immune pathways was measured continuously during the first 24 h post infection. Expression patterns were analysed relatively to several housekeeping genes, a non-injected and a sterile injected control group. The expression levels of the AMPs abaecin, defensin and hymenoptaecin showed an up-regulation in both, *E.coli* and sterile injected bees, within the first minutes up to hours post infection. Significant effects of bacterial injection vs sterile injections on AMP expression were detected after 4-8 hours, but were not linked to a significant decrease in bacterial titres. A multiple regression analysis of immune pathway related genes and expression of AMPs indicated connection between IMD-pathway activation and both, sterile and non-sterile injections. This suggests an adaptation of AMP expression towards any wounding, which, under natural conditions will rarely be sterile.

28-4 Impact of genetic diversity on disease resistance during colony founding in a socially polymorphic ant

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Polygyny (the presence of multiple queens in the same colony) increases genetic diversity within the colony but decreases relatedness among colony members. The inclusive fitness cost due to a reduction in relatedness may be mitigated by a higher disease resistance in colonies with increased genetic diversity. We evaluated this hypothesis in the context of colony founding by queens of the socially polymorphic ant *Formica selysi*. We compared the disease resistance of workers produced by either one or two mated young queens in incipient colonies. We also evaluated the resistance of workers in queenless experimental groups with various levels of diversity (one, two and four matrilines). All ants used in these experiments were reared in the laboratory, ensuring that they were of similar age and had been exposed to the same environment. The workers were challenged with the entomopathogenic fungus *Beauveria bassiana*. We found no difference in worker resistance between one-queen and two-queen incipient colonies of similar size, nor between the experimental groups of various diversity levels. In contrast, we found evidence that body size and group size were positively correlated with disease resistance. Furthermore, the survival of workers was lower when the queens were present, which might result from higher energy consumption or from workers preferentially directing their cleaning behavior towards the queens. Overall, we found no evidence that higher genetic diversity increased the resistance of *F. selysi* workers to *B. bassiana* during colony founding, but two-queen incipient colonies may still be more resistant because they tend to produce more workers during the first months of colony-founding. It should also be noted that the impact of group diversity in the field may depend on variation in environmental or social factors that are absent in the laboratory setting, such as past exposure to parasites, demography or parasite diversity.

28-5 EASIER LIFE FOR HONEY BEE PATHOLOGISTS: A NOVEL METHOD FOR ORAL INFECTION OF INDIVIDUAL WORKERS

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Honey bee pathology often requires controlled individual oral infection of workers to ensure that each bee receives the same amount of pathogens. The conventional methods are quite labour intensive and time consuming, thereby often compromising sample sizes. Here we present a fast, less labour-intense and cheap novel method, which allows for large sample sizes in honey bee pathology. Freshly emerged workers were individually transferred into standard 1.5 ml Eppendorf reaction tubes. The tubes were then inserted with the lid side into racks and fixed. The tips of the Eppendorf tubes were cut to provide air and an opening of about 0.4 mm. Then, the racks were stored at RT for 2 hours to starve the workers. Each worker was then fed with 5 µl of 50% sugar solution containing a standardised amount of the pathogen(s) (e.g. a Nosema spore solution with 2 x 105 spores). To ensure that the sugar water is consumed and not post hoc exchanged between workers via trophallaxis, the racks were stored 30 minutes. Finally, the tubes were opened and workers transferred into standard hoarding cages to monitor worker mortality and infection progress.

28-6 FACTORS INFLUENCING INFECTION RATES OF THE PARASITE *NOSEMA BOMBI* IN NATURAL POPULATIONS OF THE BUMBLEBEE *BOMBUS TERRESTRIS*

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One of the parasites which can seriously reduce the fitness of bumblebee colonies is *Nosema bombi*, an obligate intracellular parasite belonging to the microsporidia (Otti & Schmid-Hempel 2007). Given the importance of bumblebees as pollinators in natural as well as agricultural ecosystems and for the pollination of greenhouse crops (Goulson et al. 2003, Banda & Paxton 1991, van Doorn & Velthuis 2004), *N. bombi* can thus have a great ecological and economic impact. The aim of this study was to collect first data on potential factors influencing the prevalence and severeness of *N. bombi* infections in natural populations of *B. terrestris*. To do so we collected bumblebees (males and workers) from six populations in Middle Sweden. The sampling sites were either characterized by organic farming without any use of pesticides, or conventional farming with regular pesticide application. We determined the *N. bombi* prevalence in the populations and degree of infection in all collected individuals by counting *N. bombi* spore loads. Further, to be able to infer the infection rate not only at the individual, but also on the colony level, we genotyped all individuals with five microsatellite markers. Using the software “Colony 1.3” these genotypes were then used to reconstruct colony identity of the sampled individuals. Based on these results we will discuss the influence of land use type (organic vs. conventional) and the impact of the sex of an individual bumblebee on the rate and severeness of *N. bombi* infections.

28-7 Specialized pathogenic fungi from the genus *Pandora* (Entomophthorales) and their capacity to manipulate *Formica* ant hosts

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Insect pathogenic fungi from the ancient order Entomophthorales induce behavioural changes in infected host that can often be inferred from the specific localities of dead or dying hosts. Moribund individuals normally move towards the top of a vegetation structure such as a grass stem where they become fixed to express `extended' phenotypes. The exposed cadavers produce conidiophores that actively project conidial spores for horizontal transmission to new hosts. Most known species of Entomophthorales are obligate pathogens of insects and other arthropods, including Hemiptera, Diptera, Lepidoptera, Coleoptera and Hymenoptera. Only one example is known where eusocial Hymenoptera are natural hosts: *Pandora formicae* (= myrmecophaga) infecting ants of the genus *Formica*. Here we report first observations on field distributions of *P. formicae* in Denmark, variation in key phenotypic traits, host specificity and phylogeography. Our results indicate that there may be a strong relationship between *Pandora* genotype and *Formica* host species.

28-8 The prevalence of a nematode parasite and the reproductive success of *Bombus terrestris* from three populations in Europe

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Parasites of social insects can have a considerable impact on the fitness of their hosts. The nematode parasite, *Sphaerularia bombi*, infects hibernating bumble bee queens, and castrates them, preventing them from founding colonies. Previous studies of this host-parasite interaction have examined the prevalence of the parasite in various bumble bee (*Bombus*) species and at various individual sites. Parasite prevalence should influence both parasite impact and host resistance. However, the impact of *S. bombi* has rarely been quantitatively measured, although some host species have been reported to exhibit resistance to castration by the nematode. In addition to the impact on individual queens, the parasite may also impact the population if prevalence levels are high enough in comparison to other sources of mortality. *Bombus terrestris*, a common and widespread species, is known to be a major host of *S. bombi*. Here I use it to examine the prevalence and impact of *S. bombi*, at both the individual and population levels and across three European populations. Irish, UK and Swiss B. terrestris populations are believed to differ in parasite prevalence. Using queens collected from these populations in 2010, and maintained in captivity, I will report the prevalence, infection intensity and impact of the parasite on the host, at both the individual and population levels. These data will be interpreted with respect to variation in parasite prevalence.

28-9 PARASITE VIRULENCE AND HOST RESISTANCE IN A GENERALIST NEMATODE PARASITE OF BUMBLE BEE QUEENS

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Our understanding of parasites in social insects revolves around studies of single-species interactions. However, many parasites use multiple host species, and in such situations it is unclear what our expectations should be for parasite virulence and host resistance across host species. Preliminary theoretical models suggest that transmission routes and host quality should determine how virulence and resistance evolves. However, hardly any empirical work has examined these predictions. Bumble bees have become the main study system for studying the evolutionary ecology of parasites in social insects, and many of their parasites infect multiple host species. This makes them an excellent system in which to examine the complexities of multiple-host parasites. Using the nematode parasite *Sphaerularia bombi*, which infects bumble bee queens, we have combined field observations and controlled laboratory experiments to determine parasite virulence, host resistance, and how they vary across host species. We collected bumble bee queens of 6 different species across two years in Dublin, Ireland. These queens were brought back to the lab where we recorded parasite virulence (the impact on host longevity and reproduction), parasite fitness (reproductive output) and host resistance (the inverse of virulence). Using a common garden background (commercial colonies of *Bombus terrestris*), we conducted laboratory experiments to determine the virulence and fitness of parasites taken from different host species. Our results suggest that the nematode impacts species differentially, and that this depends upon host life-history. We discuss these results with reference to bumble bee ecology and multiple-host theory.

28-10 A QUANTITATIVE MODEL OF HONEY BEE COLONY POPULATION DYNAMICS

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Mature honey bee colonies normally maintain a relatively stable population size, but recently there have been widespread reports of bee colonies exhibiting rapid and catastrophic population decline to the point of colony death. This has been called colony collapse disorder. As an aid to testing hypotheses for the causes of colony failure we have developed a compartment model of honey bee colony population dynamics using established principles of honey bee population structure and behavioural development. We used the model to explore the impact of different death rates of forager bees on colony growth and development. The model predicted a critical threshold forager death rate beneath which colonies regulate a stable population size, but if death rates are sustained higher than this threshold, rapid population decline was predicted and colony failure was inevitable. The model also predicted that high forager death rates drew hive bees into the foraging population at much younger ages than normal, which acted to accelerate colony failure. The model suggests that colony failure can be understood in terms of observed principles of honey bee population dynamics, and provides a theoretical framework for experimental investigation of the problem of honey bee colony collapse.

28-11 COLONY LEVEL COSTS OF SEQUENTIAL EXPOSURE OF ANT SOCIETIES TO DIFFERENT PATHOGENS

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Similar to long-lived individual organisms, also long-lived societies face the problem of multiple infections with parasites and pathogens. Exposure to multiple parasites can occur simultaneously or sequentially, both within individuals and within colonies (between individuals). Here we report on the colony level effects of sequential infections of colonies of the invasive garden ant, *Lasius neglectus*, with different fungal pathogens. Infections of these types are highly likely to occur in nature, as spores of the studied entomopathogenic fungi are common in the soil surrounding ant nests, as well as in form of sporulating insect cadavers. Spores of different fungi can thus be easily picked up by foragers of the colony. We studied the susceptibility of ant workers in the presence of multiple pathogens in the nest, and found that colonies pay an unexpectedly high cost of these multiple infections.

