



ISBA9

9th International Symposium on Biomolecular Archaeology

June 1st – 4th 2021

(Toulouse, FRANCE)



illumina®



Toulouse



TABLE OF CONTENTS

TALKS

Plant evolution and domestication	
Cheryl Makarewicz et al. Biomolecular identification of the Bronze Age spread of millet into the Altai	p12
Philipp W. Stockhammer et al. Proteins and combustion markers in human dental calculus from the 2nd millennium BCE Eastern Mediterranean	p12
Mélanie Roffet-Salque et al. Dairying, diseases and the evolution of lactase persistence in Europe	p13
Oscar Estrada et al. VINICULTURE: grapes and wines in France from the origins of viticulture to the Middle Ages	p14
Jazmin Ramos Madrigal et al. The journey of maize into Eastern North America	p14
Isabelle Gaffney et al. Investigating drought stress markers in archaeological maize using a novel paleometabolomics approach.	p15
Contributions about ancient diet and cuisine	
Christina Cheung et al. Fish for the babies - a reappraisal of the role of protein-based weaning food in human prehistory	p17
Jasmin Lundy et al. Cuisine in Medieval Sicily: insights from organic residue analysis of ceramics containers and other archaeological evidence	p17
Sylvia Soncin et al. Diet at 79AD Herculaneum: a compound specific stable isotope approach	p18
Vika Efrossini et al. Autarchy on an island? A multi-method reconstruction of diets in Late Bronze Age Kefalonia, Greece.	p19
Pierre-Jean Dodat et al. Isotopic calcium biogeochemistry: dietary reconstruction of two Neandertals from Regourdou site (Dordogne, France) and comparison with one Neandertal from La Grotte du Bison (Yonne, France).	p19
Florinda Notarstefano et al. Feasts and drinks in Iron Age communities of southern Apulia: residue analyses in indigenous decorated pottery	p20
Innovative methods developed to optimize the recovery and analysis of ancient biomolecules	
Alexandra Morton-Hayward et al. Ancient preserved brains: a multi-omics approach to neural tissues in the archaeological record, and insights from molecular taphonomy	p21
Ismael Rodriguez Palomo Site specific deamidation of Asn and Gln assessed by analysis of ancient collagen	p21
Tomasz Suchan et al. Ancient DNA capture using RNA hyRAD probes	p22
Torsten Günther. Estimating ancestry proportions and adjusting genotype likelihoods in the presence of mapping bias	p23
Harald Ringbauer et al. Identification of long shared haplotypes in ancient DNA - Inferring close and distant relatives	p23
Viviane Slon et al. Ancient DNA from the Levantine Paleolithic is within reach: Results from sedimentary DNA analysis at Sefunim Cave, Israel	p24

Human evolution and group interactions, from the Paleolithic to the Middle Neolithic	
Cosimo Posth et al. Genomic analyses of Zlaty kun reveal the oldest modern human skull from Europe	p25
Laurits Skov et al. Genetic Analysis of 17 Neandertals from two sites in the Altai Mountains	p25
Carlos S. Reyna-Blanco et al. Danubians: A local complex history towards Neolithisation	p26
Ainash Childebayeva et al. Genomic portrait of an early Neolithic farming community in Central Europe	p27
Céline Bon et al. Don't judge a book by its cover: cultural evidence of Mesolithic resurgence in Middle Neolithic Paris Basin not linked to genetic admixture.	p27
Refoyo Martinez et al. Reconstructing ancient traits and recovering variants under positive selection at the Mesolithic-Neolithic transition using 1,490 ancient imputed genomes	p28
The challenges and new developments in the field of sedimentary ancient DNA (sedaDNA), paleoecology and paleoclimate reconstruction	
Hazel Reade, Rhiannon Stevens. Nitrogen palaeo-isoscapes: Exploring drivers of change in Late Pleistocene environments using archaeological isotope data	p30
Robin Allaby et al. sedaDNA of Doggerland: a reconstruction of the early Holocene palaeoenvironment using emerging taxonomic assignment, ecological, and authentication methods	p30
Rosie Everett et al. From theoretical processes to the reality of the depositional environment: The challenges of understanding taphonomic processes for sedimentary DNA (sedaDNA) interpretation	p31
Diyendo Massilani et al. Microstratigraphic preservation of ancient DNA in sediment	p31
Ainara Sistiaga et al. Did hot pot make us humans? A geochemical approach to the paleolandscape of Olduvai Gorge at the emergence of the Acheulean, 1.7ma.	p32
Rhiannon Stevens et al. Records of past permafrost thaw preserved in fossil bone sulphur isotope signatures?	p33
Large-scale statistical analyses to explore the impact of transitions on human phenotypes and population structures	
Inigo Olalde et al. Human mobility at the Roman Danubian Limes before and after the fall of the Empire	p34
Evan Irving-Pease et al. Ancestry-aware modelling of the evolution of GWAS variants in a large cohort of ancient genomes	p34
Ruoyun Hui et al. Validating polygenic risk prediction using ancient DNA	p35
Sandra Penske et al. An interdisciplinary study of the social structure from the early Neolithic to the early Bronze Age in central Germany	p36
Anna Szecsenyi-Nagy et al. Unraveling the genetic network of Bronze Age populations: complex genomic structures in the 3000-800 BC East-Central Europe	p36
Fernando Racimo et al. Inferring and simulating spatiotemporal movement using ancient and sedimentary DNA	p37

Human evolution, mobility and social change outside western Europe	
Xiaoran Wang et al. Multiple isotopic and aDNA analyses of Nevali Cori shed light on a socio-section of ancient southeast Anatolia	p39
Selina Carlhoff et al. Population history of ancient Wallacea	p39
Victor Moreno-Mayar et al. Ancient Rapanui genomes reveal pre-European trans-Pacific contact with Indigenous Americans	p40
Stefanie Eisenmann et al. Immigration and Locality at Alalakh, a 2nd millennium BC city in the northern Levant	p41
Marcel Keller et al. Genetic characterization of an Iron Age community from Tuva, Southern Siberia	p42
Yue-Chen Liu et al. Genomic Evidence for At Least Four Prehistoric Migrations to Micronesia	p43
Research on extinct animals and conservation genetics	
Sabhrina Aninta et al. Do different islands shape different babirusa?	p44
Natalia Przelomska et al. Archaeogenomics of Atlantic sturgeon from the Chesapeake - a population recovering from a perilous decline	p44
Marta Maria Ciucani et al Genomic analyses of the extinct Sardinian dhole (<i>Cynotherium sardous</i>) reveal its evolutionary history	p45
Michael V. Westbury et al. Unlocking the evolutionary past of sabre-toothed cats through a palaeogenomic lens	p46
Marianne Dehasque et al. Studying selection in the last woolly mammoth population using genome-wide time series data	p46
Michael Richards et al. Biomolecular studies of mammoths from Late Pleistocene British Columbia, Canada	p47
The origins and evolutionary trajectory of domestic, exploited and/or commensal animals	
Marie Balasse et al. Sheep birth seasonality in European past herds. The Neolithic biological and cultural heritage	p49
He Yu et al. Palaeogenomic analysis of the black rat reveals multiple European introductions associated with human economic history	p49
A. M. Eikeset et al. Genomic stability through time despite decades of exploitation in cod on both sides of the Atlantic ocean	p51
Tatiana Feuerborn et al. Where you lead I will follow: Shaping Siberian dog ancestry through trade and dispersal	p52
Anne Kathrine Runge et al. Palaeoproteomic analyses of dog palaeofaeces reveal a preserved dietary and host digestive proteome	p52
Pablo Librado et al. The genomic foundation and spread of domestic horses	p53
The application of ancient proteomic techniques to molecular archaeology	
Jorune Sakalauskaite et al. Shell palaeoproteomics: from biomineralization to characterisation of ancient proteins preserved in archaeological mollusc shells	p55
Tuuli Kasso et al. Combing Honeycombs - Palaeoproteomics Applied on Historical Beeswax	p56
Patrick Ruether et al. SPIN - Species by Proteome INvestigation	p56
Laura Vinas Caron et al. Proteomic analysis provides insights into the history and organisation of a medieval palimpsest	p57

Marc Dickinson et al. Amino acid dating of mammalian tooth enamel and its potential for building geochronologies	p57
Manasij Pal Chowdhury et al. A machine learning approach in developing an ATR-FTIR-based screening system for collagen (Zooms analysis) and mtDNA in archaeological bones	p58
Ancient microbes and pathogens and their contribution to understanding past epidemics and health (1)	
Kisten Bos et al. A treponemal genome from an historic plague victim supports a recent emergence of yaws and its presence in 15 th century Europe	p60
Kerttu Majander et al. Unravelling the History of Treponema pallidum with Ancient DNA Investigations	p60
Elizabeth Nelson et al. Mycobacterium tuberculosis diversity across the precolonial Andes	p61
Verena Schuenemann et al. New ancient Mycobacterium leprae genomes reveal leprosia as a potential source of high strain diversity in medieval Europe	p62
Lena GraneHäll et al. Metagenomic analysis of ancient dental calculus reveals unexplored diversity of oral archaeal Methanobrevibacter	p63
Magdalena Haller et al. Mass burial genomics reveals outbreak of enteric paratyphoid fever in the Late Medieval trade city Luebeck	p64
Ancient microbes and pathogens and their contribution to understanding past epidemics and health (2)	
Alexander Hübner et al. Paleofeces reveal recent loss of diversity and microbial symbionts in the Western gut microbiome	p65
Christiana Scheib et al. Investigating human-pathogen evolution using paired ancient genomics and proteomics	p66
Frank Maixner et al. Molecular and microscopic analysis of human paleofeces from the salt mines in Hallstatt, Austria, provide precious insights into dietary habits and gut microbiome composition of Iron Age miners	p67
Irina Velsko et al. Tracing human evolution through oral microbiome gene content	p67
Aida Andrades Valtueña Insights into the genomic evolution of Yersinia pestis through comparative pangenomics and de novo assembly	p68
Maria Spyrou et al. Towards an understanding of the early history of the Second Plague Pandemic	p69

ISBA9 FUTURE FELLOWS

Mateja Hajdinjak et al. Genetic history of the oldest Upper Palaeolithic modern humans in Europe	p70
Megan Michel et al. Coevolution and Cariogenicity: Analysis of 80 Ancient <i>Streptococcus mutans</i> Genomes Spanning the Neolithic Transition	p71
Evelyn Todd et al. Tracking the origins and spread of donkey domestication using ancient genomes	p72
Anders Bergström et al. 100,000 years of wolf population dynamics	p72
Alice Rose et al. A whole-town approach to understanding mobility: an isotopic investigation of Medieval Cambridge, UK	p74
Miriam Bravo-Lopez et al. Evolutionary insights into <i>Tannerella forsythia</i> in the Americas through paleogenomics	p74
Lethi Saag et al. Genetic ancestry changes in Stone to Bronze Age transition in the East European plain	p75
Kendra Sirak et al. Ancient DNA reveals admixed genetic ancestry and no differences between socioeconomically stratified burial groups at the Christian Period Nubian site of Kulubnarti	p76
Elena Zavala et al. Identification of hominin and faunal turnovers at Denisova Cave from sediment DNA	p76

POSTERS

Eleftheria Orfanou et al. A Window into Late Bronze Age Central Europe: Multiproxy investigations at Kuckenbug	p78
Silvia Valenzuela-Lamas et al. Analysing animal mobility at a large scale: challenges and possibilities	p78
Xavier Mata et al. CASCADE: A Custom-Made Archiving System for the Conservation of Ancient DNA Experimental Data	p79
Maria Zicos et al. First insights into the population genetics of the extinct Darwin's ground sloth (<i>Mylodon darwini</i>) from Cueva del Milodon, Chile.	p79
Gwenaëlle Goude et al. Identifying biological and behavioural diversity among European Neolithic farmers	p80
Pere Bover et al. Ancient DNA analysis of feathers from funerary bundles at the pre-Hispanic religious center of Pachacamac (Peru)	p81
Perle Guarino-Vignon et al. Genetic continuity in Central Asia since Iron Age	p81
Cheryl Makarewicz et al. Identifying the geospatial origins of Altai Neolithic and Bronze Age communities in bone collagen $\delta^{2}\text{H}$ and $\delta^{18}\text{O}$	p82
Akshyeta Suryanarayan et al. Organic networks and foodstuffs in Bronze Age south-eastern Arabia: preliminary results of lipid residue analysis of local and imported vessels	p82
Bruno Ariano et al. Inbreeding in ancient Malta	p83
Éadaoin Harney et al. A minimally destructive protocol for DNA extraction from ancient teeth	p83
Morgane Ollivier et al. Thousands of years of human-dog relationships: uncovering adaptation to dietary changes of dogs, resulting from the transition to farming	p84

Corrie Hyland et al. Interpreting Life Events using Multi-Tissue Stable Isotope Analysis, Viru Valley Peru	p85
Emma Falkeid Eriksen et al. Ancient DNA analyses of Neolithic Atlantic Bluefin Tuna from Scandinavia	p85
Samantha Leggett. Thinking Outside the Box with Human Mobility Studies: a case study from early medieval Europe	p86
Karen Giffin et al. Methodological advancements in molecular technologies for uncovering the presence of concurrent diseases in past populations	p86
Danijela Popović et al. Ancient genomes reveal long range influence of the site and culture of Tiwanaku	p87
Marlon Bas et al. Insights from amelogenin peptide analysis on sex, gender, and childhood in prehistoric societies	p88
Zandra Fagnäs et al. Potential of archaeogenetic studies of dental calculus to shed light on past human migrations in the Pacific	p88
Eleanor Green et al. Dredging up genomes: leveraging submerged human remains to understand life and death around the River Thames	p89
Ioannis Kontopoulos et al. Adsorption behaviour of single and double stranded DNA on mineral surfaces: implications for DNA survival in sedimentary archives	p90
J. Camilo Chacón-Duque et al. Using museum specimens to assess the genomic impact of recent population decline on Andean bears	p90
Beatriz Fonseca et al. Conformational analysis and water dynamics: a study on the survival of beta-lactoglobulin peptides.	p91
Alexander Verry et al. Pleistocene population expansion of the extinct eastern moa (<i>Mea crassus</i>) revealed by ancient mitochondrial genomes	p91
André Elias Rodrigues Soares et al. A novel study of the history of domesticated sheep in the Bronze and Iron Age Central Asia	p92
Irina Dambueva. Investigation of ancient humans from Buryatia burial grounds	p93
Gunnar Neumann et al. Genetic analysis of a c. 4,800 year old <i>Brucella abortus</i> genome isolated from a calcified nodule	p93
Isabel Alves et al. Population structure of Brittany provides new insights on the introduction of steppe ancestry in western Europe	p94
Fabiana Di Gianvincenzo et al. Palaeoproteomics identifies unexpected components in the ground layer of paintings from the Danish Golden Age	p95
Xuexue Liu et al. Tracking the transformation of the domestic horse during the Bronze and the Iron Age	p96
Mehmet Somel et al. Runs of homozygosity suggest a reduction in inbreeding through the Holocene	p96
Meriam Guellil et al. An invasive <i>Haemophilus influenzae</i> serotype b infection in an Anglo-Saxon juvenile Plague victim.	p97
Peter Seeber et al. Assessing approaches to tracing terrestrial herbivorous mammals using ancient DNA from lake sediments	p97
Maxime Borry et al. Pydamage: automated ancient damage identification and estimation for contigs in ancient DNA de novo assembly	p98

Maria Valentina Aquilino et al. The pharmaceutical content of some Late roman unguentaria from Hierapolis of Phrygia (Turkey): residue analyses and literary sources	p99
Ayshin Ghalichi et al. Genomic signals of continuity and admixture in the Caucasus	p99
Tina Saupe et al. The Enigma of Hyksos: An ancient DNA study	p100
Joanna Bonczarowska et al. Reduced virulence of late medieval Yersinia pestis strains could have contributed to the disappearance of plague from Europe.	p101
Nada Salem et al. Insights into ancient Egyptian genomes in the first Millennium BC	p101
Aurélie Manin et al. A new look at the evolutionary and cultural history of the pig in Corsica, from its Neolithic introduction to modern populations.	p102
Christina M. Balentine et al. Exploring the population history of Patagonia & Tierra del Fuego, Chile, using ancient DNA	p102
Fanny Mendisco et al. Genomic ancestry during Antiquity in France	p103
Abigail Breidenstein et al. Paleogenomic insights into Nubian ancestry from ancient Middle Nile populations	p104
Sanni Peltola et al. Ancient DNA links genetic admixture to language shift in the medieval Upper Volga	p105
Pauline Raimondeau et al. Tracking the spatio-temporal spread of the cultivated olive with archaeogenomics	p105
Valentina Zaro et al. Genomic and anthropological analysis on the human skeletal remains recovered in the House with Garden in Pompeii, Italy	p106
Lorelei Chauvey et al. Improving ancient DNA extraction from waterlogged grape pips	p107
Nikolay Oskolkov. Detecting Modern Contamination in Ancient DNA Samples with Deep Learning	p107
Balázs Gyuris et al. Population genomic analyses of ancient Hungarians and related peoples in the Volga-Kama and Ural regions	p107
Miranda Pedro Morell et al. Archaeogenomics of prehistoric Iberian sheep: insights on early European sheep populations and their relationship with modern sheep.	p108
Paola Campos et al. Deciphering the evolutionary history of a bacterial crop pathogen: insights from historical herbarium specimens	p109
Yi Wang et al. Surface sediment DNA metabarcoding reveals DNA distribution patterns in lake	p109
Catherine Gilbert et al. Study of collagen crosslinking and associated modifications in bones using proteomics	p110
Reyhan Yaka et al. Variation in Genetic Relatedness Patterns among Co-burials in Anatolian Neolithic Societies	p110
Sophy Charlton et al. The Ancient American Dog Project: Understanding the History of Dogs in the Americas	p111
Constanza De La Fuente et al. Human demographic history of the western Trans-Himalayan region	p112
Robert Maier et al. Rapid inference of demographic history with ADMIXTOOLS 2	p112
Bharath Nair. Modelling protein decay	p113

Pierre Clavel. Assessing the predictive taxonomic power of the bony labyrinth 3D shape in horses, donkeys and their F1-hybrids	p113
Lane M. Atmore et al. Using extremely low-coverage sequence data to accurately assign structural variation haplotypes in ancient specimens	p114
Esha Bandyopadhyay et al. Ancient DNA investigation at Photangkhu Longkhap, Nagaland, India	p114
Tammy Buonasera et al. Comparison of proteomic, genomic, and osteological methods of archaeological sex estimation at two ancestral Ohlone sites in Central California (2440 – 100 cal BP)	p115
Ezgi N. Altınışik et al. First Genomic Insights into Pre-pottery Neolithic of Upper Mesopotamia	p116
Annabelle De Vries et al. Oceanic island museomics: human impact and the natural laboratory paradigm	p116
Jack Sharpen et al. Modelling and profiling the biomolecular mechanisms of mummification	p117
Hugh McColl et al. 15th Century Genomic Evidence of Indian Ocean Slave Trade beneath Kota Melaka	p117
Yvette Collin, Ludovic Orlando. Bridging the Divide: Mutually Beneficial Collaboration Between Indigenous and European Scientists in aDNA Research	p118
Bárbara De Sousa Mota et al Imputation of ancient genomes	p118
Chloë Bartlet et al. Resolving the timescale of South-Central African palaeoenvironments and their impact on human behaviour and evolution	p119
Maité Rivollat et al. Insights from unprecedentedly large family trees from the Neolithic site of Gurgy in France	p119
Miranda Evans et al. Palaeoproteomics and Diet at the Romano-British Site of Northstowe	p120
Nathan Wales. Grapevine palaeogenomics across the Mediterranean Basin	p121
Dániel Gerber et al. Uncanny genetic proportions from Hungary suggest a long lasting Hunter-Gatherer ancestry in Central Europe at the Bronze Age	p121
Valentina Zaro et al. Unraveling the genetic history of Italians: a genome-wide study of Iron Age Italic populations	p122
Beatrice Demarchi et al. Can ancient biomolecules resolve controversy surrounding the identity of the extinct Australian thunderbirds (<i>Genyornis</i>)?	p122
Guido Alberto Gnecci-Ruscione et al. Origin and spread of the Iron Age eastern Scythians revealed through a one-thousand-year transect of ancient genomes across the Central Asian steppe.	p123
Elissavet Ganiatsou et al. Women and children in Roman Thessaloniki: an investigation of breastfeeding and weaning through delta-13C and delta-15N incremental analysis.	p124
C. Eduardo Guerra Amorim et al. Genetic Structure and Pathogen Diversity of a Moche Tomb in Northern Peru	p124
Jeremy Courtin et al. Palaeoreconstruction of East Siberian Pleistocene glacial and interglacial biodiversity based on ancient DNA analyses of sediments from the Batagay Megaslump exposure - Providing a picture of past ecosystems	p125