28-12 CHARACTERISTICS OF HONEY BEE COLONIES (*APIS MELLIFERA*) IN SWEDEN SURVIVING *VARROA DESTRUCTOR* MITE INFESTATION

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A population of European honey bees (*Apis mellifera*) surviving *Varroa destructor* mite infestation in Sweden for over ten years without mite control treatment, demonstrate that a balanced host-parasite relationship may evolve over time through a natural selection process. Colony level traits linked to *Varroa* tolerance were investigated in this population to identify any possible characteristics that may be responsible for colony survival in spite of mite infestations. Hygienic behaviour measured as the brood removal rate, grooming behaviour measured as the proportion of damaged mites in colony debris, and brood attractivity determined as the mite distribution between brood and adult bees, were not significantly different in the untreated surviving population compared to control colonies. However, reduced colony size as an adaptation of this population as well as the suppression of mite reproductive success, were significant in the surviving colonies. Our data suggest that colony level adaptive traits may limit mite population growth by reducing mite reproduction opportunities (reduced colony size) and by suppressing the mite reproductive success. Virus infection dynamics in these surviving colonies compared to control colonies will be discussed, as well as preliminary results that compare and contrast mite reproduction data in a population of surviving honey bee colonies in France with the surviving population in Sweden. The data suggests that the selection parameters influencing the development of this trait and the mechanisms behind it may be different in different population surviving mite infestation.

28-13 Relationship between nest size of the hornet *Vespa analis* and prevalence of its parasite *Xenos moutoni*

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An intriguing question concerning the interactions between social insects and their parasites is how and to what extent nest size is related to the prevalence of parasitism (=proportion of parasitized individuals). We explored this problem in the hornet *Vespa analis* and its strepsipteran parasite *Xenos moutoni*. First instar larvae *X. moutoni* sneak into nests of the hornet by attaching themselves to foraging hornets and enter larval bodies of the host. The parasite do not kill but inactivates workers, thus is expected to affect colony performance or nest size. On the other hand, nest size is possibly related with defensive potential of colony or other characteristics that affect parasite prevalence. In our three-year sample of *V. analis* nests collected in Nagoya, central Japan, 36 to 48% nests contained more than one parasitized adult hornet. The number of cells did not differ between nests with and without parasitized workers. Therefore, the effects of parasite-caused losses within the worker force on nest size appear to be negligible. However, the prevalence of parasitized workers tended to decrease with an increase in the number of cells. Our analysis indicated that the number of parasitized workers was consistently low and did not increase with nest size, resulting in a relatively high prevalence in small nests. We then concluded that higher parasite prevalence in smaller nests is a result, rather than a cause, of small size.

28-14 Preserving pollinator health in East Africa

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Honey bee populations worldwide have been decimated by the introduction of *Varroa mites*, yet efforts to control them have had limited success. Kenya represents a unique opportunity to investigate the bases for the resistance of African races of bees to diseases and pests that have plagued managed honey bees throughout the rest of the world. We have used molecular, chemical, and behavioral approaches to fully characterize the impacts of *Varroa* on honey bee populations in East Africa, and any existing mechanisms of bee resistance. We will use this new knowledge to develop protocols for sustainable – nonchemical - beekeeping practices, thus minimizing the impacts of *Varroa* on this ecologically and agriculturally critical species. Our specific aims are 1) to determine the geographic distribution , *Varroa* and disease loads, and behavioral characteristics of the four *Apis mellifera* subspecies in East Africa, 2) to characterize the impact of *Varroa* infestation and honey bee behavioral traits on colony health and productivity and 3) to develop and implement a short course to disseminate the results of our research and, if *Varroa* control is necessary, train African beekeepers on non-chemical *Varroa* management techniques.

28-15 Temporal variation of parasitism in bumblebees (*Bombus* spp.) by *Crithidia bombi* (Trypanosomatidae) on a local scale

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Annual colonies of social insects show extreme differences in the number of individuals during the year. This population dynamics might influence the host-parasite interactions, especially for horizontally transmitted parasites. Throughout the season, the increasing number of workers in colonies in a population leads to increased contacts and, as a consequence, to the spread of parasites. To analyze this, we use an established model system in evolutionary biology - bumblebees (*Bombus* spp.) and their gut parasites *C. bombi*. The prevalence of the parasite in field caught bumblebees was analyzed in the course of one season. Moreover, the parasites were genotyped, in order to analyze the occurrence of multiple infections and changes in genotype frequencies. General parasitic prevalence increases from June, being highest in July and then decreases again in August in two different species, *B. terrestris* and *B. lapidarius*. A similar pattern was found in the case of multiple infections. Analog to this, single infections decreased from June, being lowest in July and then increased later in the year. Several clones of *C. bombi* appeared twice during the year, but could not be detected throughout the whole season. In July, when the population density is at its maximum, prevalence and number of multiple infections is also at highest level. During this period selection on parasite genotypes occurs, as we observed significant changes in allele frequencies. Most likely within host competition between parasite genotypes would explain these observed patterns.

28-16 Overview of USDA research on colony collapse disorder in honey bees and funding opportunities

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Managed honey bees in the world are suffering major losses. In 2006, the phenomenon known as Colony Collapse Disorder (CCD), became a significant threat to the pollination industry in the USA and several other countries. CCD continues to be of paramount concern and honey bee losses have increased steadily over the past 3 years. Several fruit, nut and vegetable crops depend on pollination services by honey bees. CCD and other issues impacting bees have raised the costs of pollination services to growers. Also at risk, are wide spread crop losses, which could threaten global food security. The US Department of Agriculture has played a key role in developing intramural and extramural programs for fundamental and applied research relevant to honey bee health. Important new discoveries have been made, and will potentially offer novel solutions to mitigate CCD. The leading research programs will be highlighted in this presentation. In addition, USDA investments in current and future competitive research will be reviewed.

28-17 GENETIC VARIATION IN RESISTANCE TO *NOSEMA* PARASITES IN THE EUROPEAN HONEYBEE *APIS MELLIFERA*

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Since the widely reported population declines of the European honeybee *Apis mellifera* in America there has been increased public interest and funding for research, in order to assess possible causes of the decline. *A. mellifera* is also of interest as a model system, as it provides an excellent semi-natural system for parasite studies, allowing biologically relevant interactions, transmission modes and doses to be investigated. *A. mellifera* is known to host a wide range of parasites, including two microsporidia. The originally described *Nosema apis*, a parasite known to decrease colony productivity and in severe cases longevity, and the more recently described *Nosema ceranae*, thought to have jumped from its original host, *Apis ceranae* the Asian honeybee. *N. ceranae* has been reported as being more virulent than its sister species, and has been linked to colony losses in Spain. Understanding the host-parasite dynamics of infections is crucial to detangling the pathology and control of both species. Honeybees are known to be highly polyandrous and therefore have genetically diverse colonies, increasing the potential for within colony variation in resistance. However, heritable resistance to *Nosema* has yet to be fully investigated. Here we investigate whether there is genetic variation in honeybees to *Nosema apis* and *Nosema ceranae*. We used hand-dosing of individual workers to carefully control the precise numbers of parasite spores that bees ingested, then determined individual survival, infection prevalence and intensity using both spore counts and quantitative real-time PCR, before genotyping in order to assess whether infection status is determined by patriline. The results could give insight into breeding of honeybee lines more resistant to current and emerging pathogens. They also aid our understanding of variation in virulence and susceptibly between colonies, parasite species and populations.

28-18 NEMATODE-PARASITIZED HORNETS VISIT OVERWINTERING SITES IN SUMMER TO RELEASE JUVENILE PARASITES FOR TRANSMISSION

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The nematode *Sphaerularia vespae* was recently found in the hornet *Vespa simillima* and described as the second species of the genus *Sphaerularia*. It parasitizes only hornet gynes (potential queens) and deprives them of fertility. To find the infection route of the nematode, we observed the behaviors of overwintered *V. simillima* gynes that were parasitized by the nematode and new gynes that left their nest for overwintering. We found that the parasitized gynes frequently visited decayed wood in summer (late June to mid-August), probably to seek potential hibernacula instead of building nests. The parasitized gynes visiting decayed wood on the forest floor walked on the surface of the wood and/or entered holes made by beetles. Typically, parasitized gynes repeatedly visited the same overwintering sites. We also confirmed that clumped juvenile nematodes were released from the tip of the gyne's abdomen and transferred to the surface or inner wall of decayed wood. The timing of expression of this host behavior corresponded well with the hatching of nematode eggs in the body of hosts. Yet another finding was that new gynes visited the decayed wood where nematodes had been released and entered the wood for overwintering in autumn (mid-September to late October). In early winter, we collected several overwintering gynes in the decayed wood and found that most of them were infected with the nematode. These results strongly suggests that the parasitic nematodes manipulate its host to visit decayed wood, which is a preferred overwintering site of the hornet for its own transmission, and that transmission of the parasite occurs in the wood. The parasitized and new gynes showed similar behavior on decayed wood; this suggests that the parasite could induce pre-existing host behavior such as the quest for an overwintering site rather than cause aberrant behavior of the hosts.

28-19 Links between *Varroa destructor* infestation and honeybee immunity

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Varroa destructor is an external parasite of the honeybee (*Apis mellifera*) and is the cause of the decimation of colonies, which is a worldwide phenomenon. During the last decade, the social immunity of the honeybee was extensively investigated demonstrating that hygienic behaviour is a key factor helping colonies in the fight against varroasis. In contrast, little is known about the role of individual immunity against varroasis. However, there is much evidence of its implication in the struggle against the disease: *Varroa destructor* synthesizes immunosuppressive protein in saliva, uses molecular mimicry to escape the immune response and transmits viruses which also disrupt honeybee immunity. Taken together, these mechanisms indicate that *V. destructor* faces an immune response which impinges on its lifecycle. The aim of this work is to assess the link between the immune competences of honeybees at the individual level and the degree of infestation of the colony. For this purpose, foragers from nine Varroa tolerant colonies from an apiary untreated against *V. destructor* and naturally infested at different levels (ranging from 0.29 to 6.5 %) were examined for several parameters including body mass, hygienic behaviour, antimicrobial peptide, enzymatic, hormonal and antioxidant transcription levels and enzymatic and antimicrobial activities. The results showed that the weight of the foragers is positively correlated with the infestation level. In contrast, transcription levels of hymenoptaecin, glucose deshydrogenase, phenol oxidase and microsomal glutathione S-transferase and antimicrobial activity against *Escherichia coli* are negatively correlated with the mite level found in colonies. Overall, these results indicated that less infested colonies involved light weight bees with higher immune competences comparing to more infested ones. These results are of great interest in the development of prophylactic strategies against varroasis.

28-20 Genetic diversity in a trypanosome parasite infecting key pollinators

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Pollinators, such as bumblebees, are crucial for the functioning of natural ecosystems and also for agriculture. However recently, there has been an observed decline of managed and non-managed pollinators. This has, in part, been attributed to parasites. Among the bumblebee parasites, *Crithidia bombi* is a highly prevalent gut parasite of *Bombus* spp. Through its transmission via faeces *C. bombi* spreads either in the nest within the colony or on flowers from colony to colony and from species to species. *C. bombi* is a flagellated protozoa that belongs to the order of Trypanosomatidae. This order contains parasites that have been intensively studied as they cause serious diseases in humans, crops and domestic animals. Several of these parasites show genetic peculiarities and some have been reported to undergo genetic exchange. Nevertheless, in most of the Trypanosoma parasites, not much is known about the population dynamics of the parasite in its host.Genetic markers and the ability to clone *C. bombi* give us an overview of the population structure of *C. bombi* from distinct geographic areas with different ecological background. We show that the flagellate occurs almost globally, and seems to be restricted to bumblebees. Further we examine the species differentiation and the evolution of multiple infections, phenomena that have important implications for genetic diversity, and could influence host-parasite evolution.

28-21 VARIATION IN HONEYBEE MORTALITY INDUCED BY PHYLOGENETICALLY DIFFERENT CHALKBROOD (*ASCOSPHAERA APIS*) STRAINS

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Antagonistic coevolution between pathogens and hosts requires genetic variation for both pathogen specificity and host susceptibility. *Ascosphaera apis* is the causative agent of chalkbrood disease, a major pathogen in honeybees that reduces brood number and honey production. Genetic variation for honeybee susceptibility to chalkbrood has recently been demonstrated, but corresponding genetic variation for chalkbrood virulence has not been previously studied. We used four *A. apis* strains from two phylogenetically distinct clades to infect honeybee larvae from three hives with naturally mated queens. We found that chalkbrood strain and larval colony origin both affected mortality rates after infection. The two chalkbrood strains from one clade caused relatively low host mortality (12% and 14%), whereas the two strains from the second clade induced much higher larval mortality rates (71% and 92%). In addition, larvae from one of the three hives showed significantly higher susceptibility to chalkbrood infections compared to the other two hives. Our results demonstrate genetic variation between strains for virulence, which may together with genetic variation in host susceptibility drive coevolutionary dynamics between pathogen and host.

28-22 DIVERSITY AND PATHOGENESIS OF ASCOSPHAERACEOUS FUNGI ASSOCIATED WITH CAVITY NESTING MEGACHILIDS AND *APIS MELLIFERA*

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The Ascosphaeraceae (Onygenales) is an ascomycetous family of only three genera, two of which are monotypic. The diversity of the family lies within *Ascosphaera*, a genus of less than thirty species found only in association with bees. Ascosphaeraceous fungi are pathogens of bee larvae, infecting their host through the gut, or are saprophytic on provisions and/or nesting material. We examine the diversity and intraspecific genetic variation of these fungi in association with cavity nesting solitary bees and *Apis mellifera*. We also look at the role host plant pollen might play in chemically protecting oligolectic pollinators against pathogenic Ascosphaerae.

28-23 EFFECT OF VIRULENCE AND ODOR OF *METARHIZIUM ANISOPLIAE* ON THE RESISTANT-BEHAVIORS OF THE TERMITE, *COPTOTERMES FORMOSANUS*

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Entomopathogen-resistant behaviors of termites are known as commonly as other social insects, and often account for the difficulty in controlling termites by fungi. We studied the behavioral response of *Coptotermes formosanus* to an entomopathogenic fungus, *Metarhizium anisopliae* with different levels of virulence for better understanding of the disease-resistant nature of termites. When fungus-uninfected termites were allowed to contact fungus-infected nestmates at a mixing ratio of 4:1, the frequency of grooming was higher than that of control assay group of termites free of fungal infection. This was particularly prominent with the higher virulent fungi. Another series of experiments were conducted at the same mixing ratio to demonstrate the ability of termites in the detection of foreign organisms, using termites treated with solutions of odor substances from conidia. The results showed the close relation between fungal virulence and attachment ability of conidia to termites, the high ability of termites in detecting fungus-infected nestmates, and of the importance of olfactory cues in pathogen-induced behavior (e.g. attack, grooming or burial).

Oral Presentations

The living past of insect sociobiology

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A BRIEF HISTORY OF DISTRIBUTED DECISION-MAKING IN SOCIAL INSECTS

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My talk will review the relatively recent introduction and great success of the modern concept of distributed decision-making in social insects and how this has replaced a more mystical view of how societies actually make choices. In this way, I will both sample the quirky treasure trove of the historic literature and show how massive progress has been made through the use of beautiful experimental designs, cutting-edge technologies and pioneering analyses. Hard science has made mysticism retreat to the shadows but this “unweaving of the rainbow” should only sharpen our appetite for further wonder.

LASIOGLOSSUM, CHARLES MICHENER, AND SOCIAL BEHAVIOR IN BEES

Michael Breed

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Much of current thought about the evolution of eusociality and the role of primitively eusocial species in illuminating our understanding of the cusp between solitary and eusocial life first bloomed in the 1970’s. The laboratory of Charles Michener served as an incubator for a generation of scientists, for empirical studies of primitively eusocial Hymenoptera, and for ideas that made Lawrence, Kansas, a hub for the nascent field of sociobiology. Charles Michener has characteristics as a mentor that we would all do well to emulate. These include patience and understanding, but also high standards and unwavering integrity. The laboratory was more driven by shared curiosity than by competition, a positive atmosphere that was enhanced by his mentoring. *Lasioglossum* bees serve as an excellent model system, providing windows into the evolution of colonial life, reproductive division of labor, and division of labor among workers. By championing work on *Lasioglossum*, Michener fostered an appreciation for the value of studying the less obvious eusocial species. *Polistes* was also an important part of the mix at Kansas at that time, and studies of *Polistes* broadened the perspective of the laboratory. The influence of William Hamilton’s ideas were strongly felt, and led to insights that details of mechanisms, such as reproductive suppression and nestmate recognition, could be used to test larger evolutionary theories. Trivers’ key papers, published during that decade, provided a strong impetus for discussion. The presence of other scientists in Lawrence with research foci on eusocial insects, particularly Taylor, Jander, and Stockhammer, brought important perspectives to the the work on primitively eusocial insects. In sum, a discussion of *Lasioglossum* and the influence of the Michener laboratory on the present-day study of eusocial organisms is as much about models of how scientific ideas and scientists are fostered and tested as it is about specific findings.

THE GROWTH IN UNDERSTANDING OF THE BIOLOGY OF THE SWARM-FOUNDING WASPS

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The swarm-founding wasps will be defined and placed taxonomically. They are worthy of special attention for several reasons: they are ecologically the most dominant social wasps; they are speciose, especially in the New World; and their colony organization has achieved a level of complexity substantially greater than that of independent-founding social vespids such as *Polistes* and the vespines. Study of this group has lagged behind work on the independent founders for two main reasons. First, they are largely tropical in distribution, and second, their nesting habits make access to behavior more difficult. An acceleration in work on them is likewise due to two factors: travel to the tropics has become easier in recent decades and technological advancements have facilitated study of these large and complex groups. I will highlight the key players in the study of this group, from the nineteenth century up to the present.

PERCEPTION OF INSECT SOCIOBIOLOGY IN THE COUNTRY OF LAMARCK

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France was a special case concerning the perception of insect sociobiology, influenced by the perception of general sociobiology, which early became a taboo under the influence of at least four cultural features. First, concerning the question of determinism, there is a strong tradition, in France, to pay more attention to proximate than to ultimate causes, to the 'how' than to the 'why', to mechanisms than to adaptive correlations. Several historically famous French biologists illustrate this tradition like Georges Cuvier, Claude Bernard, René-Antoine Ferchault de Réaumur, or the self-taught Jean-Henri Fabre. This cultural tradition was related to the reluctance for speculative aspects of evolutionary theories in general and for sociobiology, perceived as a by-product of Darwinism. Second, there was in France, during the Lyssenko episode and after, a convergence of interest between influent Catholic and Marxist intellectuals (even biologists) to try to disqualify Darwinism; the former (e. g. Pierre-Paul Grassé or Rémy Chauvin) because there were opposed to natural selection (Nature is too perfect to result from chance), and the latter because they opposed the pertinence of genes. From both sides they instrumented the political dispute about sociobiology in the US in order to demonize Darwinian ideas and slow down their penetration in France. The third specific feature was a strange reluctance of French ecologists for ethology (including prominent population geneticists who accepted the role of genes for everything except behavior). Last but not least is the French traditional posture to mistrust the so-called 'anglo-saxon' influence and to maintain a so-called 'cultural exception'. All these French specificities made easier to reject the sociobiological agenda for at least one generation, more successfully than was the attempt, in 1973, to block the deliverance of the Nobel Prize to Konrad Lorenz in 1973 and to depict ethology as a dangerous science.

FROM BACTERIOPHAGE MOLECULAR BIOLOGY TO WASP SOCIOBIOLOGY - DRIVING AGAINST THE TRAFFIC ON A ONE-WAY STREET

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Modern Science is a strange profession – to survive you need to embrace the current fashion but to be remembered tomorrow you need to be ahead of your times. For a while I led a double life practising fashionable molecular biology as a profession during the week and chasing the then little-known sociobiology as a hobby during the week-ends. Before I was nearly consumed by molecular biology, I managed to drive against the current and embrace animal behaviour and sociobiology as a full-time profession. Having thus had the great fortune of pursuing my hobby as a profession, I have attempted on the one hand to use the Indian paper wasp *Ropalidia marginata* to advance our understanding of the origin and evolution of cooperation and on the other hand to use organismal and evolutionary biology to make the study of the local fauna and flora fashionable. In this talk I will briefly illustrate my joys and frustrations of working in an area which was unfashionable, compounded by the fact I was not formally trained for it. I will also reflect on the importance of and prospects for the sustained study of ecology and organismal biology in India and other similar biodiversity-rich developing countries.