James A. Fellows Yates et al. nf-core/eager: reproducible, portable, and efficient ancient genome reconstruction	p126
Christian Urban et al. Human mitochondrial haplogroups and ancient DNA preservation across Egyptian history	p126
Alina N. Hiss et al. Extraction and Analysis of ancient DNA from Mineralized Tissue Formations	p127
Katterinne Mendez et al. Estimating age at death from ancient DNA methylomes	p127
Binia De Cahsan et al. Shedding light on the evolutionary past of the world's largest tropical lake using sedaDNA	p128
Moritz Muschick et al. Tropical cichlid fish subfossils as source of ancient DNA and tool to study adaptive radiation	p129
Donikë Sejdiu et al. Koch's bacillus genome, literally?	p129
Léa Drieu et al. Improving the taxonomic identification of lipids preserved in pottery: towards a detailed characterisation of ancient culinary practices through MALDI MS?	p130
Andrea A. Cabrera et al. Ancient DNA traces the history of some of the southernmost polar bear fossil remains	p130
Gutierrez Eléa et al. Exploring the Influence of Caloric Restriction (CR) on Nitrogen and Carbon Stable Isotope Ratios on Mouse Bones	p131
Clio Der Sarkissian et al. Advancing Ancient Mollusk Shells as Multi-Proxy Archives of the Past	p131
Thiseas Lamnidis, Stephan Schiffels. Assessing the resolution of rare variation 8 for inferring human history from ancient genomes	p132
Andaine Seguin-Orlando et al. No particular genomic features underpin the dramatic economic consequences of 17th century plague epidemics in Italy	p132
Xavier Roca-Rada et al. Ancient mitochondrial genomes from the Argentinian Pampas inform the peopling of the Southern Cone of South America	p133
Jolijn Erven et al. Evaluating the effect of genotype imputation of ancient whole genome <i>Sus scrofa</i> DNA	p134
Olaf Thalmann et al. Epigenetic variation in Neolithic hunter-gatherer and farmer populations from Scandinavia	p134
Lord Edana et al. Population dynamics and demographic history of Eurasian collared lemmings	p135
Thibault Clauzel et al. Variations in the stable isotope ratios (d18O, d13C, d15N, d34S) of human bone tissue at the individual scale: is one bone enough?	p136
Meaghan Mackie et al. Characterisation of Bronze and Iron Age Italian Wool Textiles by Palaeoproteomics	p136
Giada Ferrari et al. The preservation of ancient DNA in archaeological fish bone	p137

TALKS

Plant evolution and domestication

Biomolecular identification of the Bronze Age spread of millet into the Altai **Makarewicz Cheryl (1), Roffet-Salque Melanie (2), Casanova Emmanuelle (2),** **Grushin Sergey (3), Tishkin Alexey (3), Evershed Richard (2)**

1 - University of Kiel (Germany), 2 - University of Bristol (United Kingdom), 3 - Altai State University (Russia)

*The westward spread of broomcorn millet (*Panicum milaceum*) out of China into the western Eurasian steppe was facilitated by mobile pastoralists in the Inner Asian Mountain Corridor by the mid-third millennium BCE evidenced by the presence of carbonized millet in mortuary contexts and winter foddering of livestock with ¹³C enriched millet at Early Bronze Age sites located in the Dzhunghar Mountains. Millet was likely present further to the north in the Minusinks Basin (southern Siberia) by the mid-second millennium BCE as suggested by ¹³C enrichment of human bone collagen over faunal $\delta^{13}\text{C}$ values. However, direct and unambiguous evidence for millet in the form of carbonized paleobotanical remains for Bronze Age contexts in southern Siberia remain lacking. Here, we present biomolecular and isotopic evidence for millet processing in the Altai through the identification of the 'millet biomarker' miliacin in ceramic pottery and carbon isotope ratios of organic residues in pottery. New approaches involving direct compound-specific dating of lipid residues were used to ascertain the antiquity of millet use in the region. Through biomolecular analyses of organic residues, it is possible to better track the spread of millet and other cultivars into regions where the burial environment is not conducive to the preservation of carbonized seed remains and the dietary origins of the human skeletal isotopic record is ambiguous.*

Proteins and combustion markers in human dental calculus from the 2nd millennium BCE Eastern Mediterranean

Stockhammer Philipp W (1, 2), Scott Ashley (1,2), Buckley Stephen (3), Power Robert (1), Andreadaki-Vlazaki Maria (4), Akar Murat (5), Hallager Birgitta (6), Ingman Tara (7), Maran Joseph (8), Martin Mario A. S. (9, 10), McGeorge Photini (11), Protopapadaki Eftychia (12), Schmidt-Schultz Tyede (13), Shafiq Rula (14), Stuijts Ingelise (15), Yegorov Dmitry (16), Milevski Ianir (16), Yener K. Aslihan (17), Finkelstein Israel (18), Schultz Michael (13), Spiteri Cynthia (19), Warinner Christina (2, 20)

1 - Ludwig Maximilian University Munich (Germany), 2 - Max Planck Institute for the Science of Human History, Jena (Germany), 3 - University of Tübingen (Germany), 4 - Systematic Excavations in Minoan Kydonia (Greece), 5 - Department of Archaeology, Mustafa Kemal University, Alahan-Antakya (Turkey), 6 - Danish Institute at Athens (Greece), 7 - Koç University Research Center for Anatolian Civilizations (ANAMED), Istanbul (Turkey), 8 - Institut für Ur- und Frühgeschichte und Vorderasiatische Archäologie, University of Heidelberg (Germany), 9 - Tel Aviv University, Tel Aviv (Israel), 10 - University of Haifa, Haifa (Israel), 11 - British School at Athens (Greece), 12 - Ephorate of Antiquities of Chania (Greece), 13 - Institute of Anatomy and Embryology University Medical School, Göttingen (Germany), 14 -

Anthropology Department, Yeditepe University, Istanbul (Turkey), 15 - The Discovery Programme (Ireland), 16 - Israel Antiquities Authority (Israel), 17 - New York University (United States), 18 - Tel Aviv University (Israel), 19 - University of Tübingen (Germany), 20 - Harvard University, Cambridge (United States)

In the last years, human dental calculus has been recognized as a key source of biomolecular and micro traces informing us about past culinary practices. In the framework of the ERC Starting Grant Project «FoodTransforms: Transformations of Food in the Eastern Mediterranean Late Bronze Age», we have extracted and analysed proteins, lipids, microremains and further markers from the dental calculus from 69 individuals who lived in the 2nd millennium BCE Eastern Mediterranean with the help of Thermal Desorption/Pyrolysis-Gas Chromatography-Mass Spectrometry, Tandem-Mass Spectrometry and Polarizing Microscopy. Here, we present the results of our study of dental calculus with regard to food related proteins and phytoliths as well as combustion markers and related micro charcoal fragments. We are able to show that besides already known food resources (like wheat, sesame, dates) also soy, banana and turmeric were consumed at least by some individuals in the Southern Levant during the Middle Bronze (Megiddo) and the Iron Ages (Tel Erani). Moreover, we were able to identify evidence for the inhalation of fires made on the basis of wood from Pinaceae and sometimes also oak and/or dung. Individuals from the Aegean centers at Tiryns and Chania also show the inhalation of fires made by using lignite (brown coal) from sources possibly in the northwestern Peloponnese and Crete, respectively, which constitutes the first evidence of the exploitation of brown coal in Europe.

Dairying, diseases and the evolution of lactase persistence in Europe

Roffet-Salque Mélanie (1), Evershed Richard P. (1), Davey-Smith George (2, 3), Timpson Adrian (4), Diekmann Yoan (5), Lyon Matthew S. (2, 3, 6), Mark Thomas (4, 7)

1 - Organic Geochemistry Unit, School of Chemistry, University of Bristol (United Kingdom), 2 - MRC Integrative Epidemiology Unit, University of Bristol (United Kingdom), 3 - Population Health Sciences, Bristol Medical School, University of Bristol (United Kingdom), 4 - Department of Genetics, Evolution and Environment, University College London (United Kingdom), 5 - Department of Genetics, Evolution and Environment, University College London, London (United Kingdom), 6 - NIHR Bristol Biomedical Research Centre, University of Bristol (United Kingdom), 7 - UCL Genetics Institute, University College London (United Kingdom)

In European and many African, Middle Eastern and Southern Asian populations lactase persistence (LP) is the most strongly selected monogenic trait to have evolved over the last 10,000 years. While LP selection and prehistoric milk consumption must be linked, considerable uncertainty remains concerning their spatiotemporal configuration and specific interactions. We provide detailed distributions of milk exploitation across Europe over the last 9k years using >6,700 pottery fat residues from almost 500 archaeological sites. European milk use was widespread from the Neolithic period onwards but varied in intensity spatially and temporally. Surprisingly, comparison of model likelihoods indicates that selection varying with levels of prehistoric milk exploitation provides no better explanation of LP allele frequency trajectories than uniform selection since the Neolithic. In the UK Biobank cohort of ~500K contemporary Europeans, LP genotype was only weakly associated with milk consumption and did not show consistent associations with

improved fitness or health indicators. This suggests other hypotheses on the beneficial effects of LP should be considered for its rapid frequency increase. We propose that lactase non-persistent individuals consumed milk when it became available, but that under particular conditions and microbiological milieux this was disadvantageous, driving LP selection in prehistoric Europe. Comparison of model likelihoods indicates that population fluctuations, settlement density and wild animal exploitation - proxies for these drivers - provide better explanations of LP selection than milk exploitation alone. These findings offer new perspectives on prehistoric milk exploitation and LP evolution.

VINICULTURE: grapes and wines in France from the origins of viticulture to the Middle Ages

Estrada Oscar (1), Chauvey Lorelei (1), Mata Xavier (1), Bouby Laurent (2), Wagner Stefanie (1, 3), Nathan Wales (4), ANR-Viniculture consortium (5), Orlando Ludovic (1)

1-CAGT, Centre for Anthropobiology & Genomics of Toulouse, France 2-ISEM, Institut des Sciences de l'Evolution Montpellier, France 3-INRAE, Institut National de la Recherche Agronomique, France 4-BioArCh, UK 5-ANR, Agence Nationale de la Recherche, France

*The grapevine (*Vitis vinifera* L.) is one of the oldest and economically important fruit crops. Archaeological and genetic evidence suggests that the domestication of the grapevine began 6,000-8,000 years ago in Southwest Asia, with subsequent spread through Southern Greece to the Mediterranean region. However, the domestication history of grapevine remains largely unknown. Several scenarios have been proposed including possible secondary domestication in Western Europe, various origins of varietal lineages, and various introgression levels from local wild grapevines. The extent to which millennial changes in viticultural practices and vineyard management have shaped present-day genomic diversity is also largely debated. The ANR-funded VINICULTURE program aims to investigate the diversity of grapevine in France from the origins of viticulture to the Middle Ages. To achieve this objective, we applied high-throughput sequencing to an assemblage of 155 waterlogged ancient grape pips from archaeological sites spread all over France. We characterised 49 new ancient grape genomes dating from the Bronze age to Roman times. Comparison to whole-genome sequence data from a diversity panel of ancient and present-day grape accessions revealed the diversity range in the ancient genetic pool. It also demonstrated long-distance exchange and temporal lineage maintenance as common ancient viticulture practices in France.*

The journey of maize into Eastern North America

Ramos Madrigal Jazmin (1, 2), Smith Bruce (3), Sánchez-Barreiro Fátima (1), Carøe Christian (1), Shroeder Bryon (4), Vieira Filipe G. (1), Da Fonseca Rute R (5), Liu Shanlin (6), Guo Chunxue (6), Zhang Guojie (6, 7), Petersen Bent (1, 8), Sicheritz-Pontén Thomas (1, 8), Gopalakrishnan Shyam (1, 2, 9), Gilbert M. Thomas P. (1, 2, 10), Wales Nathan (11)

1 - Section for Evolutionary Genomics, The GLOBE Institute, University of Copenhagen, Copenhagen (Denmark), 2 - Center for Evolutionary Hologenomics, The GLOBE Institute, University of Copenhagen, Copenhagen (Denmark), 3 - The Santa Fe Institute, Santa Fe, NM, USA (United States), 4 - Center for Big Bend

Studies, Sul Ross State University (United States), 5 - Center for Macroecology, Evolution and Climate (CMEC), The GLOBE Institute, University of Copenhagen, Copenhagen (Denmark), 6 - China National Genebank, BGI-Shenzhen, Shenzhen (China), 7 - Section for Ecology and Evolution, Department of Biology, Faculty of Science, University of Copenhagen, Copenhagen (Denmark), 8 - Centre of Excellence for Omics-Driven Computational Biodiscovery (COMBio), Faculty of Applied Sciences, AIMST University, Kedah (Malaysia), 9 - Bioinformatics, Department of Health Technology, Technical University of Denmark (Denmark), 10 - University Museum, Norwegian University of Science and Technology, Trondheim (Norway), 11 - Department of Archaeology, University of York, York (United Kingdom)

After its initial domestication in Mesoamerica, maize (Zea mays ssp. mays) dispersed to the north and south of the continent, where it became the main subsistence economy of many indigenous cultures. The woodlands of eastern North America are one of the last stops in maize's journey from its domestication center; however, the hypothesized dispersal routes remain contentious. By the time maize reached Eastern North America ~1700BP, it had already been present in the US Southwest for two millennia. So far, archaeological evidence has not revealed whether maize was transported across the Great Plains or via the Gilmore corridor—a possible trade route from northeastern Mexico through coastal Texas. Understanding the route and associated adaptive pressures have implications for commercially important modern varieties that descend from these ancient lineages. To investigate the origin of maize in eastern North America, we sequenced the genomes of 27 archaeological maize specimens from Northeast Mexico, West Texas, the Southwestern USA, and the Ozark region, ranging in age from 2,750 years ago to historic periods. We show maize initially dispersed into Eastern US from the US Southwest rather than the Gilmore corridor, and we find that ancient maize from different sites in the US Southwest carry different proportions of West Mexican maize ancestry, suggesting multiple entries of maize into the region. Finally, by co-analyzing our data together with other ancient maize genomes, we detect a decline in genetic diversity as maize dispersed north from its domestication center, in particular in domestication associated genes.

Investigating drought stress markers in archaeological maize using a novel paleometabolomics approach.

Gaffney Isabelle (1), Wilson Julie (2), Craig Oliver (3), Thomas-Oates Jane (1)
1 - Department of Chemistry, University of York (United Kingdom), 2 - Department of Mathematics, University of York (United Kingdom), 3 - Department of Archaeology, University of York (United Kingdom)

Maize (Zea mays. L) has been cultivated for 10,000 years and has become the most produced crop globally, supporting some of the world's poorest countries. Understanding how maize farmers have adapted their agricultural practices in the past, and how maize has responded to past climate change, could make a vital contribution to ensure food security during the current climate crisis. Many desiccated samples of maize were discovered preserved in Tularosa Cave in New Mexico, and have been attributed to two temporally distinct occupations (ca. 1850 BP and 750 BP). Palaeoclimate data show the region underwent periods of drought and that one severe episode corresponded with the cave's later occupation. Genetic studies have shown that maize from the latter period may have adapted to drought stress. Here,

we show that high-resolution mass spectrometric analysis of the extractable plant metabolome can be used to evaluate the response of crops to multiple agroecosystem stressors. First, we show the impact of drought stress on both drought tolerant and susceptible maize hybrids and then used this approach to investigate the metabolome of archaeological maize. Intriguingly, we have observed signals in the data from the archaeological maize samples that are consistent with a range of accepted drought stress markers.

Contributions about ancient diet and cuisine

Fish for the babies - a reappraisal of the role of protein-based weaning food in human prehistory

Cheung Christina (1, 2), Tacail Theo (3), Balter Vincent (4), Herrscher Estelle (1), Thomas Aline (2)

1 - Laboratoire méditerranéen de préhistoire Europe-Afrique (France), 2 - Éco-Anthropologie (France), 3 - Institut für Geowissenschaften, Mainz (Germany), 4 - Laboratoire de Géologie de Lyon - Terre, Planètes, Environnement, Lyon (France)

Weaning is a critical and perilous developmental stage for infants that has broad biological, anthropological, and sociological relevance to our understanding of past societies. We present the first study to employ compound specific isotope analysis (CSIA) to investigate weaning practices in human prehistory. In addition, we explore whether calcium isotope analysis can be used to help distinguish the consumption between (breast)milk and high trophic-level resources, as the two are often obscured in terms of stable nitrogen isotope compositions. This study reconstructs the weaning practices at two middle Neolithic communities in the Paris Basin Region: Balloy «Les Réaudins» (BLR) and Vignely «La Porte aux Bergers» (VPB), using compound specific carbon and nitrogen isotope analysis in conjunction with enamel calcium isotope data, and bulk collagen carbon, nitrogen, and sulfur isotope data. Our results demonstrated that instead of a starch-based weaning food such as cereal gruel - as expected for most agricultural groups - a protein-based weaning food that incorporated freshwater resources was used in these two communities. Our data has demonstrated that protein-based weaning food was likely more prevalent in the past than previously thought, even among agricultural societies. It also shows that the use of protein-based, high trophic-level weaning foods can skew the $\delta^{15}\text{N}$ weaning curve and produce an erroneously late estimation for weaning ages. Thus, the common assumption that weaning foods and adult diets share similar isotopic compositions can be problematic, given the importance of protein-based weaning food in human prehistory has likely been underestimated.

Cuisine in Medieval Sicily: insights from organic residue analysis of ceramics containers and other archaeological evidence

Lundy Jasmin (1), Drieu Léa (1, 2), Meo Antonino (3), Orecchioni Paola (3), Aniceti Veronica (3), Fiorentino Girolamo (4), Primavera Milena (4), Alexander Michelle (1), Ughi Alice (1), Talbot Helen (1), Molinari Alessandra (3), Carver Martin (5), Craig Oliver E (1)

1 - BioArCh, Department of Archaeology, University of York (United Kingdom), 2 - Culture et Environnements, Préhistoire, Antiquité, Moyen-Age (Nice France), 3 - University of Rome 2 (Tor Vergata), Rome, Italy (Italy), 4 - Laboratory of Archaeobotany and Palaeoecology – Department of Cultural Heritage – University of Salento (Italy), 5 - University of York, UK (United Kingdom)

Organic residue analysis (ORA) of absorbed residues provides direct chemical evidence of the contents of ceramic vessels and yields insight into pottery use and commodities that were prepared, combined and consumed, thus reflecting cuisines of

the past populations that utilized these ceramic containers. Here absorbed residues of 300 cooking pots and other domestic containers from 9th-14th century contexts in Sicily were analysed, using a multi-faceted ORA approach. Through this large scale, multi-faceted approach a range of commodities including animal products, vegetables, beeswax, plant resins and fruit products were identified, with a complex mixing of resources observed in many cases. Through a temporal approach, this analysis enabled us to identify specific uses of ceramic containers in different socio-economic settings, such as the processing of dairy products in rural settlements. Furthermore, a diachronic approach yielded important insights into culinary habits through the multi-transitional period from the 9th-14th century AD, where in some cases continuity was observed in the use of pottery over a 500 year period as well as some interesting chronological specificities. These results were interpreted within a highly collaborative framework, utilizing other archaeological evidence of food consumption in medieval Sicily. Among these; zooarchaeological and archaeobotanical remains enabled an in-depth interpretation of the contents of pottery vessels and provided evidence of a wider range of resources available. Whilst, stable isotope analysis broadened our understanding of culinary habits, by providing direct evidence of diet. Here, through a multidisciplinary approach, a wide range of questions concerning medieval cuisine in Sicily are addressed.

Diet at 79AD Herculaneum: a compound specific stable isotope approach
Soncin Sylvia (1), Talbot Helen (2), Fernandes Ricardo (3, 4, 5), Colonese André (6), Bondioli Luca (7, 8), Craig Oliver (2)

1 - BioArCh, Department of Archaeology, University of York. (BioArCh - University of York Environment Building, Wentworth Way, Heslington York YO10 5NG, UK United Kingdom), 2 - BioArCh, Department of Archaeology, University of York (United Kingdom), 3 - Department of Archaeology, Max Planck Institute for the Science of Human History, Jena (Germany), 4 - School of Archaeology, University of Oxford (United Kingdom), 5 - Faculty of Arts, Masaryk University, Brno (Czech Republic), 6 - Department of Prehistory and Institute of Environmental Science and Technology, Universitat Autònoma de Barcelona (Bellaterra, 08193, Spain Spain), 7 - Section of Bioarchaeology, National Museum of Prehistory and Ethnography Luigi Pigorini, Museum of Civilizations, Rome (Italy), 8 - Dipartimento dei Beni Culturali, Università di Padova, Padua (Italy)

Carbon and nitrogen stable isotope analysis (SIA) of human bone collagen has been extensively applied in archaeology allowing to gain meaningful insights into ancient human dietary practices. However, several studies have now exposed the important limitations of SIA regarding how carbon and nitrogen from different food sources and macronutrients are registered in collagen, potentially resulting in misleading interpretations. As a consequence, many researchers are turning to the isotope analysis of amino acids (CSIA-AA) in bone collagen where the relationship to dietary sources is better understood. Here, we apply CSIA-AA to the extraordinary assemblage of the victims of the AD 79 Vesuvius eruption at Herculaneum deploying two Bayesian models that incorporate knowledge of protein synthesis. By doing so, we were able to reduce the number of assumptions and quantify dietary differences between sexes that were difficult to discern using SIA, further opening up the

potential for studying gender based inequalities in ancient societies. Crucially, for the first time, we provide datasets that can be meaningfully compared with nutritional records of modern Mediterranean populations, that are likely to be of additional interest to the broader community of nutrition, health and environmental scientists.

Autarchy on an island? A multi-method reconstruction of diets in Late Bronze Age Kefalonia, Greece.

Efrossini Vika (1), Presslee Samantha (1), Talbot Helen (1), Von Tersch Matthew (1), Alexander Michelle (1)

1 - Department of Archaeology, University of York (United Kingdom)

The archaeology of Late Bronze Age Greece (c. 1600-1100 BC) is largely concerned with the investigation of social hierarchies in the sphere of the elite, an approach instigated by the domination of cultural historical paradigms and perpetuated by the discoveries of the palatial structures on the South mainland and Crete.

Unquestionably illustrious, the elite became the omnipresent focus of research, leaving a disproportioned place for the actions of non-elite agents. Kefalonia is an island located off the west coast of Greece, territorially removed from palatial influence. Its relationship to the elite is a matter of heated debate, fueled significantly -but inconclusively- by the presence of 'royal' tombs on the island. Resilient economically and culturally, the island sees a population expansion after the collapse of the palatial system, argued to be an influx of 'immigrants' created by the widespread destructions on the mainland. In this study, we present for the first time the application of a multi-method approach (bulk bone collagen $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, collagen amino acid $\delta^{13}C$ and $\delta^{15}N$, bone carbonate $\delta^{13}C$) to fully characterize diets. Our aim is twofold: firstly, we present a methodological suite that overcomes the limitations of single-method dietary reconstructions posed by the idiosyncrasies of the Mediterranean landscape, and secondly, we characterize the variable use and distribution of natural resources as part of a regional political economy that formulates the role of non-elite polities in the Late Bronze Age world.

Isotopic calcium biogeochemistry: dietary reconstruction of two Neandertals from Regourdou site (Dordogne, France) and comparison with one Neandertal from La Grotte du Bison (Yonne, France).

Dodat Pierre-Jean (1,2), Maureille Bruno (1), Couture-Veschambre Christine (1), Hardy Maurice (3), Balter Vincent (2)

1 - De la Préhistoire à l'Actuel : Culture, Environnement et Anthropologie (France), 2 - Laboratoire de Géologie de Lyon - Terre, Planètes, Environnement [Lyon] (France), 3 - Archéologies et Sciences de l'Antiquité (France)

Recent developments on non-traditional stable isotope systems open new research perspectives in archaeological contexts, even in highly diagenetic environments when organic matter is destroyed. The calcium isotopic composition ($\delta^{44}/^{42}Ca$) of bone and tooth enamel can be used as dietary indicator. Here, we present Ca isotopes analysis of fossil bone samples of the fauna from Regourdou and La Grotte du Bison Mousterian sites as well as from bone samples of 3 Neandertal individuals. Comparison between the Regourdou Neandertals and the one of La Grotte du Bison shows a potential different feeding behavior. All the $\delta^{44}/^{42}Ca$ values of

Regourdou 1 Neandertal are low, placing this individual amid carnivores, contrary to the Neanderthal of La Grotte du Bison which has values between that of the reindeer and that of the bovid and mammoth. Using a bone-muscle Ca isotopic offset determined on extant animals, we show that the low $\delta^{44}/^{42}\text{Ca}$ value of carnivores cannot be accounted for by the consumption of meat only, as plants and meat have indistinguishable $\delta^{44}/^{42}\text{Ca}$ values. Mass balance calculations indicate that the low $\delta^{44}/^{42}\text{Ca}$ values of carnivorous are explained by the ingestion of bone in low quantity. Our results show that Regourdou 1 Neandertal consumed a mixture of various herbivorous prey as well as bone, which probably occurs when marrow was ingested, this is not the case for the Neandertal from La Grotte du Bison for which we have no clear indication of bone consumption, but for which meat consumption cannot be excluded.

Feasts and drinks in Iron Age communities of southern Apulia: residue analyses in indigenous decorated pottery

Notarstefano Florinda (1), Semeraro Grazia (1)

1 - University of Salento (Lecce, Italie) (Dipartimento Beni Culturali Via D. Birago 64 73100 Lecce Italy)

Fermented and alcoholic beverages played an important role in feasting and social events in past agricultural societies, helping to establish and maintain social relationships. The study of indigenous societies of southern Italy between the 8th and the 6th century BC is revealing interesting aspects related to the ceremonial sphere and its relevance in defining the identity of local communities. An indirect evidence of ritual and social activities within the Japygian culture can be found in the particular incidence of decorated pottery, whose morphological repertoire is characterised mostly by liquid containers: narrow necked vessels, small jugs and askoi. The current project incorporates experimental study, use-wear and residue analyses of matt-painted local pottery from different Iron Age settlements of southern Apulia with the aim of investigating their possible use for the production and consumption of alcoholic beverages. Residue analyses on potsherds are carried out by gas chromatography-mass spectrometry (GC-MS) with different extraction procedures, allowing the identification of both lipid substances and fermentation markers. The preliminary results indicate that the vessels contained fermented beverages coming from sugar-rich fruits and cereals, thus giving first insights on the real function of decorated pottery and alcohol consumption in ritual and ceremonial activities among the local communities of southern Apulia during the Iron Age.

Innovative methods developed to optimize the recovery and analysis of ancient biomolecules

Ancient preserved brains: a multi-omics approach to neural tissues in the archaeological record, and insights from molecular taphonomy

Morton-Hayward Alexandra (1), Hansen Anders Johannes (1), Hansen Martin (2), Collins Matthew (1, 3)

1 - Globe Institute, University of Copenhagen (Denmark), 2 - Department of Environmental Science, Aarhus University (Denmark), 3 - McDonald Institute for Archaeological Research, University of Cambridge (United Kingdom)

As one of the first organs to decompose post-mortem, brains are far more numerous than they should be in the archaeological record. >1600 have been reported by generations of baffled archaeologists in the last 400 years; yet deep-time brain preservation is stubbornly described as a unique or exceptionally rare phenomenon, and remains little-investigated. One of the foremost areas of current neuropathological research is the time-dependent change in the amino acid chemistry of the brain. As we age, spontaneous protein modifications promote molecular misfolding, polymerisation and aggregation; but tissue deposition of these neurotoxic fibrils need not necessarily end with the cessation of life. Might the formation of organically insoluble, non-hydrolysable plaques, so devastating in a plethora of dementias, perversely act to preserve the brain after death, stabilising otherwise labile biomolecules during long-term diagenesis? This multi-omics approach to the study of preserved neural tissues is the first of its kind, unravelling the means by which the brain persists when other organs perish. Exploiting cutting-edge strategies to maximise the recovery of ancient proteins, lipids, aDNA and metabolites, >200 brains excavated from a 19th C. workhouse cemetery site (UK) have yielded a wealth of biomolecular data, mined with advanced bioinformatic pipelines. Most particularly, biological and diagenetic patterns of molecular modification suggest that, not only do preserved brains shed light on health and disease in the long-dead, but probing the utmost extent of neural plaque deposition might also illuminate the clinical trajectory of living tissues in the aged.

Site specific deamidation of Asn and Gln assessed by analysis of ancient collagen

Rodriguez Palomo Ismael (1)

1 - Globe Institute (Denmark)

The need for authentication of proteins in ancient samples is becoming a central issue in palaeoproteomics as risks of contamination from modern proteins are increasingly recognised. Moreover, dating based upon the level of decay is important due to the necessity of building chronological scales in archaeological studies. Glutamine deamidation has been proposed before as an authenticity and chronological marker. Using Python, we mined published and in-house MS2 data analyzed with MaxQuant from collagen type I rich ancient samples from bone, dental calculus, parchment and leather. We derived the site-specific asparagine (Asn) and

glutamine (Gln) deamidated fraction and calculated the relative rates of deamidation for different tripeptide combinations of the form Xxx-Asn/Gln-Zzz in order to sort them. The order of Xxx-Asn-Zzz tripeptides in our bone and dental calculus samples is correlated with that in previously studied solubilized Gly-Xxx-Asn-Zzz-Gly pentapeptides, while Xxx-Gln-Zzz patterns do not show correlation with their corresponding pentapeptides. In parchment and leather samples, the correlation is disrupted due to chemical treatments and environmental factors. We performed a principal component analysis on the tripeptides deamidated fraction data. The loadings of the tripeptides to the principal components are structured by these features. Moreover, we show a relation between the first principal component and Thermal age, resembling previous studies. We hypothesize that these patterns are due to different deamidation reaction mechanism domination, either via a fast cyclic intermediate formation or a slow direct hydrolysis, in mineralized versus solubilized collagen, which is rapidly lost due to leaching.

Ancient DNA capture using RNA hyRAD probes

Suchan Tomasz (1, 2), Wagner Stefanie (1,3), Calvière-Tonasso Laure (1), Lorelei Chauvey (1), Schiavinato Stéphanie (1), The ERC Pegasus Consortium, Orlando Ludovic (1)

1 - Centre for Anthropobiology and Genomics of Toulouse (CAGT) - UMR5288 (France), 2 - W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, Poland (Poland), 3 - BIOGECO, INRA, Univ. Bordeaux, Cestas (France)

DNA hybridization capture techniques allow to focus sequencing efforts on pre-selected parts of the genome, thus reduce analytical costs and improve sensitivity. They also provide an alternative to shotgun sequencing to obtain genome-wide data from DNA extracts showing low endogenous content, which represent the vast majority of archaeological remains. However, the design of probes requires extensive genome panels for SNP discovery and their production cost can be prohibitive. Here, we explore the efficacy of a modified hyRAD protocol aimed at the cost-effective production of RNA capture probes providing scalable reduced genome representations. The technique proved to be especially effective on DNA extracts with low endogenous content (~1%), that could be enriched up to 53-fold. The aDNA sequences mapped preferentially on the genomic regions targeted by the probes, resulting in on-target enrichment of up to 146-fold. Performing two rounds of capture increased the specificity of the experiment (up to 3.60-fold increase of on-target reads, relative to one round of capture), even despite much higher proportion of PCR duplicates. This work also demonstrated the capacity of hyRAD to provide shared orthologous variants across multiple samples, attesting hyRAD as a promising tool in ancient population genomics. As probe library constructs do not simply allow for RNA production but also include Illumina sequencing adapters, they provide an especially effective tool for discovering SNPs, even in non-model organisms.

Estimating ancestry proportions and adjusting genotype likelihoods in the presence of mapping bias

Günther Torsten (1)

1 - Human Evolution, Dep. Organismal Biology, Uppsala University (Sweden)

A common part of many demographic analyses in ancient DNA studies is the estimation of ancestry proportions. We have previously shown that mapping ancient DNA sequencing reads to a linear reference genome can bias population genetic inference. One possibility to mitigate the effects of such mapping bias would be to directly incorporate it into population genomic analysis. Genotype likelihoods are a powerful approach to accommodate uncertainty about genotypes as they can be directly used in downstream analysis. Here, we introduce an approach to empirically adjust genotype likelihoods for mapping bias which we then test for estimating ancestry proportions in the presence of mapping bias. We simulate NGS data with ancient DNA damages under a demographic model and then map the sequencing reads to a linear reference genome sequence. We show that estimated ancestry proportions can differ up to 20% depending on population divergence and the origin of the reference sequence. Adjusting genotype likelihoods for mapping bias leads to similar estimates of admixture proportion than when mapping to a reference genome from an outgroup population. Furthermore, we compare the estimates using corrected and uncorrected genotype likelihoods to established methods based on pseudohaploid data. In some cases the effect of using different methods for estimating admixture proportions is stronger than the impact of mapping bias. Finally, corrected genotype likelihoods can have many more applications beyond the estimation of ancestry proportions, they can, for example, be used for more accurate estimates of allele frequencies at individual loci when searching for signatures of selection

Identification of long shared haplotypes in ancient DNA - Inferring close and distant relatives

Ringbauer Harald (1), Akbari Ali (2), Mallick Swapan (3), Mitnik Alissa (4), Patterson Nick (5), Reich David (6, 7, 8, 9)

1 - Department of Human Evolutionary Biology, Harvard University (United States), 2

- Harvard Medical School, Boston, (United States), 3 - Harvard Medical School,

Boston (United States), 4 - Department of Genetics, Harvard Medical School (United

States), 5 - Broad Institute of Harvard and MIT, Cambridge (United States), 6 -

Department of Genetics, Harvard Medical School, Boston (United States), 7 -

Department of Human Evolutionary Biology, Harvard University, Cambridge (United

States), 8 - Broad Institute of MIT and Harvard, Cambridge (United States), 9 -

Howard Hughes Medical Institute, Harvard Medical School, Boston (United States)

We present a new method to identify long shared haplotypes, so called Identity-By-Descent (IBD) blocks, from low-coverage human ancient DNA data. These genomic signposts of recent relatedness allow one to robustly identify pairs of relatives up to 6th degree. Beyond that, this new method also enabled us to infer rates of more distant relationships from rates of IBD sharing. I will give an overview of this new method and how we applied it to the published ancient DNA record to gain insight

into past mobility and demography via inferring and analyzing direct genealogical links.

Ancient DNA from the Levantine Paleolithic is within reach: Results from sedimentary DNA analysis at Sefunim Cave, Israel

Slon Viviane (1,2,3), Clark Jamie L. (4), Friesem David E. (5, 6, 7), Orbach Meir (7), Porat Naomi (8), Meyer Matthias (3), Kandel Andrew W. (9), Shimelmitz Ron (7)

1 - The Dan David Center for Human Evolution and Biohistory Research, Tel Aviv University, Tel Aviv (Israel), 2 - Department of Anatomy and Anthropology and Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv (Israel), 3 - Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology, Leipzig (Germany), 4 - Department of Sociology and Anthropology, George Mason University, Virginia (United States), 5 -Haifa Center for Mediterranean History, University of Haifa, Haifa (Israel), 6 - Recanati Institute for Maritime Studies, Department of Maritime Civilizations, School of Marine Sciences, University of Haifa, Haifa (Israel), 7 - Zinman Institute of Archaeology, University of Haifa (Israel), 8 - Geological Survey of Israel, Jerusalem (Israel), 9 - The Role of Culture in Early Expansions of Humans, Heidelberg Academy of Sciences and Humanities at the University of Tübingen, Tübingen (Germany)

DNA tends to degrade faster in warm climates, thus far hindering the recovery of DNA from prehistoric populations in the Levant. As part of a larger study aimed at investigating the properties of DNA preservation in archaeological sediments throughout Eurasia, we collected 33 sediment samples from Sefunim Cave in Israel for genetic analyses. The samples were taken from freshly excavated areas, while taking precautions to minimize contamination introduced by handling, and spanned all five Paleolithic archaeological horizons of the site. Four of the samples contained traces of ancient mitochondrial DNA from Cervidae and/or Hyaenidae, coinciding with the zooarchaeological record at the site. A combination of luminescence-based dating of the sediments and radiocarbon dates indicates that the DNA comes from layers at least 30,000 years old. Furthermore, a geoarchaeological study suggests little movement of the sediments after their deposition. All four positive samples were taken from the same area within the cave, suggesting that local conditions there were particularly conducive to the long-term preservation of DNA. We hypothesize that a large limestone boulder, which covered that area for millennia and which was removed shortly prior to sampling, helped reduce the loss of DNA in the underlying sediment. The recovery of DNA from sediments at Sefunim Cave pushes back the current limit of DNA preservation in the Levant by more than 15,000 years. The analysis of DNA from Upper Paleolithic populations in the Levant may therefore be closer in reach than previously thought.