Oral Presentations

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THE EFFECT OF INTER-GROUP COMPETITION ON INTRA-GROUP COOPERATION IN *POLISTES DOMINULUS*

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The balance between cooperation and conflict within colonies of primitively eusocial insects, such as *Polistes dominulus*, is affected by within-group factors, e.g. relatedness and policing by other group members, and extra-group factors, e.g. competition from other nests. We hypothesize that more cooperative groups are better able to compete for resources with other nearby groups, and thus expect that inter-group competition will increase within-group cooperation, as predicted by Reeve & Hölldobler's (2007) game theoretic "nested tug-of-war" model for the emergence of cooperation within groups. In the first explicit test of this model in social insects, we manipulated the distance between early worker-phase *P. dominulus* nests to create two experimental treatments: high competition (nests close together) and low competition (nests far apart). We took video recordings before and after transplanting the nests, and scored cooperative behaviors (e.g. foraging, trophallaxis) and aggression (e.g. darts, lunges). We found that the per capita rate of foraging was significantly higher with decreased nearest neighbor distance, supporting our main prediction. The rate of within-nest aggression was also significantly higher with decreased nearest neighbor distance, contrary to our expectations. We suggest that this increased rate of aggression was a response to the increased probability of encountering non-nestmates at a given nest when nests are in closer proximity, and we are conducting a laboratory experiment to investigate this hypothesis further. By showing when it pays individuals to be cooperative, our results help explain the transition to sociality in insects, and give support to general theoretical principles that underlie cooperative behavior in all taxa, including primate societies.

WHEN TO ACQUIRE NEW INFORMATION? HOW PERSISTENCE AND REWARD AFFECT SAMPLING, TRACKING AND CONSTANCY IN BUMBLEBEES

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Tracking changes in resources requires foraging animals to make decisions on when to sample resources and how to apply the information gained through sampling in future decisions. Theory suggests that the value of sampling depends on the rate of environmental persistence, and on the relative costs of making errant decisions; and that there are many instances in which sampling and using new information is not optimal. We tested these predictions with bumblebees (*Bombus impatiens*), which show robust learning, but also tend to show a well-described constancy in their choices. Using a serial Y-maze offering sucrose solution rewards, we gave bumblebees repeated choices between a resource providing a steady, mediocre reward and a resource fluctuating between a bad reward and a good reward. We varied the persistence of the fluctuating resource and the relative value of the good reward in a 4 x 3 factorial experiment. Bumblebees were not completely constant and did sample, adjusting their behavior to experienced changes in persistence. Bees sampled the fluctuating resource as predicted at moderate values of persistence, but showed surprisingly suboptimal tracking except when the fluctuating resource was very persistent and the potential rewards high. Bees also showed differences in their patterns of choice, for instance, lose-stay choices increased under low persistence and choice constancy as a whole changed with the potential good reward. Thus bumblebees acquire, but do not always use new information.

RESOURCE ALLOCATION DURING COLONY FISSION IN THE ANT *CATAGLYPIS CURSOR*

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Organisms face trade-offs that are likely to constrain their reproductive strategies, in particular the amount of resources they invest in offspring and how they partition resources among offspring. Social insects that reproduce by colony fission are particularly well suited to study these allocation strategies as parental investment and offspring quality are readily measured as the number of individuals allocated to new nests. A colony that fissions may maximise the number of colonies it produces or their size, and it may produce colonies of equal or of varied size. We studied the fission of 19 colonies in the ant *Cataglyphis cursor* in the field. Results show that fissioning colonies were not larger than average but varied noticeably in size (731±456 workers, range 252 to 1,808). They produced a variable number of nests (4.0±1.3, range 2 to 7) which markedly differed in size (183±214 workers, range 30 to 1,284). Larger colonies did not produce more nests but they produced larger nests. This suggests that new colonies benefit from being larger. In addition, there was a tendency for larger colonies to disperse more their propagules. The mother queen was replaced by a daughter queen in 10 of 19 colonies, and daughter queens were produced by thelytokous parthenogenesis (14 colonies), sexual reproduction (4 colonies) or a combination of both (1 colony). Overall, colony fission appears to be more variable in *C. cursor* than in honeybees or army ants, where life-history traits severely constraint the minimal size of colonies. One possible explanation for such variability is that *C. cursor* follows a complex bet-hedging strategy (Olofsson *et al*. 2009 *Proc R Soc B*).

RECLAIMING THE CROWN: QUEEN TO WORKER PUNISHMENT IN THE ANT *APHAENOGASTER COCKERELLI*

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In animal societies, reproductive rights are a central source of conflict. Cheaters in these societies attempt to reproduce on their own and may usurp the position of the established reproductive individual(s). In ants, this usually results in these workers being policed (attacked) by their nestmates, preventing successful reproduction. Direct queen to worker aggression over reproduction or “queen policing” has seldom been reported in ants, and is limited to species which can be characterized by having a low level of queen to worker reproductive dimorphism. In the desert ant *Aphaenogaster cockerelli*, a species with high queen to worker reproductive dimorphism, we show that queens police reproductive daughter workers. Through an analysis of hydrocarbon fertility signals, we demonstrate that reproductively active daughter workers are singled out and attacked. Furthermore, we show by transfer of gland contents that queens use their Dufour's gland contents to mark workers and elicit aggression from nestmates. Analysis of the Dufour's gland contents of mature queens, alate queens, reproductive workers, and nest workers shows the contents of the queen's Dufour's gland to be queen specific. The resulting combined aggression from the queen and the workers she recruits can lead to the death of the cheating worker. The potential reasons why, in a species with a high level of reproductive dimorphism, queens resort to costly physical aggression will be discussed.

Poster Presentations

Open session - Social insect behaviour

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30-1 DEFENCE AGAINST WORKER REPRODUCTIVE PARASITISM IN *APIS MELLIFERA*

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When a honey bee (*Apis*) colony becomes queenless and broodless its only reproductive option is for some of its workers to produce sons before the colony perishes. However, for this to be possible the policing of worker-laid eggs must cease. The cessation of worker-policing renders the queenless colony susceptible to reproductive parasitism by workers from other colonies. Such reproductive parasitism is known to occur in *A. florea*, *A. cerana* and *A. mellifera*. Genetic analysis of worker populations has demonstrated that the proportion of non-natal workers present in *A. mellifera* declines after a colony is made queenless, but the remaining non-natal workers have higher reproductive success than natal workers. We compared rates of nestmate and non-nestmate acceptance in both queenright and queenless *A. mellifera* colonies using standard assays. We showed that queenless colonies of *A. mellifera* differentiate between natal and non-natal workers, while queenright colonies do not. Queenright colonies rejected significantly more non-natal workers from queenright colonies than they did those from queenless colonies, indicating that queenright guards can discern the likelihood that a non-natal worker is a potential reproductive parasite. However, the guards of queenless colonies treated queenright and queenless non-natal workers equally. We suggest that this is because all non-natal workers are potential reproductive parasites in queenless colonies.

30-2 GROUP NAVIGATION LEADS TO INCREASED PATH EFFICIENCY IN THE WOOD ANT *FORMICA RUFA*

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In social insects the mechanisms by which individuals are able to accurately navigate through their environment are relatively well understood. However, many species do not forage alone and individuals therefore have the opportunity to obtain information about the presence and location of food sources from their conspecifics. This is exemplified by the recruitment and orienting properties of the pheromone trails employed by many ant species. Here we present data demonstrating how visual navigation in the wood ant, *F. rufa*, is affected by the presence of other navigating nestmates. Ants were trained to search for a sucrose food source at the base of a black cardboard landmark. In tests where ants searched for the learnt food location in a group their routes were shorter and straighter than the routes they showed when foraging individually. This was the case both when ants were trained individually and tested as a group and the converse; ants were trained in groups and tested as individuals. Interestingly the increase in performance due to being in a group appeared to decline as an individual's level of experience increased.

30-3 DIGGING EFFORT IN LEAF-CUTTING ANT QUEENS AND ITS EFFECTS ON SURVIVAL AND COLONY GROWTH DURING THE CLAUSTRAL PHASE

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Nest foundation in *Atta* leaf-cutting ants is claustral, and the single founding queen completely relies on its body reserves throughout several weeks until the first workers emerge and initiate foraging. Nest digging is very time- and energy-consuming, and it is an open question how queens decide about the length of the tunnel they dig and therefore the depth of the initial chamber. Shallow founding nests may be energetically cheaper to dig, but queens may be more exposed to environmental variables. Deeper nests, on the other hand, may be climatically more stable and suitable, but more expensive to dig. We hypothesized that the maximal nest depth excavated by *Atta* founding queens may represent the outcome of an evolutionary trade-off between maximizing nest depth and minimizing energy expenditure during digging, so as to save energy for the long claustral phase. We tested this hypothesis by comparing the fitness consequences of increased digging effort in queens that were experimentally stimulated to excavate a complete founding nest once, twice or three times consecutively, compared to control queens that did not dig. Fitness was quantified as mortality rates, rates of egg-laying and offspring production, and size of the fungus garden until the emergence of the first workers. Results showed that, in contrast with the initial expectations, fungus growth, oviposition and offspring production were not affected by the increased digging effort in the successive initial excavations. However, a significant increase in mortality was observed in queens that dug two or three nests consecutively. It is argued that a behavioural mechanism for the control of nest depth during nest founding has evolutionary been selected for as a trade-off between maximizing nest depth, to favour protection of the queen against environmental variables, and minimizing energy expenditure during digging, which significantly affects survival.