Human evolution and group interactions, from the Paleolithic to the Middle Neolithic

Genomic analyses of Zlatý kun reveal the oldest modern human skull from Europe

Posth Cosimo (1, 2), Prüfer Kay (1, 3), Yu He (1), Stoessel Alexander (1, 4), Spyrou Maria (1, 2), Deviese Thibaut (5), Mattoni Marco (6), Ribechini Erika (6), Higham Thomas (5), Velemínský Petr (7), Brůžek Jaroslav (8), Krause Johannes (1, 3)

1 - Archaeogenetics Department, Max Planck Institute for the Science of Human History, Jena (Germany), 2 - Archaeo- and Palaeogenetics, Institute for Archaeological Sciences, University of Tübingen (Germany), 3 - Archaeogenetics Department, Max Planck Institute for Evolutionary Anthropology, Leipzig (Germany), 4 - Institute of Zoology and Evolutionary Research, University of Jena (Germany), 5 - Oxford Radiocarbon Accelerator Unit, Research Laboratory for Archaeology and the History of Art, University of Oxford (United Kingdom), 6 - Department of Chemistry and Industrial Chemistry, University of Pisa (Italy), 7 - Department of Anthropology, National Museum, Prague (Czech Republic), 8 - Department of Anthropology and Human Genetics, Faculty of Science, Charles University, Prague (Czech Republic)

Following their dispersal out of Africa, modern humans admixed with Neandertals ~60,000-50,000 years ago, and then expanded into Europe a few thousand years before Neandertals disappeared. The genetic composition of these first Europeans is, so far, not well characterized since only a very limited number of individuals older than 40,000 years have been genetically analyzed. Here, we report both genome-wide capture and shotgun data generated from the temporal bone of an almost complete skull from a female individual found at Zlatý kůň in present-day Czechia. This genome represents a deeply divergent out-of-African lineage that did not contribute genetically to either Europeans or Asians but carries a similar proportion of Neandertal ancestry like other Upper Paleolithic hunter-gatherers. Several radiocarbon dating attempts resulted in inconclusive results though the length of the introgressed Neandertal blocks are longer than those observed in the 45,000-year-old Ust'-Ishim genome from Siberia. This suggests that Zlatý kůň is at least as old as Ust'-Ishim and thus is the earliest modern human skull sequenced from Europe to date.

Genetic Analysis of 17 Neandertals from two sites in the Altai Mountains

Skov Laurits (1), Peyregne Stephane (1), Popli Divyaratan (1), Iasi Leonardo (1), Hajdinjak Mateja (1), López David (1), Zavala Elena I. (1), Slon Viviane (1), Grote Steffi (1), Kharevich Aliona (2), Kolobova Kseniya (2), Markin Sergey (2), Viola Bence (3), Krivoshapkin Andrey (2), Derevianko Anatoly (2), Kelso Janet (1), Meyer Matthias (1), Pääbo Svante (1), Peter Benjamin (1)

1 - Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology, Leipzig (Germany), 2 - Institute of Archaeology and Ethnography, Russian Academy of Sciences, Novosibirsk, Russia (Russia), 3 - Department of Anthropology, University of Toronto, Toronto, Canada (Canada)

Neandertals occupied Eurasia from around 400kya until around 40kya. However, there are few locations with multiple, contemporaneous Neandertals and we therefore know little about the structure of their social groups. We present genetic data from 17 Neandertal specimens from two sites in the Altai Mountains in Siberia; 15 from Chagyrskaya Cave and two from Okladnikov Cave. Using hybridization capture, we generate full mitochondrial genomes from all 17 specimens, and 0.4- to 12-fold coverage in the nuclear genome of 712,373 single nucleotide polymorphisms selected to be informative about archaic hominin diversity. We also generate between 0.02- and 42-fold coverage of 6.9Mb of the Y chromosome for the 10 male individuals. The genomes of the Chagyrskaya and Okladnikov individuals are all more similar to the genomes of a 60,000-80,000-year-old from Chagyrskaya Cave and late Neandertals in Europe than to the genome of an older Neandertal found in Denisova Cave, less than 130 km away. The Neandertals from Chagyrskaya Cave represent a closely related group of 7 males and 5 females and include a father-daughter pair. Extended tracts of homozygosity exceeding 2.5 cM in length cover 20-30% of the genomes indicate that the ancestors of these Neandertals lived in small groups with an effective population size between 30-110 individuals, smaller than the population size estimated for early modern human groups. The diversity of the Y chromosomes is lower than of the mitochondrial genomes suggesting that either fewer men than women contributed to the next generation, or that women moved more frequently between groups.

Danubians: A local complex history towards Neolithisation

Reyna-Blanco Carlos S (1, 2), Hofmanová Zuzana (1, 2), Link Vivian (1, 3), Schulz Ilektra (1, 4), Winkelbach Laura (5), De Becdelièvre Camille (6), Stefanovic Sofija (7), Burger Joachim (8), Wegmann Daniel (1, 2)

1 - Swiss Institute of Bioinformatics (SIB) (Switzerland), 2 - Departement de Biologie, Université de Fribourg (Switzerland), 3 - Université de Fribourg (Switzerland), 4 - Departement de Biologie, Université de Fribourg (Switzerland), 5 - Palaeogenetics Group, Institute of Organismic and Molecular Evolution (iomE), Johannes Gutenberg University (Germany), 6 - University of Belgrade, Department of Archaeology (Serbia), 7 - Biosense Institute, University of Novi Sad/Department of Archaeology, Faculty of Philosophy, University of Belgrade. Serbia. (Serbia), 8 - Paleogenetics Group, University of Mainz (Germany)

The Danube Gorges boast more than 25 prehistoric sites that attest to the gradual behavioral transformations of mobile groups during the Epipaleolithic (ca 13000-9500 cal BC), to sedentary fisher-hunter-gatherers of the Mesolithic (ca. 9500-6300 cal BC) and early pottery users who practiced animal husbandry during the Neolithic (ca. 6300-5500 cal BC). Here we present a new collection of temporal ancient samples that, together with a series of available samples, shed light on the genetic footprints of these cultural changes. We document these footprints by characterizing patterns of genetic diversity, which we quantify accurately by using a probabilistic framework (ATLAS) that accounts for post-mortem damage and allows for reference-free recalibration of base quality scores. We observed striking differences between the two main sites: At Lepenski-Vir, we found multiple pulses of introgression of Aegean farmers into the Mesolithic community, including individuals of both pure

Aegean ancestry and of recently admixed origin that adopted a fisher-hunter-gatherer diet. In contrast, no inclusion of immigrating farmers was observed at Vlasac. Benefiting from our probabilistic framework, we also explicitly contrasted the genetic diversity between the X and autosomes as well as between functional classes on the autosomes. These results are consistent with an elevated population size and stronger patrilocality of Neolithic farming communities and showcase the possibility to quantify behavioural processes in past societies.

Genomic portrait of an early Neolithic farming community in Central Europe

Childebayeva Ainash (1), Rivollat Maïté (1, 2) Barquera Rodrigo (1), Aron Franziska (1), Rohrlach Adam Ben (1, 3), Nicklisch Nicole (4, 5), Alt Kurt W. (4, 5), Meller Harald (5), Friederich Susanne (5), Krause Johannes (1), Haak Wolfgang (1)

1 - Department of Archaeogenetics, Max Planck Institute for the Science of Human History, Jena, Germany (Germany), 2 - University of Bordeaux, PACEA-UMR5199, Bordeaux, France (France), 3 - ARC Centre of Excellence for Mathematical and Statistical Frontiers, School of Mathematical Sciences, the University of Adelaide, Adelaide, SA, Australia (Australia), 4 - Danube Private University, Krems-Stein, Austria (Austria), 5 - State Office for Heritage Management and Archaeology Saxony-Anhalt - State Museum of Prehistory, Halle (Saale), Germany (Germany)

The Neolithic expansion from Anatolia to Europe has been one of the major topics in ancient DNA research over the last ten years. Multiple studies to date have shown that the cultural spread of agriculture from Anatolia and the Near East was accompanied by large-scale expansions of early farmers. This study presents genome-wide single nucleotide polymorphism (SNP) data from a Linear Pottery Culture (LBK) site Derenburg Meerestieg II (DER) (n=32 individuals) in today's Germany. Based on a population genetics analysis, we show that DER individuals had high levels of Anatolian Neolithic ancestry, together with a limited degree of local hunter-gatherer admixture. A comparative analysis of DER individuals with other early European farmers (EEF) shows similar levels of hunter-gatherer admixture, low levels of inbreeding, and relatively large effective population size, which suggest a swift expansion from a comparatively large source deme, or alternatively, a selective but extensive social and/or mating network. Based on a scan for signatures of selection, our study reports loci associated with immune function and metabolism indicating selective pressures linked to the process of Neolithization, such as infectious disease and a changing diet, that were faced by the early farmers.

Don't judge a book by its cover: cultural evidence of Mesolithic resurgence in Middle Neolithic Paris Basin not linked to genetic admixture.

Bon Céline (1), Thomas Aline (2), Dessarps Françoise (1), Lafosse Sophie (3), Sauvage Juliette, Delvigne Marine

1 - Eco-Anthropologie et Ethnobiologie (France), 2 - éco-Anthropologie (Musée de l'Homme - 17, place du Trocadéro - 75016 Paris France), 3 - Eco-Anthropologie et Ethnobiologie (France)

The spread of agriculture in Europe is an emblematic episode of prehistoric

migrations. While the material data from the early Neolithic testify to an off-ground culture from exogenous origin, the paleogenetic data record the diffusion of Anatolian populations to the western reaches of the European continent. Recent research clarifies the relationships between migrant farmers and local hunter-gatherers. At the European scale, ancestry from Western European Mesolithic Hunter Gatherer (WEHG) increases in Neolithic farmer genomes through time, with various proportions in local populations. In the Paris Basin, the Cerny Culture (5th mill BC) flourishes during Middle Neolithic. Despite an overall farmer context, Cerny graves exhibits items linked to the wild world and hunting. Scholars interpret this contrast as a «Mesolithic resurgence» suggesting a late acculturation of hunter-gatherers. For better understanding Cerny's demographic dynamics, we obtained whole genome data from a dozen of individuals from different archaeological sites and compared to previously published genomes from European Mesolithic and Neolithic individuals. On a biological point of view, Cerny individuals are strongly related to Early Neolithic farmers (especially from LBK contexts) with a low amount of WEHG ancestry. According to our data, the changes in the burial ideology at the beginning of the Middle Neolithic in the Paris Basin do not coincide with a biological admixture with descendants of Mesolithic hunter-gatherers. If these changes were, however, linked to a late «Mesolithic resurgence» phenomenon, this implies that the acculturation was carried out without crossbreeding.

Reconstructing ancient traits and recovering variants under positive selection at the Mesolithic-Neolithic transition using 1,490 ancient imputed genomes

Martinez Refoyo (1) Racimo Fernando (1), Willerslev Eske (1, 2, 3, 4), Nielsen Rasmus (1, 5, 6), Kristiansen Kristian (7), Delaneau Olivier (8, 9), Allentoft Morten E. (1, 10), Sikora Martin (1), Irving-Pease Evan K (1), Consortium Geogenetics Mesoneo (1)

1 - Lundbeck Foundation GeoGenetics Centre, Globe Institute, University of Copenhagen, Copenhagen, Denmark. (Denmark), 2 - The Wellcome Trust Sanger Institute, Cambridge, UK (United Kingdom), 3 - The Danish Institute for Advanced Study, University of Southern Denmark, Odense, Denmark (United Kingdom), 4 - Department of Zoology, University of Cambridge, Cambridge, UK (United Kingdom), 5 - Department of Statistics, UC Berkeley, Berkeley, CA, USA (United States), 6 - Department of Integrative Biology, UC Berkeley, Berkeley, CA, USA (United States), 7 - Department of Historical Studies, University of Gothenburg, Gothenburg, Sweden (Sweden), 8 - Swiss Institute of Bioinformatics (SIB), University of Geneva, Geneva, Switzerland. (Switzerland), 9 - Institute of Genetics and Genomics in Geneva, University of Geneva, Geneva, Switzerland (Switzerland), 10 - Trace and Environmental DNA (TrEnD) Laboratory, School of Molecular and Life Sciences, Curtin University, Perth, Western Australia, Australia (Australia)

Between 12,000 and 5,000 years ago, Western Eurasia underwent a dramatic social transformation: the development of agricultural practices in the Middle East was followed by a mass movement of people from Anatolia into Europe. This process - known as the Mesolithic-Neolithic transition - had major consequences for human societal organization, cultural practices, health and genetics. To understand its genomic impact across time and space, we generated a dataset of 318 ancient

genomes from this period at an average of 0.75X coverage. The dataset was combined with other published sequences, and a total of 1,490 ancient genomes were imputed and phased using present-day reference panels. We then reconstructed polygenic scores for phenotypes of ancient hunter-gatherers, farmers and steppe nomad populations, using effect size estimates from genome-wide association studies conducted on the UK Biobank. We found that the most significantly over-dispersed scores correspond to variants associated with traits related to pigmentation, anthropometric traits and disorders associated to diet and sugar levels, suggesting strong population trait differences preceding the transition, followed by trait homogenization via subsequent admixture. We also looked for strong episodes of positive selection on particular genetic variants, and recovered several novel candidate genes involved in cardiovascular disorders, and glucose and lipid metabolism. Overall, this dataset provides a highly detailed picture of changes in genetic variation over several millennia, and of ancestral relationships between ancient humans and humans living in Western Eurasia today.

The challenges and new developments in the field of sedimentary ancient DNA (sedaDNA), paleoecology and paleoclimate reconstruction

Nitrogen palaeo-isoscapes: Exploring drivers of change in Late Pleistocene environments using archaeological isotope data

Reade Hazel (1), Stevens Rhiannon (1)

1 - UCL Institute of Archaeology, London (United Kingdom)

A significant decline in bone collagen $\delta^{15}\text{N}$ during the late glacial has been observed in multiple herbivore species from a wide range of mid and high latitude environments. The decline, recently termed the Late Glacial Nitrogen Excursion (LGNE), has been linked to increased moisture based on an observed inverse relationship between foliar $\delta^{15}\text{N}$ and rainfall in the modern environment. However, the terrestrial nitrogen cycle, and in turn $\delta^{15}\text{N}$ values in biological material, are influenced by a range of complex and often interconnected environmental and climatic drivers. Further, herbivore bone collagen $\delta^{15}\text{N}$ represents an integrated record of these climatic and environmental drivers, mediated by species ecology and niche partitioning. As the body of late Pleistocene faunal $\delta^{15}\text{N}$ data continues to grow at an exponential rate, significant new opportunities to investigate the LGNE in greater detail are becoming available. Here, we present results from a previously unused method of investigation; time-sliced palaeo-isoscape mapping. We combine newly generated and previously published herbivore bone collagen $\delta^{15}\text{N}$ data from late Pleistocene and early Holocene European contexts (c. 50,000 - 8,000 years BP, 38°N - 58°N, 10°W - 20°E) and assess changing spatial gradients of $\delta^{15}\text{N}$ through time. Integrating this analysis with high resolution palaeoclimatic, environmental and ecological data, we explore potential drivers of the LGNE, with implications for understanding the landscapes in which Late Palaeolithic populations operated within.

sedaDNA of Doggerland: a reconstruction of the early Holocene palaeoenvironment using emerging taxonomic assignment, ecological, and authentication methods

Allaby Robin, Cribdon Becky, Ware Roselyn, Everett Rosie, Gaffney Vincent

1 - University of Warwick, Coventry (United Kingdom)

Until ~7000-8000 years ago, much of the southern North Sea was a coastal plain now named Doggerland. Evidence from surrounding countries and occasional artefact finds suggest that Doggerland was inhabited, and it is expected to be a rich source of archaeological information. We are part of Europe's Lost Frontiers, a multi-disciplinary project reconstructing the early Holocene landscape. Our work focuses on shotgun sequencing of sedimentary ancient DNA (sedaDNA) from seabed cores. DNA data was interpreted using three emerging methods. First, we assigned sequence reads to taxa using Phylogenetic Intersection Analysis (PIA). Designed for non-microbial shotgun data, PIA works from standard BLAST output while accounting for the limitations of BLAST and DNA databases, achieving 96% accuracy when the original organism is not represented. Second, we use estimated genome size to

convert read counts into a more ecologically-relevant proxy, termed biogenomic mass. Finally, we authenticate metagenomic reads using age-associated DNA damage patterns with the new tool MetaDamage. This finds appropriate reference sequences for individual input reads, removing the need to align all input reads to a single reference genome. Plant and animal DNA profiles suggest the cores have captured environments through the inundation process. There are woodland and freshwater profiles; some are grass-dominated and may represent reed beds; others combine seagrasses and small terrestrial signals to imply a brackish coastal environment. We also present individual taxa indicative of wider environmental changes, such as climate and potentially human activity.

From theoretical processes to the reality of the depositional environment: The challenges of understanding taphonomic processes for sedimentary DNA (sedaDNA) interpretation

Everett Rosie (1), Gearey Benjamin (2), Cribdon Becky (1), Gaffney Vince (3), Allaby Robin (1)

1 - University of Warwick (United Kingdom), 2 - University College Cork (Ireland), 3 - University of Bradford (United Kingdom)

The process for extracting, sequencing, and taxonomic analysis of DNA extracted from sedimentary sequences (sedaDNA) is a well-established discipline within ancient DNA research. As a complementary tool to conventional proxy analysis used in palaeoecological reconstruction (e.g. pollen), sedaDNA has not only demonstrated its capabilities to support and improve on resolution of plant taxa, but also to extend the questions that can be addressed by palaeoenvironmental research. However, understanding the role of taphonomic processes - the processes which determined depositional and post-depositional mechanisms that allow for preservation of a proxy archive - is poorly understood for sedaDNA within certain depositional environments. In this paper, we examine two case-studies of sequences from fluvial and intertidal depositional environments analysed using conventional proxies (pollen and diatoms) with supporting sedaDNA analysis, and attempt to examine the output within the framework of key processes that drive taphonomic research - i.e source, deposition, post-depositional movement. Conventional proxy taphonomy has been a discipline in the making over the last 50 years and pre- and post-depositional processes are better understood with ongoing research. This paper does not attempt to answer all questions in relation to the preservation potential of sedaDNA within specific depositional environments. However, it does aim to highlight some of the key issues in analytical approaches for identifying processes of taphonomy in sedaDNA assemblages, and the nuances in trying to develop models to predict sedaDNA preservation within complex, depositional environments.

Microstratigraphic preservation of ancient DNA in sediment

Massilani Diyendo (1), Morley Mike (2), Mentzer Susan (3), Aldeias Vera (4), Miller Christopher (3), Goldberg Paul (5), Roberts Richard (6), Stahlschmidt Mareike (1), Essel Elena (1), Nagel Sarah (1), Richter Julia (1), Nickel Birgit (1), Slon Viviane (7), Meyer Matthias (1)

1 - Max Planck Institute for Evolutionary Anthropology (Germany), 2 - Flinders University of South Australia (Australia), 3 - Tübingen University (Germany), 4 - University of Algarve Campus de Gambelas, Faro (Portugal), 5 - Boston University, Boston (United States), 6 - University of Wollongong (Australia), 7 - Tel Aviv University, Tel Aviv (Israel)

The recovery of ancient hominin DNA from Pleistocene cave sediments indicates that sediment DNA analysis may help to complete the map of past human occupation and reveal where and when different hominin groups may have overlapped and interacted. However, little is known about the sources of ancient mammalian DNA, and in particular hominin DNA, in archaeological sediments, and its mode of preservation. To investigate the microstratigraphic preservation of ancient DNA in sediment we take advantage of resin-impregnated sediment blocks, used by geoarchaeologists to study soil and sediment micromorphology (microstratigraphy) in thin section, to reconstruct site formation processes and to micro-contextualise anthropogenic remains. We assess the extent to which the process of resin impregnation interferes with ancient DNA recovery and combine micromorphological and genetic analyses to investigate patterns of aDNA preservation in sediment. We observe a strong heterogeneity in the taxonomic composition of mammalian DNA in sampling spots located only a few millimeters apart, indicating that the DNA is primarily concentrated in localized particles rather than being uniformly distributed throughout the sediment. Sampling sediment from micromorphology blocks allows us to combine micromorphological and genetic data, make predictions about microstratigraphic characteristics more conducive for aDNA preservation and provide means for evaluating whether DNA leaching or other disturbances such as bioturbation confound genetic time series data. Our work opens new possibilities for studying ancient DNA preservation in intact archaeological sediments and may help to develop approaches to optimize the recovery of ancient DNA from specific taxa from sediment.

Did hot pot make us humans? A geochemical approach to the paleolandscape of Olduvai Gorge at the emergence of the Acheulean, 1.7ma.

Sistiaga Ainara (1, 2), Husain Fatima (1), Uribelarrea David (3), Martin-Perea David (3), Diaz-Martin Fernando (4), Baquedano Enrique (5), Mabulla Audax (6), Domínguez-Rodrigo Manuel (7), Summons Roger (1)

1 - Department of Earth, Atmospheric and Planetary Science, MIT, Cambridge (United States), 2 - University of Copenhagen, Københavns Universitet (Denmark), 3 - Complutense University of Madrid (Spain), 4 - Universidad de Valladolid, Valladolid (Spain), 5 - Museo Regional de Madrid (Spain), 6 - National Museums of Tanzania (Tanzania), 7 - Universidad de Alcalá, University of Alcalá (Spain)

Disentangling the influence of environment on human evolution is essential for understanding early hominin behavior. Traditional landscape reconstructions at Olduvai Gorge have revealed the presence of precession-driven wet-dry cycles atop a general aridification trend, but might underestimate the effect of local-scale conditions, likely more dynamic. Here, we employ fossil lipid biomarkers to study the paleolandscape at Olduvai Gorge at the emergence of the Acheulean technology, 1.7 Ma. Our analysis reveals the presence of a rich mosaic ecosystem, with

groundwater-fed rivers, aquatic plants, and hydrothermal features, which may have supported remarkable biodiversity in an increasingly arid environment. The study of hydrothermalism in ancient settings and its impact on hominin evolution has not been addressed before, although the association of thermal springs in the proximity of archaeological sites documented here can also be found at other localities. Our data supports the presence of an aquatic-dominated landscape with hydrothermal features that offered hominins new opportunities to hunt and cook readily available tubers and herbivore prey at the emergence of the Acheulean technology. Although use of fire at this time is controversial, to cook the only requirement is heat. In areas with strong geothermal and tectonic activity hot springs may have provided an alternative way to thermally process food that would have required minimal effort, which, at the same time, would have decreased digestibility-reducing components of starches. Cooking may, thus, have had a simple pre-fire stage during human evolution.

Records of past permafrost thaw preserved in fossil bone sulphur isotope signatures?

Stevens Rhiannon (1), Reade Hazel (2), Sayle Kerry (3), Tripp Jennifer (4), Higham Tom (5)

1 - UCL Institute of Archaeology (31-34 Gordon Square, London WC1H 0PY United Kingdom), 2 - UCL, Institute of Archaeology (United Kingdom), 3 - NERC Life Sciences Mass Spectrometry Facility, Glasgow (United Kingdom), 4 - University of San Francisco (United States), 5 - RLAHA, school of archaeology, University of Oxford (United Kingdom)

Sulphur isotope ratios ($\delta^{34}\text{S}$) in plant, human and animal tissues are increasingly used to explore food provenance, present and past diets, and ancient human and animal mobility. Our research, however, suggests that environmental conditions can sometimes be the primary driver of plant and therefore animal $\delta^{34}\text{S}$ values. Our data shows high-magnitude temporal changes in animal bone collagen $\delta^{34}\text{S}$ at the end of the last ice age that are unidirectional, occur in multiple species (with differing dietary niches and mobility behaviours) and in multiple different geographic areas. These records are observed in regions of known permafrost presence during the Last Glacial Maximum, and the excursions coincide with the suspected timing of localised permafrost development and thaw. The magnitude of change observed is well beyond that reported from other environmental settings. The commonly invoked interpretations of changing animal diet and mobility fail to adequately explain the data; the continental wide pattern can only be adequately explained through regional scale environmental drivers. We hypothesise the high-magnitude changes in faunal $\delta^{34}\text{S}$ from these palaeo-permafrost environments are driven by biogeochemical cycling related to soil redox and permafrost thaw. Faunal collagen preserves well in the fossil record and for samples from the last c. 50,000 years can be directly radiocarbon dated, offering an unrivalled prospect for developing high-resolution, tightly chronologically-constrained records of past permafrost change. Furthermore, our findings indicate terrestrial herbivore $\delta^{34}\text{S}$ values can reflect local hydrological conditions, complicating the use of sulphur isotopes as a tool for food origin, animal migration, and archaeological mobility research.

Large-scale statistical analyses to explore the impact of transitions on human phenotypes and population structures

Human mobility at the Roman Danubian Limes before and after the fall of the Empire

Olalde Inigo (1), Carrión Quilis Pablo (1), Rohland Nadin (2, 3), Grbic Miodrag (4), Miladinović-Radmilović Nataša (5), Tomanović Željko (6), Keckarević Dušan (6), Mikić Ilija (5), Lalueza-Fox Carles (1), Reich David (2, 3, 7, 8)

1 - Institut de Biologia Evolutiva, Barcelona (Spain), 2 - Department of Genetics, Harvard Medical School (United States), 3 - Broad Institute of Harvard and MIT (United States), 4 - University of Western Ontario (United States), 5 - Institute of Archaeology, Belgrade (Serbia), 6 - University of Belgrade (Serbia), 7 - Department of Human Evolutionary Biology, Harvard University (United States), 8 - Howard Hughes Medical Institute, Harvard Medical School (United States)

At its peak, the Roman Empire unite all Mediterranean shores under the same rule and law. This, together with great improvements in long-distance communications, brought human mobility across the Mediterranean to an unprecedented scale. From all the areas under Roman control, the Balkans is particularly interesting as it was the midpoint connecting the Western and the Eastern parts of the Empire. In this project, we have extracted and analyzed genome-wide from ancient Roman and post-Roman individuals (n=69) from 3 settlements located in present-day Serbia; most importantly the capital of Moesia Superior Roman province, Viminacium. Genetic and radiocarbon dating analyses results point to a high degree of cosmopolitanism in Viminacium during the early imperial period. We observe two major groups of individuals: one with a local ancestral signature likely deriving from Balkan Bronze and Iron Age populations, and other with Near Eastern ancestral origin, suggesting strong population movements from the Eastern parts of Empire impacting not only Rome, but also other major cities like Viminacium. Moreover, we detect remarkable cases of human mobility across the Saharan and the Mediterranean, such as a young male, possibly a legionary, whose ancestral origins lie in Eastern Africa. Beginning in the IV century CE, we detect a clear influx of Eastern European ancestry that continues through the Early Medieval period and represents the final step in the formation of present-day Balkan populations. These results highlight how dense samplings at specific sites can provide a detailed view on both individual and large-scale human mobility patterns.

Ancestry-aware modelling of the evolution of GWAS variants in a large cohort of ancient genomes

Irving-Pease Evan (1), Pearson Alice (2), Stern Aaron J. (3), Sikora Martin (1), Allentoft Morten E. (1), Nielsen Rasmus (4, 5), Durbin Richard (2), Willerslev Eske (1) (6), Racimo Fernando (1)

1 - Globe Institute, University of Copenhagen (Denmark), 2 - Department of Genetics, University of Cambridge (United Kingdom), 3 - Graduate Group in Computational Biology, University of California, Berkeley (United States), 4 - Department of Integrative Biology, University of California, Berkeley (United States), 5 - Department

of Statistics, University of California, Berkeley (United States), 6 - Department of Zoology, University of Cambridge (United Kingdom)

The Neolithic transition was one of the most significant changes in human society, with far-reaching consequences for the diet, health and social organisation of affected populations. These dramatic changes in lifestyles were likely concomitant with shifts in the selective pressures exerted on a wide range of traits and newly emergent diseases. Whilst genome-wide association studies (GWAS) of present-day populations have identified large numbers of genetic variants associated with phenotypes of interest, the extent to which these variants have been under positive selection during recent human evolution remains unclear. Here, we reconstruct the allele frequency trajectories and selection coefficients of tens of thousands of GWAS variants through time, using a panel of >1,600 ancient phased whole-genomes-sampled from across West Eurasia throughout the Holocene-combined with thousands of published present-day genomes. To account for population structure in our samples, we applied a novel chromosome painting technique that allows us to accurately assign ancestral population labels to haplotypes found in both ancient and present-day individuals. By conditioning our selection analyses on these haplotype backgrounds, we infer selection trajectories of GWAS variants in a manner that is invariant to changes in the admixture proportions through time. Our results reveal that some variants were universally selected across ancestries, while many others were only selected in particular ancestral backgrounds. These ancestry specific trajectories reveal many novel aspects about the differing contributions made by hunter-gatherers, Neolithic farmers and steppe pastoralists to present-day phenotypes, and highlight the interplay between selection and admixture in Holocene West Eurasia.

Validating polygenic risk prediction using ancient DNA

Hui Ruoyun (1, 2), Scheib Christiana (1, 3), Inskip Sarah (1, 4), Cessford Craig (5), Robb John (1), Kivisild Toomas (6)

1 - University of Cambridge (United Kingdom), 2 - The Alan Turing Institute (United Kingdom), 3 - Institute of Genomics [Tartu, Estonia] (Estonia), 4 - University of Leicester (United Kingdom), 5 - Cambridge Archaeology Unit (United Kingdom), 6 - KU Leuven (Belgium)

Anthropometric traits in the human skeleton can reveal rich information about the experience of the individual. For example, stature estimation obtained from long bones have been widely used as an indicator for population health and nutrition. It is also well known that these traits are influenced by genetics: in modern populations, the heritability of height and bone mineral density is estimated to be around 0.8 and 0.5, respectively. Although transferring the results of modern genetic association studies into historical populations faces many difficulties, anthropometric analysis could benefit from incorporating genetic information. We have shown that common variants in the genome can be imputed with high accuracy from very low-coverage shotgun sequencing data. We compare various methods of polygenic prediction regarding their accuracy and sensitivity to imputation errors, using height in UK Biobank as an example. The result suggests that the best-performing polygenic prediction can explain >30% of variation in height even if the genetic sequencing

coverage is as low as 0.05x. Using ancient DNA sequences and skeletal measurements from sites in medieval Cambridge, we explore how they can be combined to model the disparities between social groups. Although our sample size is limited compared to the complexity of the question, we hope it illustrates the benefits of combining evidence and provides inspiration for future studies.

An interdisciplinary study of the social structure from the early Neolithic to the early Bronze Age in central Germany

Penske Sandra (1), Küßner Mario (2), Nováček Jan (2), Friederich Susanne (3), Meller Harald (3), Krause Johannes (1), Haak Wolfgang (1)

1 - Max Planck Institute for the Science of Human History, 07745 Jena (Germany), 2 - Thüringisches Landesamt für Denkmalpflege und Archäologie, 99423 Weimar (Germany), 3 - Landesamt für Denkmalpflege und Archäologie Sachsen-Anhalt, 06114 Halle(Saale) (Germany)

Past archaeogenetic studies have revealed two major genetic turnover events during Europe's prehistory. The first event is described by a change of the genetic makeup at the transition from a hunter-gatherer to a farming subsistence. The second one is linked to the arrival of 'Steppe ancestry' during the early Bronze Age. However, the fine-scale population processes, timing and speed of these major changes, are not well understood. In a novel interdisciplinary approach, we integrate genetics, archaeology, anthropology, isotope analysis and new modelling approaches to answer detailed questions about mobility, migration, social structure, kinship and pedigree of prehistoric societies in Central Germany. By analyzing new genome-wide data for an additional 138 individuals from central Germany, ranging from the early Neolithic to the late Bronze Age (6290-2960 calBP), we aim to address these questions. The genetic results are consistent with previous findings about German early and middle Neolithic individuals. During the Late Neolithic, Yamnaya-related steppe ancestry arrives in Central Europe and leads to the spread of the Corded Ware Cultural Complex. Here, we present the earliest dated Corded Ware-associated individuals known from Central Germany (~2700 calBP) and can confirm a rapid spread of this ancestry profile. In addition, we also investigated closed late Neolithic and early Bronze Age burial sites by integrating genetic, isotopic, archaeological and anthropological data. We observe an unexpectedly high amount of first-degree genetic relatives. The resulting demographic profile and kinship structure suggests a patrilocal society with a low variability in male Y-haplogroups in contrast with more varied mt-haplogroups.

Unraveling the genetic network of Bronze Age populations: complex genomic structures in the 3000-800 BC East-Central Europe

Szecsényi-Nagy Anna (1), Eszter Melis (2), Cheronet Olivia (3), Hajdu Tamás (4, 5), Szeniczey Tamás (4, 5), Dani János (6), Ernée Michal (7), Dobeš Mirek (7), Velemínský Peter (8), šefčáková Alena (9), Teschler-Nicola Maria (10, 11), Pinhasi Ron (11), Haak Wolfgang (12), Mende Balázs (1), Kulcsár Gabriella (2), Kiss Viktória (2), Reich David (13, 14)

1 - Institute of Archaeogenomics, Research Centre for the Humanities, Eötvös

Loránd Research Network, Budapest (Hungary), 2 - Institute of Archaeology, Research Centre for the Humanities, Eötvös Loránd Research Network, Budapest (Hungary), 3 - Department of Evolutionary Anthropology, University of Vienna, Vienna (Hungary), 4 - Eötvös Loránd University, Faculty of Science, Institute of Biology, Department of Biological Anthropology, Budapest (Hungary), 5 - Hungarian Natural History Museum, Department of Anthropology, Budapest (Hungary), 6 - Déri Museum, Debrecen (Hungary), 7 - Czech Academy of Sciences, Institute of Archaeology, Praha (Czech Republic), 8 - Department of Anthropology, The National Museum, Praha (Czech Republic), 9 - Department of Anthropology, Slovak National Museum - Natural History Museum (Slovakia), 10 - Natural History Museum Vienna (Austria), 11 - Department of Evolutionary Anthropology, University of Vienna, Vienna (Austria), 12 - Department of Archaeogenetics, Max Planck Institute for Evolutionary Anthropology, Leipzig (Germany), 13 - Department of Genetics, Harvard Medical School, Boston (United States), 14 - Department of Human Evolutionary Biology, Harvard University, Boston (United States)

After 3000 years of Neolithic and Copper Age periods, the Early Bronze Age societies of East-Central Europe developed new cultural contacts and experienced drastic population transformations in the 3rd millennium BC. Little is known however about these newly developed communities of the Carpathian Basin from population genetic aspects: Beaker Complex associated population brought diversification to the central part of the Carpathian Basin between 2500-2200 BC (Olalde et al. 2018), and the southern fringe of the region experienced new steppe-related genetic ancestry as well (Mathieson et al. 2018). We present in this paper, through genome-wide SNP analyses of over 240 unpublished individuals, how the steppe-related ancestry radiated, and how it reached the different communities of the Early Bronze Age Carpathian Basin from the northwest, east and the south. We observe strong regionalization in certain parts of the study area, whereas others show long-distance connections to various directions. In addition, intensive sampling of the Early Bronze Age graveyards in the northwestern part of the study region sheds light on the social structure of the communities. Between 2000-1500 BC the cremation funeral rite became predominant in the central Carpathian Basin, and this fact only let us observe scattered signs of the populations' genetic composition. Despite such obstacles, differences and clines of steppe-related ancestry are still observable in this period, whose populations show continuity to the Late Bronze Age and subsequent Iron Age population of the region.

Inferring and simulating spatiotemporal movement using ancient and sedimentary DNA

Racimo Fernando (1), Petr Martin (1), Wang Yucheng (2), Sikora Martin (1), Woodbridge Jessie (3), Fyfe Ralph (3), Kristiansen Kristian (4), Vander Linden Marc (5), Willerslev Eske (1), Lindgren Finn (6)

1 - Lundbeck GeoGenetics Centre, Globe Institute, University of Copenhagen (Denmark), 2 - University of Cambridge (United Kingdom), 3 - Plymouth University (SoGEES) (Drake Circus, Plymouth, PL48AA United Kingdom), 4 - Department of Historical Studies, University of Gothenburg (Sweden), 5 - Department of Archaeology and Anthropology, Bournemouth University (United Kingdom), 6 -

School of Mathematics, University of Edinburgh (United Kingdom)

The sequencing of ancient biomolecules provides the opportunity to study population genomic processes as they unfolded in time and space. Here, I will describe several methods developed in our group to relate spatiotemporal genomic observations to informative parameters about migration, mobility and changes in species ranges. First, I will present a way to model the spread of ancestry in ancient genomes through time and space, along with a new simulation framework for modeling admixture processes in both time and space. We have recently applied these methods to a new dataset containing thousands of ancient human genomes and inferred the geographic spread of major population movements in the past 13 millennia of Western Eurasian history. I will also present a model to jointly infer changes in species distributions, using a combination of sedimentary ancient DNA data, fossil data and paleo-climate records. We have applied this model to an arctic Pleistocene mega-fauna dataset and demonstrate that incorporating different types of data allows us to evince patterns in species distributional changes that would not be visible if these were studied in isolation.

Human evolution, mobility and social change outside western Europe

Multiple isotopic and aDNA analyses of Nevali Cori shed light on a socio-section of ancient southeast Anatolia

Wang Xiaoran (1), Skourtanioti Eirini (2), Schultz Michael (3), Roberts Patrick (2), Krause Johannes (4, 5), W. Stockhammer Philipp (1, 6)

1 - Institute for Pre- and Protohistoric Archaeology and Archaeology of the Roman Provinces, Ludwig Maximilian University, Munich (Germany), 2 - Department of Archaeology, Max Planck Institute for the Science of Human History (Germany), 3 - Department of Anatomy, University of Göttingen (Germany), 4 - Faculty of Biological Sciences, Friedrich Schiller University (Germany), 5 - Department of Archaeogenetics, Max Planck Institute for Evolutionary Anthropology (Germany), 6 - Department of Archaeogenetics, Max Planck Institute for the Science of Human History (Germany)

Nevali Cori in Southeast Anatolia, the eastern wing of the Fertile Crescent, is associated with a socio-economic system of Pre-pottery Neolithic B (PPNB) of the 10th-8th millennium BC. Together with other sites in the eastern upper Tigris region, southeast Anatolia became another independent region with one of the earliest Neolithic lifestyles in sedentism and domestication. The site was repeatedly settled also later during the Halaf period, the Early Bronze and the Iron Age. In my paper, I will first present the results of strontium and oxygen isotopic analyses conducted on human and animal remains recovered from Nevali Cori. The results indicate a decline of mobility already during early PPNB. The carbon and oxygen data also revealed the seasonal hunting times of gazelles in that time. The carbon and nitrogen analysis informs about nutritional practices through time indicating changes in subsistence strategies. This enables a better understanding of the underlying mechanisms and motivations of Neolithization in west Asia. The genetic analyses of the PPNB people in Nevali Cori shed new light on the genomic structure of aceramic southeast Anatolian farmers and their genetic relations to adjacent Anatolian and Levantine regions. The post-PPNB inhabitants of Nevali Cori showed a different genetic makeup compared to the PPNB people related to the admixture event of regional gene pools across Anatolia and the Southern Caucasus during the Late Neolithic.