30-4 THE DAILY ACTIVITY RHYTHM OF A SUBTERRANEAN TERMITE, *RETICULITERMES SPERATUS*

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Almost all organisms, including insects, have circadian timing systems, which generate circadian rhythmicity in their acivity. The rhythmicity usually synchronizes with environmental daily cycles on the earth mainly by natural day-night light change. Most social insects are also exposed to day-night light cycle during foraging trip at outside of the nest. However, some social insect groups, including most of termite species, live independent from natural day-light cycles because they live under soil or within trunk through their whole lives. So we had two questions 'Do they have a daily rhythm in their activity?' 'Given they have a rhythm, how the timings of their works are allocated temporally?' In order to answer these questions, at first, we set up the device to measure automatically an individual behavioural rhythm by infra-red photosensors in the termite *Reticulitermes speratus*. The results showed that the workers had no rhythmicity in their behavioural activity, on the other hand, some allates showed behavioural rhythmicity. Moreover, we measured the rhythmicity of colony activity in field through analyzing the sound which comes from feeding trunk by termite workers. The results showed that the patterns of activity in feeding trunk varied among colonies. These results suggest that the workers of the termite *R. speratus* work around the clock in both individual and colony level. In addition, we report our novel approach to investigate how behavioural activity patterns of an individual termite worker vary when it coexists with other individuals.

30-5 Landmarks characterization in orientation of honey bee males (*Apis mellifera*) in Puerto Rico

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The orientation of the male drones of honey bees (*Apis mellifera* L.) has not been investigated thouroughly. A worker bee has a high probability of disorientation if it moves further than 2.5 Km from its hive. It is important to study the way populations act in order to understand the spatial behavior of organisms. The purpose of this study is to measure the return rate and orientation of drones released at different distances from their hives (1, 2 and 4 Km.), from the four cardinal points (from the apiary). Landscape characteristics of the release sites and the apiary were analyzed using a high definition aerial image (Ikosnos® 2004) for Gurabo (town) to detect any potential land marks that may help drones orient better when released at different locations. Landscape characteristics such as, single trees, line of trees, group of trees, rivers or canals, grasses, tracks or trails, urban areas, topographic aspect and slope; also geomagnetism index were described and digitalized on the study area. The observations were made in the apiary of the "Estación Experimental Agrícola" in Gurabo, Puerto Rico. We captured and marked 1013 drones (120 per distance per cardinal point) and observed the number of drones that returned to their hives after being released. It was expected that more drones would return from the closer releasing sites but not to find differences between the orientations of release. We found a statistically significant difference for rate of return at different release distances ($F=4.2213$, $df=2$, $P=0.0149$), but not for the cardinal point of release ($F=0.7910$, $df=3$, $P=0.4989$). In a non-metric multidimensional scale (NMS), Axis 1 explained 74.8 percent of the variation of data that show the origin of drones and was correlated with magnetism, while Axis 2 explained 48.2 percent and was correlated with aspect.

30-6 Where are the drone congregation areas of the honey bee *Apis mellifera*?

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Drone Congregation Areas (DCA's) of honey bees are places where males and queens meet to mate. Honey bees in Puerto Rico have a wide distribution, we hypothesized that many of the places appropriate to serve as a DCA, would be used as a DCA. Here we describe physical characteristics of areas where DCA's were found using an easy method: sexual queen pheromone, a helium balloon, and satellite image analysis. We found eight DCA's in 36 candidate areas with characteristics thought to be important based on previous studies. Physical characteristics of the landscape are compared across candidate areas found to be with or without a DCA. We discuss how aspect or compass direction from high to low of an area, trails or other linear markers, land cover, and winds influence the presence/absence of a DCA.

30-7 WORKER-CASTE DIFFERENTIATION INDUCED BY PARENTS IN THE SUBTERRANEAN TERMITE *RETICULITERMES SPERATUS*

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Division of labor among castes increases ergonomic efficiency of social insect colonies and colony-level fitness, contributing to ecological success of social insects. Because ergonomic efficiency depends on caste ratio of colonies, colonies are likely to adaptively adjust caste ratios according to changing environments and developmental stages of colony life cycle. Factors that determine caste fates of individuals play an important role on control of caste ratio. Thus, it is important for understanding the social organization of insects to solve the issue of what factors regulate caste fates.Our previous study in the termite *Reticulitermes speratus* showed genetic influence on caste determination of larvae, that is, developmental bifurcation into nymphs (immature alate) and workers. In the previous study environmental effects on the caste determination was also suggested: all of the offspring produced through parthenogenesis differentiated into nymphs when the offspring were reared in experimental colonies that were composed of only workers, while some of them became workers in experimental colonies that contained workers and a pair of nymphoids (neotenic reproductives that are differentiated from nymphs). In the present study, to examine effects of different numbers and forms (nymphoid or ergatoid, which is worker-derived neotenic reproductives) of neotenic reproductives on the induction of worker caste differentiation, we reared parthenogenetically-produced offspring in experimental colonies with a single and a pair of neotenics, and compared the caste ratios of the offspring between the experimental colonies. We found that all neotenics but male ergatoids induced worker-caste differentiation of offspring and the induction effects of pairs of nymphoids were greater than those of single neotenics. The results suggested that high proportion of reproductives strongly induces worker differentiation and increase workforce in their colonies.

30-8 BEHAVIOURAL VARIATIONS IN COLONY GROW RATES AND WORKER OVIPOSITION IN STINGLESS BEES' QUEENLESS COLONIES.

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It is well known for highly eusocial bees the importance of queens' presence in the nest for social regulation tasks. In honey bees, workers start laying eggs few hours after queen removal. In stingless bees, some species show workers with developed ovaries even in queenright colonies. In other species, workers never develop their ovaries. Finally, in few species workers can develop their ovaries only in queenless colonies. Comparisons between queenless and queenright colonies in the stingless bee specie *Friesella schrottkyi* showed that in queenright colonies workers are completely sterile, the rate of brood cell constructions is 24.85 ± 6.28 per 12 hours and only one single egg is laid into the cell. In queenless colonies, workers start laying eggs after 2 weeks of orphanage. The rate of colony grow per 12 hours is 2.8 ± 2.4 brood cells. A few workers lay in a single brood cell and the maximum number of eggs found in a cell was nine. Observations on the eggs fate in the cell showed that the first hatched larvae eat remaining eggs. Analysis of the stages of the provisioning and oviposition process (POP) showed clear differences in queenless colonies. In these colonies, the provisioning time is statistically higher. Still, workers lay eggs quicker than queens. The return of the queen in the colony rapidly normalized the colony grow rates and the provisioning and oviposition process. Still, few hours after the queen return, no workers were observed laying eggs. These results showed the clear importance of the queen in colony grow and workers oviposition behaviour.

30-9 DOMINANCE HIERARCHIES AMONG WORKER ANTS IN QUEENLESS COLONIES OF *PSEUDOMYRMEX GRACILIS*

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Evolutionary theory predicts that after removing the queen from an ant colony conflict arises among workers about who will produce males. In many ant species, this conflict is resolved by establishing dominance hierarchies. We examined whether this is true for *Pseudomyrmex gracilis* of the ant subfamily Pseudomyrmecinae. We established eight laboratory colonies, with a subset of individually colour-marked workers. Their behaviour was observed before and after they had been separated from the rest of the colony as well as after reunion of the colony parts when egg-laying had begun in the queenless part (8-9 h of observation in each phase). Ovaries of all individuals were dissected to assess their sizes. One to five workers per colony (mean: 12.2% of marked workers) developed ovaries large enough to presume that these individuals were capable of laying eggs. After colony separation, we observed many aggressive interactions among workers in the queenless parts. Such interactions consisted of one worker quickly moving towards another (mostly twice), rubbing with the mouthparts over the recipient's body surface. Within the first hours after reunion some individuals of the former queenless colony fragment were dragged by nestmates on legs, antennae and other body parts, and mostly killed (in average 84.3% of them had large ovaries). Interaction matrices indicate that dominance hierarchies are built; 58.7% (mean) of the workers were observed acting aggressively at least once. Individuals with large ovaries did not occupy a consistent position within those hierarchies. We conclude that, in absence of the queen, workers of *P. gracilis* establish dominance orders, and one or a few workers begin to lay eggs. However, our results do not provide evidence that the hierarchies serve to regulate worker reproduction. To find out whether this is because of changes in interaction patterns over time, we are currently collecting more data on the development of the hierarchies.

30-10 THE INFLUENCE OF COLONY ENVIRONMENT ON WORKER BODY SIZE IN THE BUMBLEBEE *BOMBUS TERRESTRIS*

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Division of labor among workers is one of the main organization principles of insect societies. In bumblebees the division of labor relates to body size. Small bees typically perform in-nest activities such as brood care, whereas larger bees are more likely to forage outside the nest. Worker size is determined during pre-adult stages, but little is known about the environmental and internal factors that determinant of the final size of the bee. Two main hypotheses may account for the variation in body size: 1) body size is determined by factors in the egg, or 2) body size is determined by the environment experience by the bee, during pre-adult development. We tested these hypotheses in the bumblebee *Bombus terrestris* in which body size between workers vary up to 4-fold difference between full sister bees developing in the same colony. We first measured the body size (inferred from marginal wing cell measurement) for all workers emerging in a colony. Consistent with previous studies, we found a positive correlation between colony age and workers body size. In a following set of experiments we used a cross-fostering design, in which we transferred eggs between pairs of incipient (queen only) and mature (queen, ~10 workers and brood) colonies. In these experiments, adult workers developing from introduced eggs were of similar size to host colony nestmates and significantly different than their biological sisters developing in the source colony. These results support the second hypothesis stating that worker body size is determined by the colony environment. We suggest that this mechanism enables flexibility and optimal compatibility of worker size with colony needs and resource availability.

30-11 THE EFFECTS OF WORKER BEHAVIOURAL STATUS ON RESPONSES TO HOMOCOLONIAL BROOD IN THE RED WOOD ANT (*FORMICA POLYCTENA*)

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As they age, workers of social insects usually switch from intranidal tasks to extranidal ones. The transition nurse - forager, also known as the change of worker behavioural status, involves mainly anatomical, physiological and behavioural maturation. Worker behavioural development may be accelerated, delayed, and even reversed by the modifications of the social context. The transition forager - nurse (behavioural reversion) may be induced by exposing foragers to brood in absence of nurses. However, in honeybee foragers absence of younger workers may induce some phenotype modifications characteristic for behavioural reversion even if foragers are not exposed to brood. In ants responses to brood are known to be influenced by worker behavioural status. Nurses engage in intranidal brood care behaviour, but retrieval of brood to the nest is carried out preferentially by foragers. Reverted nurses show brood care behaviour similar to that of normal age nurses, but its quality may be very poor. We investigated the effects of worker behavioural status on responses to homocolonial brood exposed to strong light in 4 classes of workers of the red wood ant (*Formica polyctena*): nurses, foragers kept with nurses and brood, foragers kept alone, and reverted nurses. 20 workers were put together with 5 homocolonial pupae in a glass container exposed to strong white illumination (5000 lux) and containing a shadowed area. Surprisingly, foragers kept alone differed from ants from all other groups by showing very low interest in brood. Ants from all other groups showed effective brood hiding behaviour. Nurses showed the longest latencies from the start of the test to the first antennal contact with brood, but the shortest duration of interactions with brood preceding its transport. Reverted nurses were generally very similar to foragers kept with nurses and brood, but devoted more time to interact with brood before its transport than ants from all other groups.

30-12 SCANNING BEHAVIOURS IN *MELOPHORUS BAGOTI*: A WINDOW ONTO THE MIND OF A NAVIGATING ANT

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Foragers of many ant species learn long, visually guided routes between their nest and profitable feeding grounds. The mechanisms underpinning the use of visual landmarks are much studied but less is known about how insects extract visual information from a natural scene. Recently we observed that when the Australian desert ant *M. bagoti* is disoriented or in an unfamiliar scene, it scans the world by pausing and then rotating on the spot. To further investigate this scanning behaviour and its role in visually-guided navigation, we trained ants to a feeder where the only exit was along a 1m long narrow channel. Using a high-speed (300 fps) camera we could record the portion of ants' homeward paths immediately at the channel exit when the natural panaoramic scene was first available to the ant to control their homeward path. Moreover, we could provoke scanning, in the field of view of the camera, by adding large screens which significantly altered the panoramic view of the world to which the ants had become familiar. We see that the number of scans is proportional to the degree of change in the visual scene and our detailed analysis of the scanning behaviour shows it to be saccadic in nature. Further analysis will allow us to correlate the directions adopted during the scan with properties of the visual scene.

30-13 AGGRESSIVE BEHAVIOUR OF WORKERS OF THE RED WOOD ANT (*FORMICA POLYCTENA*): THE EFFECTS OF PAST AND PRESENT BEHAVIOURAL SPECIALISATION

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Social insect workers usually engage first in intranidal tasks (act as nurses) and then switch to extranidal tasks and become foragers. Worker behavioural development can, however, be reversed if foragers are exposed to brood in absence of nurses. In the honeybee behavioural reversion involves not only the reappearance of brood care behaviour, but also the return of many other phenotype traits to the nurse-like state. Some of these modifications may be induced by absence of younger workers even if foragers are not exposed to brood. Ant behavioural reversion is much less known. We compared the behaviour of 4 groups of workers of the red wood ant Formica polyctena (nurses, foragers kept alone, foragers kept together with nurses and brood, and reverted nurses) during aggression tests consisting of a 10 min encounter of a single worker with a forager of *Formica fusca*. We were mainly interested in the effects of past and present behavioural specialisation on aggressive behaviour of workers of *F. polyctena*. We also hypothesised that foragers kept alone may express more nurse-like behaviour than foragers kept with nurses and brood due to partial behavioural reversion similar as in the case of the honeybee. As expected, nurses behaved in a less aggressive way than foragers. In some respects (for instance, the occurrence of biting behaviour) reverted nurses were more nurse-like than foragers kept alone. Nurses and reverted nurses showed similarities also in respect to other activity patterns (resting behaviour). Surprisingly, contrary to our expectations foragers kept with nurses and brood showed more behavioural similarities to nurses than foragers kept alone. Our data demonstrate that behavioural correlates of present worker specialisation may also depend on other factors such as worker age, past worker specialisation and social context, although the relative importance of each of these factors remains still to be fully unraveled.

Oral Presentations

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GYNE INVESTMENT AND IMPLICATIONS FOR COLONY FOUNDING IN *POGONOMYRMEX* HARVESTER ANTS

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Reproductive strategies in ants involve individual gynes and males and the collective behavior of the worker body that invests colony resources into sexual reproduction. One variation of reproductive strategy is the degree of claustrality, where fully claustral gynes raise their first brood solely from body reserves and where semi-claustral gynes forage for external resources. In the harvester ant genus, *Pogonomyrmex*, claustral, semi-claustral, and facultative (both) variants exist. Degree of claustrality is affected by colony investment, and is indicated by a positively correlated body size. I measured the difference in per capita production cost of gynes and males across four species of *Pogonomyrmex* that differ in degree of claustrality. Analyses were done by measuring lipid content, lean mass, as well as metabolic expenditure throughout appropriate stages of development. These comparisons provide an estimate for how costly it is for parental colonies to produce gynes (relative to males) that differ in strategy and may aid in understanding how variance in claustrality evolved in ants. I will also discuss the effects of resource supplementation of parental colonies on gyne phenotype a facultative species.

EVOLUTION OF AN ALTERNATE LIFE HISTORY IN HARVESTER ANTS

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Global climate change is in the process of changing the phenology of many species through effects on both temperature and rainfall patterns. Here we report the discovery of an alternate pattern of colony founding in the harvester ant *Pogonomyrmex occidentalis*. New colonies are typically started after mating flights triggered by summer monsoonal rains, but we have documented an increasing frequency of colonies that are founded in late spring. Genetic data indicate that spring colonies result from reproductives from a relatively small subset of existing reproductively mature colonies. Spring colonies appear to have significantly higher rates of survival than summer-founded colonies, and slower rates of increase in worker size.

ENERGETIC BASIS OF COLONIAL LIVING IN SOCIAL INSECTS

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Understanding the ecology and evolution of insect societies requires greater knowledge of how sociality affects the performance of whole colonies. Metabolic scaling theory, based largely on the body mass scaling of metabolic rate, has successfully predicted many aspects of the physiology and life history of individual (or unitary) organisms. Here we show, using a diverse set of social insect species, that this same theory predicts the size dependence of basic features of the physiology (i.e., metabolic rate, reproductive allocation) and life history (i.e., survival, growth, and reproduction) of whole colonies. The similarity in the size dependence of these features in unitary organisms and whole colonies points to commonalities in functional organization. Thus, it raises an important question of how such evolutionary convergence could arise through the process of natural selection.

TOWARD A LINK BETWEEN GEOCHEMISTRY AND THE GEOGRAPHY OF SOCIAL INSECT POPULATIONS

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All living organisms are made up of about 25 chemical elements, and these elements vary in their availability in time and space. We argue that geographic trends in the abundance and behavior of social insect populations may thus have a biogeochemical component. This geography has an energetic skeleton, arising from gradients of productivity and temperature. The meat on this skeleton is the role that shortfalls of Nitrogen, Phosphorus, and Sodium place on colony density and activity. By combining metabolic with stoichiometric approaches, we can add useful tools for predicting a geography of social insect ecology, and, in consequence, a geography of ecosystem function.

Poster Presentations

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31-1 THE IBERIAN ANT *CATAGLYPHIS FLORICOLA* SPECIES COMPLEX: MOLECULAR EVIDENCE FOR GENETIC ISOLATION AND SPECIATION

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Speciation is an important process fundamental to biodiversity. Many are the mechanisms contributing to speciation, the most frequent of them being genetic divergence of geographically isolated populations. Here we analyse the role of genetic differentiation using *Cataglyphis floricola* species complex as a model system. *C. floricola* is an endemic ant found in Southwestern Spain, throughout the lower basin of the Guadalquivir river. Two forms (black and reddish) were initially described in this species whose populations are locally parapatric. MtDNA and nDNA analysis show that 1) both morphs are currently genetically isolated 2) both morphs present different genetic population structure: The reddish morph inhabits an older geological terrain and is in equilibrium, the black morph is found in a recently formed environment and is currently under expansion, 3) the reddish morph is the ancestor of the black one.The Guadalquivir delta and the surrounding coastal area is a highly dynamic system that has significantly transformed the geomorphology of this area. We argue that these recent changes, which have occurred in a few thousand years, may have affected species population structures ultimately resulting in speciation events.

31-2 EFFECT OF CO_2 TREATMENT ON THE OVARIAN DEVELOPMENT OF HONEYBEE WORKERS (*APIS MELLIFERA* L.)

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The exposure of A. mellifera workers to CO_2 has been reported to decrease life span, induce behavioral changes, inhibit the development of hypopharyngeal and wax glands and ovaries. This study was aimed to evaluate the effects of CO_2 on *A. mellifera* worker oogenesis. Newly-emerged workers were exposed to CO_2 saturated conditions for 30 seconds and the ovaries of five-day-old and 15-day-old bees were examined in light and transmission electron microscopy preparations. The ovaries of five days-old workers exhibit long ovarioles with well-developed germarium, although oogenesis showed little development. In the control group, at this age, ovaries exhibited cysts with cystocytes forming rosettes. Ovaries of 15 days-old treated workers exhibited ovarioles of various aspects. In some regions of ovarioles, a well-preserved structure was observed, although oogenesis showed little or no development, while in others, complete disorganization with swollen cells and characteristics of cell death were observed, making the identification of cell types difficult. Germ cells of ovaries of CO_2-treated workers exhibited well-structured organelles, such as the Golgi complex, disorganization of the membranes of the reticulum, and similar to the observed in queens, larger quantities of mitochondria in the cytoplasm. In general, CO_2 treatment did not have the positive effect observed in the maturation of oocytes of queens. Therefore, our findings revealed differences between castes of *A. mellifera* in the response of a reproductive system to the exposure of CO_2. In queens, CO_2 accelerates the development and preserves ovaries, while in workers, the opposite effect is observed.