Population history of ancient Wallacea

Carlhoff Selina (1, 2), Oliviera Sandra (2), Nägele Kathrin (2), Pugach Irina (2), Koesbardiati Toetik (3), Hübner Alexander (1, 2), Meyer Matthias (2), Oktaviana Adhi A. (4), Takenaka Masami (5), Katagiri Chiaki (6), Hawkins Stuart (7), Kinaston Rebecca (8), Bellwood Peter (7), Ono Rintaro (9), Powell Adam (2), Krause Johannes (2), Posth Cosimo (1, 10), Stoneking Mark (2)

1 - Max Planck Institute for the Science of Human History (Germany), 2 - Max Planck Institute for Evolutionary Anthropology (Germany), 3 - Department of Anthropology, Airlangga University Indonesia (Jl. Dharmawangsa Dalam Surabaya Indonesia), 4 - The National Research Center for Archaeology, Jakarta (Indonesia), 5 - Kagoshima

Women's College, Kagoshima (Japan), 6 - Institute of Comparative Social Culture, Kyushu University (Japan), 7 - School of Archaeology and Anthropology, College of Arts and Social Sciences, Australian National University, Canberra (Australia), 8 - Department of Anatomy, School of Medical Sciences, University of Otago, Otago (New Zealand), 9 - Department of Human and Civilization, Minpaku National Museum of Ethnology, Osaka (Japan), 10 - Archaeo- and Palaeogenetics, Institute for Archaeological Sciences, University of Tübingen (Germany)

Much remains unknown about the population history of Wallacea, where the archaeological record is sparse, and the tropical climate is adverse to the preservation of ancient DNA. Located between the Southeast Asian mainland and Near Oceania, this region has been shaped by complex demographic interactions between previously established communities and dispersals from the Asian continent. In this project, we report genomic data from ancient individuals excavated from various islands in Wallacea and found that these ancient individuals indeed carry a mixed ancestry profile. In addition to ancestry related to the first wave of anatomically modern humans arriving in the area, we find a component related to present-day Austronesian speakers. Admixture time estimates for the oldest individuals from the northern islands around 2,800 BP are close to archaeological estimates, while younger individuals show younger admixture times indicating continuous admixture or multiple pulses involving both Asian and Papuan-related groups. Our results illustrate the value of ancient DNA data and fill an important gap in the reconstruction of movement across Wallacea.

Ancient Rapanui genomes reveal pre-European trans-Pacific contact with Indigenous Americans

Moreno-Mayar Victor (1, 2, 3), De Sousa Mota Bárbara (2, 3), Klemm Signe (4), Gorman Edmunds Moana (5), Cruz Dávalos Diana Ivette (2, 3), Neuenschwander Samuel (2, 6), Stenderup Jesper (1), Friess Martin (7), Hochstetter Francisco Torres (8), Allentoft Morten E. (1, 9), Schroeder Hannes (4), Malaspinas Anna-Sapfo (2, 3)

1 - Lundbeck Foundation GeoGenetics Centre, Globe Institute, University of Copenhagen, Copenhagen, (Denmark), 2 - Department of Computational Biology, University of Lausanne, (Switzerland), 3 - Swiss Institute of Bioinformatics, 1015 Lausanne, (Switzerland), 4 - GLOBE Institute, University of Copenhagen, Copenhagen, (Denmark), 5 - Ka Haka Hoki Mai Te Mana Tupuna, (Rapa Nui), 6 - Vital-IT, Swiss Institute of Bioinformatics, 1015 Lausanne, (Switzerland), 7 - Département Homme et Environnement. Muséum national d'Histoire naturelle – Musée de l'Homme, (France) - 8 - Museo Rapa Nui, Sector Tahai, (Rapa Nui), 9 - Trace and Environmental DNA (TrEnD) laboratory. School of Molecular and Life Sciences. Curtin University, Perth, WA, 6102, (Australia)

Rapa Nui in Polynesia is one of the most isolated places in the world inhabited by humans. Archaeological and genetic evidence show that Polynesians first settled the island from the west after ~1200 AD. The possibility of trans-Pacific contact-pre-dating European presence in Rapa Nui, which started in 1722-between the Polynesian ancestors of the Rapanui and Indigenous American populations has remained contentious for decades. Recently, analyses of genome-wide data from

present-day individuals have contributed evidence for such contact. However, these findings are still disputed since they are based on genetic data post-dating the time Europeans first set foot on the island. We sequenced the genomes of 15 ancient Rapanui to an average depth between 0.4 and 5.5X. We find that ancient and present-day Rapanui are similar as both are most closely related to present-day Polynesians. Moreover, we estimate both bear, on average, 8.8% Indigenous American-related ancestry. However, in contrast to present-day individuals, we could not detect European admixture in ancient Rapanui. We leverage the genome data from ancient Rapanui to estimate the date of admixture between their ancestors and Indigenous American populations. Using methods that rely either on admixture linkage disequilibrium patterns or on the length distribution of local ancestry tracts for inference, we estimate an admixture date >20 generations before the time in which the ancient individuals lived. These results provide evidence for non-European-mediated trans-Pacific contact, an outstanding event in recent history, yet a likely possibility in the context of the Polynesian eastward expansion.

Immigration and Locality at Alalakh, a 2nd millennium BC city in the northern Levant

Eisenmann Stefanie (1), Ingman Tara (2), Skourtanioti Eirini (1), Akar Murat (3), Ilgner Jana (1), Gneccchi Ruscone Guido Alberto (1), Le Roux Petrus (4), Shafiq Rula (5), Neumann Gunnar U. (1), Keller Marcel (6), Freund Căcilia (1), Marzo Sara (1), Lucas Mary (1), Krause Johannes (1, 7), Roberts Patrick (1), Yener K. Aslihan (8), Stockhammer Philipp W. (1, 9)

1 - Max Planck Institute for the Science of Human History (Germany), 2 - Koç University Research Center for Anatolian Civilizations (ANAMED), Istanbul (Turkey), 3 - Department of Archaeology, Mustafa Kemal University, Alahan-Antakya (Turkey), 4 - Department of Geological Sciences, University of Cape Town (South Africa), 5 - Anthropology Department, Yeditepe University, Istanbul (Turkey), 6 - Estonian Biocentre, Institute of Genomics, University of Tartu (Estonia), 7 - Max Planck Institute for Evolutionary Anthropology (Germany), 8 - Institute for the Study of the Ancient World (ISAW), New York University (United States), 9 - Institute for Pre- and Protohistoric Archaeology and Archaeology of the Roman Provinces, Ludwig Maximilian University, Munich (Germany)

Tell Atchana, the location of the ancient city of Alalakh, is situated in modern day Turkey at the northernmost fringes of the Levant. During the 2nd millennium BC Alalakh became the capital of a regional kingdom and is featured in numerous textual sources uncovered from the site itself and the wider Near East. It connected the Hittite world to the north with the Egyptian sphere to the south, and Mesopotamia, Assyria, and the northern Syrian territories to the east with the Mediterranean world to the west, taking an active role in what is frequently referred to as the first 'international age'. The extensive burial record known from the site, combined with the far-reaching contacts attested in foreign objects, styles, and architectural features, make Alalakh a prime candidate for mobility studies in the ancient world. The paper presents the first large-scale tandem study of strontium isotope and genome-wide ancient DNA (aDNA) at one single site in the Ancient Near East. Out of a total of 342 burials from inside and outside the settlement excavated up to date, a

representative subset of 53 and 37 individuals were analysed for $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and aDNA respectively with the goal to gain insights into the extent and role of locality and immigration at this urban hub (ca. 2000-1300 BC). While the strontium data is a snapshot of each individual's mobility during their lifetime, aDNA data opens up complementary perspectives into their ancestral past.

Genetic characterization of an Iron Age community from Tuva, Southern Siberia

Keller Marcel (1), Caspari Gino (2, 3), Milella Marco (4), Sadykov Timur (5), Blochin Jegor (5), Szidat Sönke (6, 7), Saag Lehti (1), Kivisild Toomas (1, 8), Metspalu Mait (1), Lösch Sandra (4), Scheib Christiana L. (1, 9)

1 - Institute of Genomics, University of Tartu (Estonia), 2 - Institute of Archaeological Sciences, University of Bern (Switzerland), 3 - Department of Archaeology, University of Sydney (Australia), 4 - Department of Physical Anthropology, Institute of Forensic Medicine, University of Bern (Switzerland), 5 - Institute for the History of Material Culture, Russian Academy of Sciences, St. Petersburg (Russia), 6 - Oeschger Centre for Climate Change Research (OCCR), University of Bern (Switzerland), 7 - Department of Chemistry and Biochemistry, University of Bern (Switzerland), 8 - Department of Human Genetics, KU Leuven (Belgium), 9 - St John's College, University of Cambridge (United Kingdom)

The site of Tunnug1 (Republic of Tuva, Russia) in Southern Siberia includes one of the oldest royal 'Scythian' kurgans known to date (9th c. BCE), as well as a younger funerary complex dating to the Kokel culture (2nd-5th c. CE). The latter, completely excavated between 2018 and 2019, has returned more than 80 individuals from both single and multiple burials. Although most burials show a careful funerary treatment including grave goods, osteological analyses revealed a remarkably high incidence rate of perimortem trauma due to interpersonal violence, interpreted as signs of warfare, executions and/or rituals. Radiocarbon dates on selected burials however indicate that this burial ground is not associated with a single event but was utilized over several centuries. The objectives of this study are to explore the genetic background and internal structure of this population through ancient DNA. Genome-wide sequencing data of more than 50 individuals revealed a strikingly high level of genetic heterogeneity. The observed cline formed by varying proportions of Western hunter-gatherer vs. Han-like ancestry corresponds well with the high mobility of the nomadic Kokel culture and reflects the importance of the Eurasian steppe as a corridor for human migrations. Nevertheless, provisional analyses suggest close kinship relations among the buried individuals, supporting the interpretation as one or multiple social communities. With this in-depth case study on a single site, we gain valuable insights into the demographic history and social structure of a 'Hunno-Sarmatian' population in times of political instability after the decline of the Xiongnu Empire.

Genomic Evidence for At Least Four Prehistoric Migrations to Micronesia

Liu Yue-Chen (1, 2), Hunter-Anderson Rosalind (3), Cheronet Olivia (4), Eakin Joanne (5), Camacho Frank (6), Pietrusewsky Michael (7), Rohland Nadin (1) (8), Bernardos Rebecca (1), Mah Matthew (1, 8, 9), Mandl Kirsten (4), Adamski Nicole (1, 9), Broomandskhoshbacht Nasreen (1, 9), Callan Kimberly (1, 9), Lawson Ann Marie (1) (9), Michel Megan (1, 9), Oppenheimer Jonas (1, 9), Stewardson Kristin (1, 9), Zalzala Fatma (1, 9), Kidd Kenneth (10), Kidd Judith (10), Schurr Theodore (11), Kennett Douglas (12), Patterson Nick (2, 8), Spriggs Matthew (13, 14), Vilar Miguel (15), Lipson Mark (1, 2), Pinhasi Ron (4), Reich David (1, 2, 8, 9)

1 - Department of Genetics, Harvard Medical School, Boston (United States), 2 - Department of Human Evolutionary Biology, Harvard University, Cambridge (United States), 3 - Independent Researcher, Albuquerque (United States), 4 - Department of Evolutionary Anthropology, University of Vienna, Vienna (Austria), 5 - Independent Researcher, Albuquerque (United States), 6 - Department of Biology, University of Guam, Mangilao (Guam), 7 - Department of Anthropology, University of Hawai'i at Mānoa, Honolulu (United States), 8 - Broad Institute of MIT and Harvard, Cambridge, (United States), 9 - Howard Hughes Medical Institute, Harvard Medical School, Boston, (United States), 10 - Department of Genetics, Yale Medical School, New Haven (United States), 11 - Department of Anthropology, University of Pennsylvania, Philadelphia (United States), 12 - Department of Anthropology, University of California, Santa Barbara (United States), 13 - School of Archaeology and Anthropology, The Australian National University, Canberra (Australia), 14 - Vanuatu National Museum, Vanuatu Culture Centre, Port Vila (Vanuatu), 15 - Department of Anthropology, University of Maryland, College Park (United States)

The earliest people of Remote Oceania arrived in the Marianas of western Micronesia 3,500-3,000 BP, but the origins of these pioneers as well as the people who arrived later in Micronesia have been unclear in the absence of genome-wide studies of ancient and modern DNA. We present data for 35 ancient individuals from Guam (~2,500 BP) and Pohnpei (~500 BP), as well as 112 present-day individuals across Micronesia. We document multiple migrations into Micronesia prior to European contact. The ancient Guam individuals descend from an East Asian-related lineage that is a sister group to the one associated with the Lapita culture that entering the southwest Pacific ~3,000 BP. The matrilineal genetic drift of these two lineages is significantly greater than expected, implying matrilineal population structure in these earliest seafarers. Present-day individuals from Guam and Palau derive essentially all their pre-European-contact ancestry from the previously undescribed East Asian-related lineage, and CHamoru (indigenous people of Guam) are distinctive in being the only known Oceanians today who lack admixture from Near Oceania. Some central Micronesians do harbor Lapita-related ancestry, albeit without the New Britain-derived admixture that is widespread in the southwest Pacific and Polynesia today, suggesting the Lapita-related ancestry arrived via a Micronesian-specific process. We obtain insights into the likely source of this Lapita-related ancestry as individuals from Pohnpei and Chuuk harbor 16-33% ancestry from a New Guinea-related source that reflects a previously undescribed migration from the northern fringe of New Guinea. We also find genetic evidence of later Polynesian migration into Micronesia.

Research on extinct animals and conservation genetics

Do different islands shape different babirusa?

Aninta Sabhrina (1, 2), Drinkwater Rosie (1, 2), Priyono Dwi Sendi (3), Syarifa Athena (3), Brace Selina (1), Rossiter Stephen (2), Winarni Nurul (4), Supriatna Jatna (4), Frantz Laurent (2, 5)

1 - The Natural History Museum (United Kingdom), 2 - School of Biological and Chemical Sciences, Queen Mary University of London, London, United Kingdom (United Kingdom), 3 - Wildlife Conservation Society - Indonesia Program (Indonesia), 4 - Research Center for Climate Change, Universitas Indonesia (Indonesia), 5 - Palaeogenomics Group, Department of Veterinary Sciences, Ludwig Maximilian University, Munich (Germany)

*The Wallacea region in Southeast Asia, Indonesia, is one of the few biodiversity hotspots teeming with endemic species such as babirusa (*Babyrousa* spp.). There are currently three species acknowledged, each belongs to distinct island region: the sulawesi babirusa in Sulawesi (*B. celebensis*), the togian babirusa in Togian (*B. togeanensis*), and the hairy babirusa in Maluku (*B. moluccansis*). To understand whether this recent anthropogenic disturbance affects the long-term adaptive potential of the current population, we used whole genome sequences from 20 modern samples (~10x coverage), sampled from Sulawesi and Togian, and two museum specimens (0.2x and 4.6x coverage), originated from the Sula Islands in Maluku Islands, to detect any difference in genome-wide diversity. Using a method that jointly estimates heterozygosity and runs of homozygosity (ROH) while incorporating deamination patterns and mapping qualities, ROHan, we found that sequences from the smaller islands, Togian and Sula, exhibit similar patterns of very low genomic diversity while sequences from Sulawesi tend to have higher genomic diversity. Despite the low sample size from Togian and Sula, the diversity pattern is quite plausible for populations inhabiting small islands for a relatively long period, especially because the Sula sample is likely coming from an older generation than the other samples. Our findings indicate that different islands allow different demographic history for the babirusas all across Wallacea. As a consequence, conservation efforts should aim to properly maintain the available genomic diversity from the different islands.*

Archaeogenomics of Atlantic sturgeon from the Chesapeake - a population recovering from a perilous decline

Przelomska Natalia (1), Balazik Matthew (2), Rick Torben (1), Reeder-Myers Leslie (3), Kistler Logan (1)

1 - Department of Anthropology, National Museum of Natural History, Smithsonian Institution (United States), 2 - Virginia Commonwealth University (United States), 3 - Temple University, Anthropology (United States)

*Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) has been a food resource in North America for at least two thousand years, appearing in Mid-Atlantic Native American archaeological sites as well as sites associated with European colonists in the 1600-1700s. However, industrial-scale fishing activities that followed led to multiple collapses of sturgeon stocks, driving Atlantic sturgeon close to extinction. While census numbers in the Chesapeake area are recovering due to conservation*

efforts, *A. o. oxyrinchus* is still recognized as a species of conservation priority. Moreover, little is known about how the population collapse affected genomic health of Chesapeake Atlantic sturgeon. In our study, we aimed to characterise this in terms of population structuring, genomic diversity and mutation load. We used spinal bones and scutes (modified bony scales) from pre-historic and historic sites along the James River. We supplemented this with a contemporary panel of James River sturgeon individuals, sampled in both spring and fall spawning seasons, as well as individuals from a population in South Carolina. Using a custom designed bait capture kit, we successfully retrieved nuclear DNA sequence data from 20 ancient individuals, half of which could be placed in the context of contemporary genomic data. Our results reveal a striking degree of differentiation between contemporary spring and fall populations in the James River. This structure was likely amplified as a result of strong genetic drift associated with decline in effective population size. Conservation priority-setting for healthy population recovery can benefit from the unique insights gleaned from studies that include archaeological material.

Genomic analyses of the extinct Sardinian dhole (*Cynotherium sardous*) reveal its evolutionary history

Ciucani Marta Maria (1), Kragmose Jensen Julie (2), Sinding Mikkil-Holger S. (3), Smith Oliver (1, 4), Bartolini Lucenti Saverio (5, 6), Rosengren Erika (7), Rook Lorenzo (5), Tuveri Caterinella (8), Arca Marisa (8), Cappellini Enrico (1), Galaverni Marco (9), Randi Ettore (10), Guojie Chunxue (11), Zhang Guojie (11, 12, 13, 14), Sicheritz-Pontén Thomas (1, 15), Dalén Love (16, 17), Gilbert M. Thomas P. (1, 18), Gopalakrishnan Shyam (1, 2)

1 - Globe Institute (Denmark), 2 - Department of Health Technology, Technical University of Denmark (Denmark), 3 - Smurfit Institute of Genetics, Trinity College Dublin (Ireland), 4 - Micropathology Ltd, University of Warwick Science Park, Coventry (United Kingdom), 5 - Dipartimento di Scienze della Terra, Paleo[Fab]Lab, Università di Firenze (Italy), 6 - Sezione di Geologia e Paleontologia, Museo di Storia Naturale, Università degli Studi di Firenze (Italy), 7 - Department of Archaeology and Ancient History, Lund University (Sweden), 8 - Soprintendenza Archeologia, Belle Arti e Paesaggio per le province di Sassari e Nuoro (Ufficio Operativo di Nuoro) (Italy), 9 - WWF Italy, Science Unit (Italy), 10 - Department of Chemistry and Bioscience, Faculty of Engineering and Science, University of Aalborg (Denmark), 11 - Beijing Genomics Institute, Shenzhen (China), 12 - Villum Center for Biodiversity Genomics, Section for Ecology and Evolution, Department of Biology, University of Copenhagen (Denmark), 13 - State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences (China), 14 - Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences (China), 15 - Centre of Excellence for Omics-Driven Computational Biodiscovery (COMBio), Faculty of Applied Sciences, AIMST University (Malaysia), 16 - Centre for Palaeogenetics (Sweden), 17 - Department of Bioinformatics and Genetics, Swedish Museum of Natural History (Sweden), 18 - Norwegian University of Science and Technology, University Museum, Trondheim (Norway)

The Sardinian dhole (Cynotherium sardous) was an iconic and unique canid species of canid that was endemic of Sardinia and Corsica until it became extinct at the end

of the Late Pleistocene. Given its peculiar dental morphology, small body size and high level of endemism, several canids have been proposed as possible ancestors of the Sardinian dhole, including the Asian dhole and African hunting dog ancestor. Morphometric analyses have failed to clarify the evolutionary relationship with other canids. We sequenced the genome of a ca 21,100 year old Sardinian dhole in order to understand its genomic history and clarify its phylogenetic position. We found it represents a separate taxon from all other living canids from Eurasia, Africa and North America, and that the Sardinian and Asian dhole lineages diverged ca 885 ka. We additionally detected historical gene flow between the Sardinian and Asian dhole lineages, that ended approximately 500-300 ka, when the landbridge between Sardinia and mainland Italy was broken, severing their population connectivity. Our sample showed low genome-wide diversity compared to other extant canids - probably a result of the long-term isolation - that could have contributed to the subsequent extinction of the Sardinian dhole.

Unlocking the evolutionary past of sabre-toothed cats through a palaeogenomic lens

Westbury Michael V (1), Barnett Ross (1), Lorenzen Eline D (1), Gilbert M. Thomas P. (1)

1 - Globe Institute (Denmark)

*Sabre-toothed cats have a special place within pop culture, in part due to their impressively large sabre teeth but also due to their lack of living homologs. While there have been many different species of sabre-toothed cats throughout time, the two remnant lineages went extinct at the end of the Late Pleistocene. These included the scimitar-toothed cat (*Homotherium latidens*), and the dirk-toothed cat (*Smilodon* sp.). The relative rarity of these species and lack of closely related living species has limited understanding into their own respective evolutionary histories but also sabre-toothed cats as a whole. However, recent advances in palaeogenomic data production and analyses have enabled a greater understanding of these lost beasts. Here, I will present recent work on sequencing the scimitar-toothed and dirk-toothed cats' genomes and how analyses of these genomes have given new insights into the evolutionary relationship both within sabre-toothed cats and between sabre-toothed cats and extant cat species. More specifically, for the scimitar-toothed cat, I will present both comparative genomic and phylogenomic results giving insights into unique genetic adaptations, levels of genetic diversity, and its phylogenetic relationship to extant cats. As for the dirk-toothed cat, I will present a draft genome and how phylogenomic analyses of this individual with both extinct and extant Felidae can give new insights into the complex evolutionary origins of Felidae.*

Studying selection in the last woolly mammoth population using genome-wide time series data

Dehasque Marianne (1, 2, 3), Pečnerová Patrícia (4), Van Der Valk Tom (5), Kanellidou Foteini (1), Muller Héloïse (6), Danilov Gleb (7), Nikolskiy Pavel (8), Tikhonov Alexei (9), Vartanyan Sergey (10), Díez-Del-Molino David (1, 3),

Götherström Anders (1, 11), Dalén Love (1, 2, 3)

1 - Centre for Palaeogenetics, Stockholm (Sweden), 2 - Department of Bioinformatics and Genetics, Swedish Museum of Natural History Stockholm (Sweden), 3 - Department of Zoology, Stockholm University, Stockholm (Sweden), 4 - Department of Biology, University of Copenhagen, Copenhagen N (Denmark), 5 - Department of Cell and Molecular Biology, National Bioinformatics Infrastructure Sweden, Science for Life Laboratory, Uppsala University, Uppsala (Sweden), 6 - Master de Biologie, École Normale Supérieure de Lyon, Université Claude Bernard Lyon I, Université de Lyon, Lyon (France), 7 - Peter the Great Museum of Anthropology and Ethnography, Kunstkamera, Russian Academy of Sciences, Saint-Petersburg (Russia), 8 - Geological Institute of the Russian Academy of Sciences, Moscow (Russia), 9 - Zoological Institute of Russian Academy of Sciences, Saint-Petersburg (Russia), 10 - North-East Interdisciplinary Scientific Research Institute N.A. Shilo, Far East Branch, Russian Academy of Sciences (NEISRI FEB RAS), Magadan (Russia), 11 - Department of Archaeology and Classical Studies, Stockholm University, Stockholm (Sweden)

Examining the impact of climate change and habitat fragmentation is of fundamental interest in the study of prehistoric populations. While much theoretical work has been conducted on the interaction between selection and drift in small and isolated populations, empirical data on the effect of these genetic processes on population viability remains limited. Furthermore, to what extent populations will be able to adapt to a changing climate remains uncertain. Palaeogenomics has been proposed as a promising tool to study these questions, since genetic processes can be tracked over longer time periods. In this project we use the woolly mammoth as a model system to study real-time selection in declining populations. Woolly mammoths were one of the most common and widespread large herbivores during the last Ice Age. At the onset of the Holocene, however, mammoths became extinct across the mainland but did survive as a small and isolated population on Wrangel Island for another 6,000 years. We study selection in this final mammoth population using genome-wide time-series data from both before and during the dramatic climate changes at the end of the Pleistocene, as well as during the subsequent isolation of mammoths on Wrangel Island. More specifically, we investigate whether woolly mammoths showed signs of selection in response to climate change and the bottleneck event, as well as to living on an island, and whether we can disentangle these signs from genetic drift.

Biomolecular studies of mammoths from Late Pleistocene British Columbia, Canada

Richards Michael (1), Termes Laura (2), Kubiak Cara (2), Speller Camilla (3), Bronk Ramsey Christopher (4), Götherström Anders (5), Dalen Love (6)

1 - Simon Fraser University (Canada), 2 - Simon Fraser University (Canada), 3 - University of British Columbia (Canada), 4 - Oxford Radiocarbon Accelerator Unit, University of Oxford (Dyson Perrins Building, South Parks Road, Oxford, United Kingdom), 5 - Archaeological Research Laboratory, Stockholm University, Stockholm, Sweden (Sweden), 6 - Swedish Museum of Natural History (Sweden)

Despite being on the migration route into (and out of) Late Pleistocene North America from Beringia, comparatively little work has been undertaken on megafauna from

British Columbia in this time period. As part of our wide-ranging British Columbia Megafauna project, we have undertaken a range of analyses on mammoth remains dating from > 50,000 to 20,000 BP. These studies include bulk isotope analysis (C, N, S), compound-specific analysis of collagen amino acids (C and N), proteomic and DNA analysis. Before our project less than 10 mammoth remains were known from the province. We have now identified over 70 specimens and have direct radiocarbon dates on over 30. In this presentation we will discuss the first results of our radiocarbon dating and biomolecular studies of these Late Pleistocene mammoths and the implications for understanding mammal migrations into North America in the Late Pleistocene.

The origins and evolutionary trajectory of domestic, exploited and/or commensal animals

Sheep birth seasonality in European past herds. The Neolithic biological and cultural heritage

Balasse Marie (1), Tornero Carlos (1, 2), Fabre Mélanie (1), Bréhard Stéphanie (1), Keller Matthieu (3), Chemineau Philippe (3), Carrère Isabelle (4), Gandois Henri (5), Fiorillo Denis (1), Manen Claire (4)

1 - Archéozoologie, archéobotanique: sociétés, pratiques et environnements (AASPE) (France), 2 - Institute of Human Paleoecology and Social Evolution (IPHES) (Spain), 3 - Physiologie de la Reproduction et des Comportements (PRC) (France), 4 - TRACES (France), 5 - Trajectoires- De la sédentarisation à l'État (France)

During the course of the diffusion of Neolithic agropastoral societies across Europe, animal husbandry was adapted to local constraints and resources, involving changes in practices and in animal physiology. The timing of animal breeding was impacted, with consequences on the organization of agro-pastoral tasks and the seasonal availability of animal productions. Past sheep birth seasonality can be investigated through the reconstruction of the seasonal cycle recorded in molars, using sequential analysis of stable oxygen isotope ratios ($\delta^{18}O$) in enamel. Modern sheep serve as comparative material to define the season of birth. First, this presentation will provide a synthesis of existing modern comparative material. Those include two new reference sets for winter, spring and autumn births. Second, we will provide an overview of sheep births distribution (timing and spread of births) in European contexts dated to the 6th-3rd millennia cal BC, with a specific focus on Southern France. By contrast to late winter-spring lambing as the general rule in Europe, autumnal lambing is evidenced in Southern France as early as in the Neolithic. This character constitutes a strong identity of today's farming practices in the northern margin of the western Mediterranean, where autumnal lambing coincides with maximal pasture availability and is prized for bringing benefits in terms of out-of-season availability of products. Its occurrence in the Neolithic involved the physiological capacity of early sheep for enlarged breeding season, but also intentional management of females and males interactions by the herder, as well as adequate forage resources to support autumnal lactation.

Palaeogenomic analysis of the black rat reveals multiple European introductions associated with human economic history

Yu He (1), Jamieson Alexandra (2), Hulme-Beaman Ardern (3, 4), Conroy Chris J. (5), Knight Becky (6), Speller Camilla (7, 8), Al-Jarah Hiba (7), Eager Heidi (9), Trinks Alexandra (10), Baron Henriette (11), Crowther Alison (12), Cucchi Thomas (13), Esser Kinie (14), Gidney Louisa (15), Gladilina Elena (16), Gol'din Pavel (17), Goodman Steven M. (18), Hamilton-Dyer Sheila (19), Kallala Nabil (20), Kivikero Hanna (21), Kovács Zsófia E. (22), Kunst Günther Karl (23), Kysely René (24), Linderholm Anna (25), Maraoui-Telmini Bouthéina (26), Morales Muñoz Arturo (27), Nabais Mariana (28, 29), O'connor Terry (7), Oueslati

Tarek (30), Pasda Kerstin (31), Radbauer Silvia (32), Ramon Joan (33), Rannamäe Eve (34), Grego Joan Sanmartí (35), Treasure Edward (36), Valenzuela-Lamas Silvia (37), Van Der Jagt Inge (38), Vigne Jean-Denis (39), Walker Thomas (40), Zeiler Jørn (41), Dobney Keith (3, 42, 43, 44), Boivin Nicole (12), Searle Jeremy B. (9), Krause-Kyora Ben (45), Krause Johannes (1, 46), Larson Greger (47), Orton David (7)

1 - Department of Archaeogenetics, Max Planck Institute for the Science of Human History (Germany), 2 - Palaeogenomics & Bio-Archaeology Research Network, Research Laboratory for Archaeology and History of Art (United Kingdom), 3 - Department of Archaeology, Classics and Egyptology, University of Liverpool (United Kingdom), 4 - Research Centre in Evolutionary Anthropology and Palaeoecology, Liverpool John Moores University (United Kingdom), 5 - Museum of Vertebrate Zoology, University of California, Berkeley (United States), 6 - Department of Archaeology, University of York (United Kingdom), 7 - BioArCh, Department of Archaeology, University of York, York (United Kingdom), 8 - Department of Anthropology, University of British Columbia (United Kingdom), 9 - Department of Ecology and Evolutionary Biology, Cornell University (United States), 10 - Charité – Universitätsmedizin Berlin, Institut für Pathologie (Germany), 11 - Römisch-Germanisches Zentralmuseum, Leibniz-Forschungsinstitut für Archäologie (Germany), 12 - Department of Archaeology, Max Planck Institute for the Science of Human History (Germany), 13 - Archéozoologie, archéobotanique : sociétés, pratiques et environnements (France), 14 - Archeoplan Eco (Netherlands), 15 - Archaeological Services, University of Durham (United Kingdom), 16 - Ukrainian Scientific Center of Ecology of the Sea (Ukraine), 17 - Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine (Ukraine), 18 - Negaunee Integrative Research Center, Field Museum of Natural History (United States), 19 - Department of Archaeology & Anthropology, Bournemouth University (Visiting Fellow) (United Kingdom), 20 - Institut national du patrimoine (Tunisia), 21 - University of Helsinki, Department of Culture (Finland), 22 - Freelance archaeozoologist (Hungary), 23 - VIAS Vienna Institute for Archaeological Science, University of Vienna (Austria), 24 - Institute of Archaeology of the Czech Academy of Sciences, Prague (Czech Republic), 25 - Department of Anthropology, Texas A&M University (United States), 26 - Department of History, University of Tunis (Tunisia), 27 - Departamento de Biología, Universidad Autónoma de Madrid (Spain), 28 - Institute of Archaeology, University College London (United Kingdom), 29 - Centro de Arqueologia da Universidade de Lisboa (UNIARQ), Faculdade de Letras, Universidade de Lisboa (Portugal), 30 - Centre National de la Recherche Scientifique, University of Lille (France), 31 - Department of Philosophy, Institute of Prehistoric Archaeology, Friedrich-Alexander-University of Erlangen-Nürnberg (Germany), 32 - Austrian Academy of Sciences, Austrian Archaeological Institute (Austria), 33 - Consell Insular d'Eivissa i Formentera (Spain), 34 - Department of Archaeology, Institute of History and Archaeology, University of Tartu (Finland), 35 - Secció de Prehistòria i Arqueologia, University of Barcelona (Spain), 36 - Department of Archaeology, Durham University (United Kingdom), 37 - Archaeology of Social Dynamics, IMF-CSIC (Spain), 38 - Cultural Heritage Agency of the Netherlands, Department of Archaeology, Amersfoort (Netherlands), 39 - Muséum National d'Histoire Naturelle Paris- UMR 7209: Archéozoologie, Archéobotanique: sociétés,

pratiques, environnements. (France), 40 - Department of Archaeology, University of Reading (United Kingdom), 41 - ArchaeoBone (Netherlands), 42 - Department of Archaeology, University of Sydney (Australia), 43 - Department of Archaeology, University of Aberdeen (United Kingdom), 44 - Department of Archaeology, Simon Fraser University (Canada), 45 - Institute of Clinical Molecular Biology, Kiel University, Kiel, Germany (Germany), 46 - Max Planck Institute for Evolutionary Anthropology (Germany), 47 - Palaeogenomics & Bio-Archaeology Research Network, University of Oxford (United Kingdom)

*The distribution of the black rat (*Rattus rattus*) has been heavily influenced by its association with humans. The dispersal history of this non-native commensal rodent across Europe, however, remains poorly understood, and different populations may have been introduced from different geographic sources during the Roman and medieval periods. Here, in order to reconstruct the population history of European black rats, we generated a de novo genome assembly of the black rat, 70 ancient black rat mitogenomes and 39 ancient nuclear genomes from sites spanning the 1st-17th centuries CE in Europe and North Africa. Analyses of the mitochondrial DNA indicate that black rats were introduced into the Mediterranean and Europe from Southwest Asia, likely via an overland route. Genomic analyses of the ancient rats reveal a population turnover in temperate Europe between the 6th and 10th centuries CE, coincident with an archaeologically attested decline in the black rat population. The near disappearance and re-emergence of black rats in Europe may have been the result of the breakdown of the Roman Empire, the First plague Pandemic, and/or post-Roman climatic cooling.*

Genomic stability through time despite decades of exploitation in cod on both sides of the Atlantic ocean

Eikeset A. M. (1), Helmersen C., Bradbury I. R., Bentzen P., Morris C, Gondek A., Baalsrud H. T., Briec M. S. O., Kjesbu O. S., Godiksen J. A., Barth J. M. I., Matschiner M., Stenseth N. Chr., Jakobsen K. S., Jentoft S., Star B. (2)

1 - Department of Ecology, Evolution, and Natural Resources, Rutgers University (United States), 2 - Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo (Norway)

The mode and extent of rapid evolution and genomic change in response to human harvesting is a key conservation question. Although experiments and models have shown a high potential for both genetic and phenotypic change in response to fishing, empirical examples of genetic responses in wild populations are rare. Here, we compare whole genome sequence data of historic and modern Atlantic cod specimens that were collected before (early 20th century) and after (early 21st century) periods of intensive exploitation and rapid decline in the age of maturation from two geographically distinct populations in Newfoundland, Canada and the Northeast Arctic, Norway. Our temporal, genome-wide analyses of 112 individuals and 346,290 loci show no substantial loss of genetic diversity and high effective population sizes. Moreover, we do not find distinct signals of strong selective sweeps anywhere in the genome, although we cannot rule out the possibility of highly polygenic evolution. Our observations suggest that phenotypic change in these

populations is not constrained by irreversible loss of genomic variation and thus imply that former traits could be reestablished with demographic recovery.

Where you lead I will follow: Shaping Siberian dog ancestry through trade and dispersal

Feuerborn Tatiana (1), Carmagnini Alberto (2), Losey Robert, Nomokonova Tatiana, Askeyev Arthur, Askeyev Igor, Askeyev Oleg, Antipina Ekaterina, Appelt Martin (3), Bachura Olga (4), Beglane Fiona, Bradley Dan (5), Daly Kevin, Gopalakrishnan Shyam (1), Guo Chunxue, Gusev Andrei, Jones Carleton, Kosintsev Pavel, Kuzmin Yaroslav, Mattiangeli Valeria, Angela Perri, Mattiangeli Andrei, Madrigal Jazmín Ramos (1), Schmidt Anne Lisbeth (3), Shaymuratova Dilyara, Smith Oliver, Yavorskaya Lilia, Zhang Guojie, Willerslev Eske (1), Meldgaard Morten (1), Gilbert M. Thomas P. (1), Larson Greger (6), Dalén Love (7), Hansen Anders J (1), S. Sinding Mikkel H. (1), Frantz Laurent (2)
1 - Globe Institute (Denmark), 2 - Queen Mary University London (United Kingdom), 3 - Natural History Museum of Denmark (Denmark), 4 - National Centre for Research on Europe (New Zealand), 5 - Trinity College Dublin (Ireland), 6 - Palaeogenomics & Bio-Archaeology Research Network, University of Oxford (United Kingdom), 7 - Swedish Museum of Natural History (Sweden)

In the Siberian Arctic dogs have played a critical role in human societies starting as early as 9,500 years ago despite major changes in material culture and subsistence strategies in the last 2,000 years, dogs have remained an essential component of life. However, very little is known about the dogs of Siberia that lead to the formation of modern populations and breeds in the midst of profound changes in human societies. To test whether the ancestry of Siberian Arctic dogs reflects societal shifts seen in the archaeological record we sequenced genomes from 20 ancient and historical dogs. In contrast to genetic evidence from humans, our results indicate that dog ancestry in Northwest Siberia changed through time reflecting interactions and exchanges with distant groups to the south and west. Our analyses indicate that Siberian dogs were genetically homogenous in the Early Holocene, between 9,500-7,000 years ago. However, dogs from Bronze Age sites in the neighbouring Eurasian Steppe region, a potential source population for admixture into Siberian dogs, were found to have ancestry relating to both ancient Arctic dogs and ancient Iranian/Levantine dogs. Similarly the dogs of Northwestern Siberia reflect a mixed ancestry relating to ancient Arctic as well as Steppe or Near Eastern sources that reflect multiple events potentially relating to the introductions of metallurgy and reindeer pastoralism to the region. Furthermore, we show that modern Siberian breeds, including Siberian Huskies and Samoyeds, still possess ancestry related to ancient Siberian lineages, despite mixing with European breeds.