31-3 NEST STRUCTURE, SEX RATIO AND POLLEN DIET OF *EUGLOSSA CORDATA* (APIDAE: EUGLOSSINI)

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We have studied nest structure, sex ratio and pollen diet from seven nests of *Euglossa cordata* (Hymenoptera: Apidae: Euglossini), in São Paulo state, Brazil. Three nests were found at the *Aechmea sp.* (Bromeliaceae) stolons. The others nests were funded in wood trap-nests and bamboo canes. The diameter and height of closed cells were recorded. Analysis of larval supply was made from 23 samples taken from the brood cells. To obtain the pollen types' frequencies 300 pollen grains were counted per sample. We counted 48 brood cells, of which 25 were still closed when we opened the nests at the laboratory. All brood cells had elliptical shape and were vertical oriented. The cells had in average 8.6 ± 0.6 mm in diameter and 12.26 ± 1.26 mm in height. We have identified 32 pollen types compound the immature food, being *Myrcia*, *Genipa americana* and *Solanum* the most frequent taxa. In a single cell we found 100% pollen of *Myrcia* and in another cell from other nest and another area we counted more then 50% pollen of the same Myrtaceae. At five nests we recorded the resident female in activity, of which three had carcass of others female, at the nest entrance. Among the emergent individuals we recorded six males and five females between January-April/2010. One nest was reused by a new emergent female, which produced two new cells. The presence of old cells, carcass of other females, and reactivation of one nest, suggest that *Euglossa cordata* is possibly philopatric.

31-4 INFERRING REPRODUCTIVE ACTIVITY THROUGH THE OVARIAN DEVELOPMENT ASSESSMENT: COMPARISON OF DIFFERENT METHODS

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The reproductive division of labour is a central feature of insect societies. Various degree of reproductive skew exist in different species, from colonies formed by fully fertile reproductives and sterile individuals to societies in which almost all individuals are physiologically able to reproduce. In order to understand the species-specific life history traits and the different strategies pursued by each colony member an assessment of the individual reproductive potential and activity it is undoubtedly fundamental. Direct observation of reproductive activity can be really stiff in social insects and the most used method so far has been the specimen dissection and the evaluation of ovarian development. Several researchers have however used, even for the same species, different features to assess it. This raises the question whether different evaluation methods yield similar results and are thus comparable.In the present contribution the social wasp *Polistes dominulus* is used as a model species to compare the most used methods of ovarian development evaluation. By recording the reproductive behaviour of about 80 wasps from several nests and by their dissection we managed both to assess the similarity of results obtained with different methods and the reliability of each method as an estimator of oviposition activity. All methods yielded very similar results, indicating their good comparability. However none of them turned out to be an accurate estimator of the real oviposition activity. Our results provide evidence that indexes obtained with different methods can be quite confidently compared but at the same time claim attention on their use as estimators of egg laying activity. We suggest that ovarian index (whatever method is used to calculate it) is a better estimator of reproductive potential (fertility) rather than reproductive activity (egg laying rate).

31-5 LABORATORY AND FIELD DETECTION OF *WASMANNIA AUROPUNCTATA* (HYMENOPTERA: FORMICIDAE) MARKED WITH RABBIT IMMUNOGLOBULINS

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A protein-specific sandwich ELISA was standardised for the detection of protein-marked workers of *Wasmannia auropunctata* fed on a diet marked with rabbit inmunoglobulines (IgG) (1 mg/ml stock solution diluted in honey). Optical absorbance (OA) was read at 405 nm. A laboratory assay was performed to determine how long rabbit-IgG was retained in workers fed during two hours with a rabbit-IgG-marked honey. Single workers (n=10, plus one replicate) were assayed for the presence of rabbit-IgG. All of them were found positive, hence the anti-rabbit IgG-specific performed sandwich ELISA was 100% effective at detecting marked workers until 24 hours after feeding (OA=0.14 ± 0.06; n= 20). In a second assay, the behavior of rabbit-IgG was evaluated under field conditions. Honey baits marked with rabbit-IgG were left exposed to outdoors in a sunny day (between 11:00 and 16:00 hours) in an open field. Temperature fluctuated between 28 and 39°C. The rabbit-IgG was detected in workers of Dorymyrmex sp. (OA=1.4 ± 0.06) five hours after bait´s outdoor exposure. Results showed that the use of rabbit immunoglobulins and its detection by sandwich ELISA can be an effective alternative method for marking ants. The IgG-marked diet based on honey was stable at environmental variations suggesting its versatility for mark-release-recapture studies in field. These results are interesting considering that ant's external marking often failed due to their grooming behavior and small-sized individuals. Trials with higher IgG levels should be assessed in order to increase the persistence of the marker in the workers.

31-6 FUNCTIONAL ROLE OF THE FUNGUS SYMBIONT ON THE FUNGUS-GROWING ANTS: FOOD AND PROTECTION.

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Fungus-growing ants (Attini) have evolved an obligate dependency upon fungus that they rear as their food source. This food source is also used by many attine species to cover the eggs, larvae and pupae. Here we provide observations showing that covering the brood with fungal crop mycelia (hereafter “fungal cover”) is an active behaviour performed by workers, and we then describe some of the fundamental characteristics of the behaviour. Further we test the hypothesis that this behaviour plays a role in protection of the brood against pathogens via an interaction with worker behavior. We studied the functional role of fungal cover and hygiene task in three different workers size of *Acromyrmex echinatior* by experimentally manipulating the fungal cover on pupae and then infecting them with an entomopathogenic fungus, *Metarhizium anisopliae*. The presence of fungal cover reduced the number of pupae with *M.anisopliae* spore germination and the percent coverage of spores on pupae, when compared to pupae that had the fungal cover experimentally removed or naturally had no such cover. When pupae were exposed to contaminants, the fungal cover significantly increased survivorship, but this effect was stronger when smaller workers rather than medium or large workers cared for the brood. This suggests that the fungal cover may play a role in suppressing pathogenic fungal growth, while worker caste-specific tasks increase may enhance this prophylactic effect.

31-7 Cytoplasmic and nuclear immunolocalization of N-cadherin in ovary of *Apis mellifera* with specific C-terminal antibody

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The cadherin superfamily is a large group of proteins mediating homotypic cell adhesion in a variety of developmental processes in vertebrates and invertebrates. One of the classical cadherins, E-cadherin, is involved in several steps of gametogenesis in Drosophila, including anchoring of germ line stem cells in their respective niches of the ovary and testis, as well as the migration of somatic cells within the trophic chamber. With the aim of investigating the role of cadherins in ovaries of the honey bee we generated a polyclonal antibody against the histidine-tailed cytoplasmic region of *Apis mellifera* N-cadherin by heterologous expression in *E. coli* cells (Gateway system). After purification by affinity chromatography, the peptide was injected into rabbits for antibody production. The serum tested by Western blotting against larval testis proteins recognized a band of approximately 300 kDa, which is close to the genomically predicted molecular mass of honey bee N-cadherin (340 kDa). By means of confocal microscopy we analyzed the localization of N-cadherin in ovaries of adult queens. N-cadherin was detected as nearly homogeneously dispersed within the cytoplasm of somatic and germ cells. Furthermore, we found a strong focal association with nuclear material in both cell types, and an increase in such nuclear foci concomittant with polyploidization in nurse cells of the trophic chambers. These results indicate that N-cadherin is probably not involved in cell adhesion processes, but may play a role in intracellular signaling, including transcriptional regulation in *Apis mellifera* gametogenesis.

31-8 Microsatellites show that forest degradation is isolating subpopulations of the stingless bee *Tetragonisca angustula*

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Characterization of a populations' genetic variability is important for assessing within-species variability and for developing meaningful conservation initiatives. In Brazil, the destruction or fragmentation of biomes such as Amazon and Atlantic forest has led to a reduction in the size of bee populations and this reduction has negatively affected the pollination of some local flora. Using microsatellites as molecular markers, we investigated the genetic structure of subpopulations of the stingless bee *Tetragonisca angustula* from forest fragments and from one island (2 km distant from the mainland) in southeastern Brazil. Eighty three bees from seven subpopulations were genotyped for 11 microsatellite loci. Population genetic analysis revealed moderate genetic diversity within *T. angustula*. The number of alleles per subpopulation ranged from 2.9 to 6.9 (mean = 4.7) and values of unbiased expected heterozygosity ranged from 0.55 to 0.74 (mean = 0.62). There was no correlation between forest fragment size and the genetic variability of the bees sampled within each fragment. However, statistical calculations suggest that gene flow between subpopulations occurs only when subpopulations are connected by areas of undisturbed forest. The island subpopulation has moderate genetic variability, and is genetically as well as physically isolated from the mainland populations. Our results indicate that forest fragmentation has not yet strongly affected the genetic variability within the T. angustula population, but now acts as a significant barrier to dispersal. Thus the long term consequences of extant and future fragmentation will be increased isolation of subpopulations and a decline of genetic variance within small subpopulations.Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

31-9 NATIVE HONEY BEE BIODIVERSITY IN TURKEY AND CONSERVATION

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Anatolia having diverse climates, semi arid, Mediterranean, and subtropical, and being located at the intersection of three phytogeographic regions, Irano-Turanian, Mediterranean, and Euro-Siberian played an important role in the diversification of honey bees. Honey bees found opportunities to adapt to a diversity of ecological, climatic, and floral conditions. Five of 26 described subspecies are distributed in Turkey, *Apis mellifera carnica* type in Thrace, *A. m. anatoliaca*, *A. m. caucasica*, *A. m. meda*, and *A. m. syriaca* in Anatolia. Investigations revealed high morphometric and genetic variation among these subspecies and a number of ecotypes. Morphometric analyses have demonstrated the differentiation among the honey bee populations. Allozymes also showed genetic differentiation of subspecies though they have low variation due to haplodiploidy. A high diversity in mtDNA, microsatellites, and differences in behavior have been observed. The presence of high mean observed heterozygosity 0.52-0.67, high gene diversity 0.56-0.72, and numerous diagnostic alleles in races indicate that honey bees have been evolving for a long time in Anatolia and they are well adapted to local conditions. This high genetic diversity is needed and must be conserved to enable the species to adapt to possible environmental changes in future and to improve honey bees through breeding-selection programs. For these purposes genetic reserves in eastern Blacksea region for *A. m. caucasica* have been established. Other areas for conservation of different races are suggested. At the same time, importation of foreign bees and replacing native bees with them and the distribution of queen bees reared at a few breeding centers to all over the country have to be abandoned so that honey bees will not loose their local adaptations as a result of genetic pollution. This is the most appropriate approach for the maintenance of the genetic diversity of honey bees for the current and future utilization.

31-10 THE GLOBAL ANT PROJECT, THE ENCYCLOPEDIA OF LIFE AND WEB BASED SPECIES PAGES

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Contemporary developments in bioinformatics allow us to bring together and analyze large amounts of data in remarkable ways. Other presentations at this conference, for example, highlight some of the advances made by examining large genetic and biodiversity data sets. An even wider range of biological information will be organized through the creation of comprehensive web pages for individual organisms, e.g. the Encyclopedia of Life's (EOL; http://www.eol.org) "an electronic page for every species." Creating web pages for a large number of organisms is a challenge. Most well developed pages are created by taxon specific specialists that are synthesizing information for groups of organisms they are passionately interested in studying. One of these taxon based efforts is the Global Ant Project (GAP; http://gap.entclub.org). It began as an effort to facilitate creating an information rich web page for every ant species. As part of this work, Antweb (http://www.antwe.org) is being expanded to include more information on its species pages. This information is also freely shared and posted on EOL. I will provide details about GAP's goals and progress. I will also outline ways others can contribute, either directly or through already existing projects (e.g. the Ants of New Guinea: http://www.newguineants.org). As work on GAP progresses, and organizes more of what is already known about ants, it will transform our view of ant biology. The synthesis of data and information will yield new discoveries and also reveal areas where further research is needed.

31-11 Effect of supplemental proteins and vitamins on honey bee foraging and activities with their production in Iraq/Kuwait

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The results indicated that the highest body dimensions of mature larvae of *Apis mellifera* was 12.16 and 4.27 mm. for length and width associated with soya bean and 12.01,4.12 mm. for sugar solution(control).While in second season was 12.09 length with vitamin C. plus skim milk and 4.15 mm. width with multi-vit. plus soya bean. While, that in 30 days old workers was 12.05mm. length with skim milk and 4.11mm width with vit.c. in second season was 12.15mm length with vit.c. plus soya bean and width take 4.12mm. in associated with vit.c. plusskim milk. The highest average number of foragers per/hour was 1458 when fed on vit.c. produced 10.140 kg./colony of honey and 203 Inch.2 pollen with vit.c. while in second season was 1212forager/hour with vit.c.plus soya bean produced 9.092kg./colony of honey and 698.3o Inch.2 with vit.c. plus soya bean.Vitamin c.plus soya bean gave the highest dimentions of visceral fat bodies in mature larvae 52.4 micron length and 45.7micron width with multi.vit. Second season showed 45.9micron with vit.c plus soya bean. In mature larvae the highest dimensions of parietal fat body was 44.5 and 40.9 microns for length and width with soya bean respectively. In 30 days old worker the highest diameter of visceral fat bodies was 44.5 ,39.9 micron for length and width with soya bean and multivit .respectively.and 44.9,41.7 for parietal fat body with soya bean.Vit.c. plus soya bean gave the highest dimension 40.5 micron , while the highest width was 34.7 micron that associated with vit.c. plus skim milk.

31-12 Repellency and toxicity of three essential oils against subterranean termites

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Present study was carried out to investigate the potential of different essential oils from commonly used plants in Pakistan as an environmentally safe measure to control termites. In laboratory bioassays, three essential oils from three different plants i.e turmeric (Curcumalonga), eucalyptus (*Eucalyptus citriodora*) and lemongrass (*Cymbopogon citrates*) were tested for their anti-termitic activities against three economically important termites species, namely *Heterotermes indicola* (Wasmann), *Coptotermes heimi* (Wasmann) and *Microtermes obesi* (Holmgren), in Pakistan. When tested for repellency, it was evident from results that three tested oils were highly repellent. Termite toxicity results were different for the three termite species at different doses i.e. 1.25 mg/.6ml, 2.5mg /.6ml, 5mg/.6ml and 10mg/.6ml. It was revealed from results that 100% toxicity was observed after 24 hrs of treatment for *M. obesi* at all doses applied from *Eucalyptus citriodora*, *Curcuma longa* and *Cymbopogon citrates*. LC50 values for *H.indicola*, *C. heimi* and *M. obesi* against turmeric oil were 4.21, 5.54, and 2.66, for eucalyptus oil 5.25, 9.26, and 3.66; and for lemongrass oil were 4.95; 6.59 and 6.66 respectively.

31-13 WHAT 11,000 ANTS CAN TELL USE ABOUT PARENTAL INVESTMENT STRATEGY

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A dominant paradigm in life history theory is that from a parent's perspective there is only one optimal size of offspring that maximizes total parental fitness. This size balances a trade-off between individual survival (e.g., larger individual offspring that survive at a higher rate) and offspring numbers (e.g., more offspring produced leads to more offspring surviving). This paradigm predicts that parents that have access to different amounts of food resources should vary the number of offspring and not their size. If this is true, then the variance in size of offspring produced by an ant colony should have a normal or leptokurtic distribution. The multifaceted model of parental investment predicts that if food is variable and unpredictable for parents, mismatches between food available and offspring able to eat it should occur often. This paradigm predicts that the variance in the size of offspring produced by an ant colony should often have a skewed, multimodal, or platykurtic distribution. To test these models of parental investment we followed the reproduction of over 200 colonies of *Pogonomyrmex salinus* over two years. Each year we manipulated bursts of food that appeared early or late in the species reproductive cycle (i.e., when a numerical response may or may not be possible). The distribution of offspring sizes at the individual colony level in response to treatment will indicate the type of life history strategy this species uses in terms of matching resource availability to offspring production.

31-14 STUDY ON THE BIOLOGY AND EFFICIENCY OF TESTES DETERMINATION OF *HABROBRACON HEBETOR* SAY (HYM.: BRACONIDAE) ON LARVAE OF *CADRA CAUTELLA*

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The results of laboratory studies on the biology *H. hebetor* at 30±2 °C, 60±5% RH and ultimate larvae of *Cadra cautella* indicated that developmental duration of this parasitoid take places in 10.765 days (egg 1.575 ± 0.30 days, larvae 2.592± 0.11 days and pupa 6.598±0.15 days). Newly emerged female parasitoid responded well to host larvae. The adult lived longest when provided with both honey and water, without food they survived less than 4 days. Results showed that female parasitoid deposited eggs throughout the 24 hours, period. There were no significant difference, in reproduction and longevity between virgin and mated females. Development and survival periods of *H. hebetor* on *Cadra cautella* were studied at five temperatures ranging from 17 to 35°C. The lower developmental thresholds for eggs, larvae and pupa were extrapolated from the linear relationship between temperature and growth rate lower developmental thresholds were estimated to be 5.907 (egg), 14.749 (larvae), 15.7156 (pupa). Degree-day (DD) accumulations of 41.88, 45.05 and 89.14 were required for completion of the egg, larvae and pupa stages. Variation of trend of progeny sex ratio and sex ratio in relation to host density and effect of age parasitoid and host size on it showed that *H.hebetor* has a male biased sex ratio. The total number of eggs per female per day (mean) increased from 13.8 to with the host density increasing from 1 to 16. study coordinative effect of age parasitoid and host size on progeny sex ratio showed that age parasitoid increased sex ratio (female/ total) significantly, but host size had no effect on sex ratio and interaction between age parasitoid and size host on sex ratio of progeny have not significant effect.

31-15 ROLE OF SOCIAL INSECTS ON SOIL CARBON DYNAMICS IN FOREST ECOSYSTEMS

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Emission of carbon dioxide (CO_2) from soil surface to the air accounts nearly half of total ecosystem respiration. Therefore, small changes in this component could have considerable impacts on ecosystem carbon budget. In this presentation, I report my recent studies conducted in boreal forests in Finland and tropical forests in Thailand and Malaysia, which aimed to clarify impact of ants and termites on soil CO_2 efflux from soil surface. In Finland, CO_2 efflux from mounds of red wood ants (*Formica rufa* group) was compared to those of the surrounding soil. CO_2 efflux from ant mounds was significantly higher compared to those of the surrounding soil, increasing spatial variation of soil carbon dynamics. The difference of carbon dioxide efflux between mounds and the surrounding soil were higher when ants were active compared to the time when ants were dormant. In tropical rainforests, on the other hand, not only ants but also termites could affect on soil carbon dynamics because their high abundance in soil. Soil respiration in these forests could be highly variable i and hot spots of soil respiration, which is extremely high emission of CO_2 from soil surface (> 10 µmol m^{-2} s^{-1}), could occur sporadically at different times and locations. Therfore, new project that aims to determine sources of soil CO_2 efflux by digging up soil after the CO_2 measurements has started on 2009. Initial results suggest colony of termite could be a reason of the hot spots of soil CO_2 efflux.

31-16 HOW TO DEAL WITH SPECIES' DELIMITATION IN SOCIAL INSECTS? A CASE STUDY IN TERMITES OF AN OLD AND VERY CURRENT PROBLEM

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The aim of this presentation is to discuss taxonomic decisions when there is a huge morphological variation among individuals, either intracolonial or intercolonial. Although this is an old problem concerning all taxonomists, it is even worse when social insects are in focus, like, for instance, the need to consider different castes. Moreover, the variation may vary, that is, the soldier caste may presents a huge variation, but inconsistent with that of the worker and reproductive castes. Soldiers of South American termite genus *Spinitermes Wasmann* are characterized by having a frontal projection with three points on the dorsal surface of the head capsule, and long slashing or snapping mandibles. Considering characters of alates, soldiers and workers, and careful examination of the digestive tube of all castes, the *Spinitermes* could be divided into three major groups: *Allognathus*, *Robustus* and *Trispinosus*. In the last group, the differences in the enteric valve structure in all castes allowed recognizing two subgroups. However, inside these groups can be found an enormous morphological variation, such as, the soldier size, shape of the head and shape of the frontal projection. The frontal projection of the soldiers can point either forward or nearly up, or present large or short lateral points in individuals from the same colony. Then, specimens from the same colony may differ from each other more than have been reported for described species of these “crown Termitinae genera” from South America. Unfortunately, the discriminate analysis, using soldiers' measurements, could help species delimitation only in some cases. In light of these facts, the question arises: would we be working with a single species, with large distribution and morphological variation, or with a complex of cryptic species?

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