Palaeoproteomic analyses of dog palaeofaeces reveal a preserved dietary and host digestive proteome

Runge Anne Kathrine (1, 2), Hendy Jessica (3, 4), Richter Kristine (5), Masson-Maclean Edouard (6), Britton Kate (6), Mackie Meaghan (2, 7), Mcgrath Krista (8), Collins Matthew (2, 9), Cappellini Enrico (10), Speller Camilla (11)

1 - Bioarch/Department of Archaeology - University of York, York, UK (United Kingdom), 2 - Section for Evolutionary Genomics, The Globe Institute, University of Copenhagen, Copenhagen (Denmark), 3 - BioArCh, Department of Archaeology, University of York, York, UK (United Kingdom), 4 - Department of Archaeogenetics, Max Planck Institute for the Science of Human History, Jena (Germany), 5 - Department of Archaeology, Max Planck Institute for the Science of Human History, Jena (Germany), 6 - Department of Archaeology, University of Aberdeen (United Kingdom), 7 - The Novo Nordisk Foundation Center for Protein Research, University of Copenhagen (Denmark), 8 - ICTA-UAB, Barcelone (Spain), 9 - Department of Archaeology, University of Cambridge (United Kingdom), 10 - Center for EvoGenomics, The Globe Institute, University of Copenhagen, Copenhagen K (Denmark), 11 - Department of Anthropology, University of British Columbia (Canada)

*The domestic dog (*Canis lupus familiaris*) has inhabited the anthropogenic niche for at least 15,000 years. In particular, dogs play a central role in Arctic adaptations, where they facilitate transportation and the acquisition of resources. However, until recently, dogs in archaeological contexts have received little attention and as such there is limited available knowledge about the lives and management of dogs in the past. Furthermore, while their osteological remains are abundant in archaeological contexts, dogs leave comparatively few traces that can provide insight into their lifeways. Recent advances in biomolecular archaeology have opened up avenues for exploration of complex substrates. This includes palaeofaeces, a novel substrate which can provide holistic insight into the host, its digestive tract and diet. Here we present the successful recovery of ancient proteins from dog palaeofaeces and show that it is a viable tool for obtaining insight into ancient diet and subsistence from Arctic contexts. We identify a suite of digestive and metabolic proteins from the host species, demonstrating the utility of this material as a novel and viable substrate for the recovery of gastro-intestinal proteomes and to identify the host species of the palaeofaeces. We also recover proteins from dietary sources in permafrost-preserved dog palaeofaeces, revealing evidence of consumed salmon, providing unique evidence for short-term dietary reconstructions.*

The genomic foundation and spread of domestic horses

Librado Pablo (1), Fages Antoine (2), Chauvey Lorelei (1), Tonasso Laure (3), Schiavinato Stéphanie (1), Kahn Naveed, Consortium Erc Pegasus, Outram Alan (4), Orlando Ludovic (5)

1 - Centre for Anthropobiology and Genomics of Toulouse (France), 2 - University of Basel (Switzerland), 3 - Centre for Anthropobiology and Genomics of Toulouse (France), 4 - University of Exeter (United Kingdom), 5 - Centre for Anthropobiology and Genomics of Toulouse (France)

The domestication of the horse considerably increased our capacity to travel beyond our speed, facilitating biological and cultural exchange between human populations. It has, thus, represented a major turning point in human history. Until recently, horse domestication was assumed to have occurred in the Central Asian Steppes of Kazakhstan, based on the ~5.5kya-old Botai settlements, which provided archaeological evidence of horse management. Ancient DNA research, however,

revealed Botai horses as an independent lineage, not ancestral to modern domesticates, but to Przewalski's horses. This suggested that a second domestication process gave rise to the genomic makeup of modern domestic horses, although its geographic and temporal locations remain, however, unknown. In order to solve the mysterious foundation of modern horses, we generated 198 new ancient genomes encompassing previously unsampled regions, prior, during and after domestication. This covers potential domestication centres such as the Pontic-Caspian steppes and Anatolia, among others. Together with 88 previously published genomes, our extensive dataset unveiled considerable diversity prior to domestication and the presence of multiple lineages that are now-extinct. These lineages were related by an extremely dynamic evolutionary history, characterised by strong patterns of isolation-by-distance and multiple secondary contacts. This underlying history shaped geographic clines of genomic diversity that proved instrumental to locate the source of modern domestic horses, both in space and time. Additionally, two genomic regions were extremely differentiated in modern domesticates, revealing, for the first time, the genetic changes that were essential to the foundation of modern domestic horses and their subsequent spread until today.

The application of ancient proteomic techniques to molecular archaeology

Shell palaeoproteomics: from biomineralization to characterisation of ancient proteins preserved in archaeological mollusc shells

Sakalauskaite Jorune (1, 2, 3), Pergolizzi Barbara (4), Taurozzi Alberto (1), Mackie Meaghan (1, 5), Thomas Jérôme (3), Laffont Rémi (3), Steimetz Emilie (3), Plasseraud Laurent (6), Rigaud Solange (7), Manen Claire (8), De Cargouët Monique (9), Potier Nicolas (9), Collins Matthew (1, 10), Marin Frederic (3), Demarchi Beatrice (2)

1 - Section for Evolutionary Genomics, GLOBE Institute, Faculty of Health and Medical Science, University of Copenhagen (Denmark), 2 - Department of Life Sciences and Systems Biology, University of Turin (Italy), 3 - Biogéosciences, UMR CNRS 6282 (France), 4 - Department of Clinical and Biological Sciences, University of Turin, AOU S. Luigi (Italy), 5 - Novo Nordisk Foundation Center for Protein Research, University of Copenhagen (Denmark), 6 - Institute of Molecular Chemistry, ICMUB UMR CNRS 6302 (France), 7 - UMR 5199 – PACEA, CNRS (France), 8 - UMR TRACES, CNRS (France), 9 - Cerep-Musées (France), 10 - McDonald Institute for Archaeological Research, University of Cambridge (United Kingdom)

*Mollusc shells possess exceptional material properties and aesthetic features, which have been highly valued since prehistoric times, e.g. for making tools or shell jewellery. Archaeological shell ornaments are widespread, portable and prized for the insight they give into behaviour and exchange but the identification of shell type used is often dubious, as many are found heavily worked and/or degraded. Here I present our recent application of palaeoproteomics to intracrystalline shell proteins, a source of molecular information to determine the biological origin of shell artifacts. The application of palaeoproteomics to mollusc shells, grounded in decades of expertise in biomineralization (known as 'shellomics') and amino acid racemization research, may provide a valuable tool to securely identify archaeological ornaments. The Mediterranean spiny oyster *Spondylus gaederopus*, thanks to its dramatic colour and form, was one of the most prized raw materials throughout prehistoric times as evidenced from their presence at sites far from their range. However, shell palaeoproteomics poses many challenges: rapid but not well-known evolutionary patterns of 'shellomes', scarcity of genomics data for different species especially considering the huge phylogenetic diversity and limited knowledge about the diagenetic stability of shell proteins. Shellomics data is lacking for species that are not of commercial importance, such as *Spondylus*. I will present insights into advancing the methodological approaches for shell palaeoproteomics and to study shell protein stability in order to predict the survival in the fossil record. Our research displays that ancient mollusc shell proteins can help to address far-reaching archaeological and palaeontological questions.*

Combing Honeycombs - Palaeoproteomics Applied on Historical Beeswax **Kasso Tuuli (1), Carøe Christian (1), Taurozzi Alberto (1), Johns Samuel (2), Roffet-Salque Melanie (2), Collins Matthew (1, 3)**

1 - The GLOBE Institute, University of Copenhagen (Denmark), 3 - Organic Geochemistry Unit, University of Bristol (United Kingdom), 4 - McDonald Institute for Archaeological Research, University of Cambridge (United Kingdom)

*This paper presents results of the ArchHives project (University of Copenhagen) which aims to unlock the biomolecular records of historical and ancient beeswax to study the archaeology of beeswax as a material and past of *Apis mellifera* in the light of the current decline of honeybees. We add beeswax to the continuously growing list of a wide range of materials that high-sensitivity mass spectrometry has made accessible for palaeoproteomics. Beeswax records (i) the colony and (ii) the microbiome of the hives (iii) the pollen sampled (up to 80 km) around the hive. Applying the first of its kind extraction protocol designed specifically to extract biomolecules from beeswax, historical beeswax specimens from the Zoological Museum of the University of Copenhagen were screened for metaproteomics. Additionally, the samples were C14-dated. Pollen and palynomorphs were identified from the samples as a reference to the geographical origin of the wax and plant proteomes. The yields of pollen in wax are short of representing a biotope as a fixed fingerprint, but provide some insight to the foraging sources of the bees. The results are reviewed for the representation of original proteins, their degradation, and presence of contaminants. This is followed by discussion on the potential and the limitations of the approach on using historical beeswax in natural history museum collections as a source to study honeybees and their past environments.*

SPIN - Species by Proteome INvestigation

Ruether Patrick (1), Husic Im, Bangsgaard P, Murphy Gregersen Kristian, Pantmann P, Carvalho M, Godinho Rm, Friedl L, Cascalheira J, Jørkov Mls, Benedetti Mm, Haws J, Bicho N, Welker F, Cappellini E, Olsen Jesper (1, 2)
1 - Novo Nordisk Foundation Center for Protein Research (Denmark), 2 - Olsen (Denmark)

Genetic species determination has become an indispensable tool in forensics, archaeology, ecology, and food authentication. The available methods are either suited for detecting a single taxon across many samples or for screening a wide range of species across a few samples. Here, we introduce Species by Proteome INvestigation (SPIN), a proteomics workflow capable of querying over 150 mammalian species in 7.2 minutes of mass spectrometry (MS) analysis. Streamlined and automated sample preparation by protein aggregation capture, high-speed chromatography and data-independent acquisition, and a confident species inference algorithm facilitate processing hundreds of samples per day. We demonstrate the correct classification of known references, reproducible species identification in degraded Iron-Age material from Scandinavia, and test the limits of our methods with Middle and Upper Palaeolithic bones from Southern European sites with late Neanderthal occupation. While this initial study is focused on modern and archaeological mammalian bone, SPIN will be open and expandable with other biological tissues and taxa.

Proteomic analysis provides insights into the history and organisation of a medieval palimpsest

Vinas Caron Laura (1, 2), Rodríguez Palomo Ismael (3), Fazlic Natasha (4), Vnouček Jiří (5), Vilde Vladimir (6), Driscoll Matthew (7), Fiddymat Sarah (8), Collins Matthew (2, 8)

1 - Centre for Textile Research, University of Copenhagen (Denmark), 2 - Section for Evolutionary Genomics, GLOBE Institute, University of Copenhagen (Denmark), 3 - Section for Evolutionary Genomics, GLOBE Institute, University of Copenhagen (Denmark), 4 - Department of Nordic Studies and Linguistics, University of Copenhagen (Denmark), 5 - Department of Preservation, The Royal Library (Denmark), 6 - Department of Engineering, University of Cambridge (United Kingdom), 7 - Arnamagnæan Institute, Department of Nordic Studies and Linguistics, University of Copenhagen (Denmark), 8 - McDonald Institute for Archaeological Research, Department of Archaeology, University of Cambridge (United Kingdom)

In the Middle Ages, documents were primarily written in parchment, an animal-based writing support. A common feature of many of these texts is that they are palimpsests. Medieval scribes would run out of parchment and reuse pages of older documents, in which the original text had been removed and written over. New imaging methods are helping to recover the hidden layers of text, but some parts remain unreadable, hampering the reassembly of the different pages composing these palimpsests. Peptide mass fingerprinting, a mass spectrometry-based proteomic method, has been previously used on parchment to identify the animal source and molecular damage resulting from parchment production, indicating its quality. Nevertheless, a more in-depth analysis of the MALDI data has not been attempted, although it has the potential to provide a better understanding of the history and organisation of these manuscripts, by revealing peptide similarities or differences across pages. In this study, we explore whether proteomic methods can assist in the investigation of ancient palimpsests. As an example, we present the results of the analysis conducted on a whole manuscript, the AM795 4to from the Arnamagnæan collection. Additionally, we visually examined the parchment for traces of production and animal features. Combined with paleographical and codicological knowledge of the book, the results have the potential to help us group the pages that could derive from the same manuscript or trace different episodes of book production.

Amino acid dating of mammalian tooth enamel and its potential for building geochronologies

Dickinson Marc (1), Titov Vadim (2), Frolov Pavel (3), Tesakov Alexey (3), Patinglag, Laila (4), Shaw Kirsty (4), Herridge Victoria (5), Lister Adrian (5), Penkman Kirsty (1)

1 - Department of Chemistry, York (United Kingdom), 2 - Southern Scientific Centre, Russian Academy of Sciences (Russia), 3 - Geological Institute, Russian Academy of Sciences (Russia), 4 - Manchester Metropolitan University (United Kingdom), 5 - Department of Earth Sciences, Natural History Museum (United Kingdom)

Directly dating mammalian remains is difficult beyond the limits of radiocarbon dating. One direct dating method is to use the predictable breakdown of proteins and amino

acids in biominerals containing closed-system protein, but the application of this technique to mammalian remains has been challenging. Our novel method targets a proteinaceous fraction in enamel protected within biomineral crystals, which has mitigated contamination, leaching and environmental difficulties. Through simulated degradation experiments to investigate the protein breakdown and the intrinsic properties of enamel's inorganic crystal structure, we have found that an intracrystalline fraction of amino acids can be successfully isolated from enamel. The extent of intra-crystalline protein decomposition (IcPD) in proboscidean enamel has been tested against known age material from Europe, showing a strong correlation between extent of IcPD and age. It is now possible to provide direct age estimation for unknown age proboscidean material from the same temperature regions. The Wisdom Teeth project is developing IcPD geochronologies using a variety of mammalian taxa (e.g. bovids and equids) from African sites spanning the Plio-Pleistocene. By targeting anthropologically significant sites (e.g. in Lake Turkana and South Africa), it is hoped that these IcPD geochronologies will provide relative dating to aid existing understanding of human evolution. We are also investigating taphonomic alteration of fossil enamel using an array of techniques, to better understand amino acid preservation. Lastly, we will use microfluidic technology to develop a «lab-on-a-chip» approach for preparation of enamel samples, both to reduce sample sizes, and to allow IcPD analysis outside specialist labs.

A machine learning approach in developing an ATR-FTIR-based screening system for collagen (ZooMS analysis) and mtDNA in archaeological bones
Pal Chowdhury Manasij (1, 2), Datta Choudhury Kaustabh (3), Buckley Michael (1, 2)

1 - Manchester Institute of Biotechnology, School of Natural Sciences, University of Manchester, Manchester (United Kingdom), 2 - Interdisciplinary Centre for Ancient Life, School of Natural Sciences, University of Manchester, (United Kingdom), 3 - Independent Data Scientist, (India)

The investigation of biomolecules from faunal remains plays a major role in studying the relationships between humans and animals, phylogenetic relationships between species, and certain evolutionary pathways. Proteins and DNA are increasingly used nowadays for identifying faunal remains which are highly fragmented and/or lack identifiable morphological markers (commonly done using ZooMS; Zooarchaeology by mass spectrometry). However, despite improving costs, they remain particularly time-consuming, and as such the development of a cheap, accurate, and minimally destructive screening technique is of vital importance. Here, we develop a new approach to FTIR-based screening of bones prior to ZooMS and aDNA analysis, using three sets of bone assemblages with varying preservation conditions, ranging from Palaeolithic to modern Holocene samples. We utilize a random forest-based (RF) machine learning (ML) approach to create a predictive model; our results improves the previously reported accuracy of FTIR-based screening for ZooMS by 20-40%. We also report a similar ML-based screening approach for analysis of aDNA. Our results potentially allows for a universal screening system applicable to bones from myriad taxa across multiple sites and preservational conditions, largely independent of the spectrometer used. It also involves minimal sample preparation

and spectral data manipulation and can be easily adapted for screening in field laboratories.

Ancient microbes and pathogens and their contribution to understanding past epidemics and health

A treponemal genome from an historic plague victim supports a recent emergence of yaws and its presence in 15 th century Europe

Bos Kirsten (1), Karen Giffin (1), Lankapalli Aditya (2), Spyrou Maria (1), Sabin Susanna (1), Posth Cosimo (3, 4), Kozakaite Justina (5), Friedrich Ronny (6), Miliauskienė Žydrūnė (7), Jankauskas Rimantas (5), Herbig Alexander (1)

1 - Max Planck Institute for the Science of Human History, Jena (Germany), 2 - Department of Archaeogenetics, Max Planck Institute for the Science of Human History (Germany), 3 - Archaeo- and Palaeogenetics, Institute for Archaeological Sciences, University of Tübingen (Germany), 4 - Archaeogenetics Department, Max Planck Institute for the Science of Human History, Jena (Germany), 5 - Vilnius University (Lithuania), 6 - Curt-Engelhorn-Zentrum Archäometrie (Germany), 7 - Department of Anthropology University of Vilnius (Lithuania)

*Advancements in methods for pathogen DNA detection in archaeological samples can enhance our understanding of disease spread in time and space. Here we report on the application of a hypothesis-free method for molecular pathogen screening in 26 putative historical plague victims from post-medieval Vilnius, Lithuania. This process revealed the presence of more than one active disease in one individual: In addition to *Yersinia pestis* we detected and genomically characterized a septic infection of *Treponema pallidum pertenuis*, a subtype of the treponemal disease family recognised as the cause of the tropical disease yaws. Our finding of yaws in northern Europe was unexpected given modern epidemiology of the disease, where it is reported in equatorial regions only. Further to this, a molecular dating analysis revealed a most recent common ancestor for all circulating yaws strains in the last millennium, indicating that the disease cluster is much younger than previously thought. Its recent emergence and presence in northern Europe during this time period are interpreted within an historical framework of intercontinental trade and potential disease movements. Through this we offer an alternative hypothesis for the history and evolution of the treponemal diseases, and posit that yaws be considered a contributor to the widespread physically disfiguring disease that appeared suddenly in late 15 th century Europe.*

Unravelling the History of *Treponema pallidum* with Ancient DNA Investigations

Majnder Kerttu (1), Akgül Gülfirde (1), Pla-Díaz Marta (2, 3), Molak Martyna (4), Dabrowski Paweł (5), Oziębłowski Maciej (6), Panagiotopoulou Hanna (7), Doan Karolina (7), Arora Natasha (8), Du Plessis Louis (9), Gonzalez Candelas Fernando (3, 10), Schuenemann Verena (1)

1 - Institute of Evolutionary Medicine, University of Zürich, Zürich (Switzerland), 2 - Joint Research Unit "Infection and Public Health" FISABIO-University of Valencia, Institute for Integrative Systems Biology (I2SysBio), Valencia (Spain), 3 - CIBER de Epidemiología y Salud Pública (Spain), 4 - Centre of New Technologies, University of

Warsaw (Poland), 5 - Department of Normal Anatomy, Wrocław Medical University, Wrocław (Poland), 6 - Faculty of Biotechnology and Food Sciences, Wrocław University of Environmental and Life Sciences, Wrocław (Poland), 7 - Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw (Poland), 8 - Zürich Institute of Forensic Medicine, University of Zürich, Zürich (Switzerland), 9 - Department of Zoology, University of Oxford (Oxford United Kingdom), 10 - 4Joint Research Unit "Infection and Public Health" FISABIO-University of Valencia, Institute for Integrative Systems Biology (I2SysBio), Valencia (Spain)

Treponemal infections are currently re-emerging diseases, representing a global threat to human health. These include syphilis, responsible for a devastating epidemic in Europe starting in the late 15th century. Recently, studies on ancient treponemal DNA, long presumed irretrievable, have confirmed historical cases of both syphilis and yaws (an endemic treponematosis) in Europe and the Americas. The origins of these diseases, however, remain unresolved, including the potential introduction of syphilis to Europe on Columbus' return from the Americas. Our previous archaeogenetic research spans from colonial Mexican cases that reported the first treponemal genomes retrieved from historical remains, to the recently revealed European strains from the early modern period. According to the results there was an unexpectedly high diversity of circulating strains, with syphilis and yaws coexisting on both continents. Additionally, a previously unknown strain, with similarities to both, was detected within the European diversity. As more historical genomes are reconstructed, the genomic evolution and relationships between subspecies can be elucidated in more detail. Here, we present a new high-coverage genome of *T. pallidum* ssp. *pallidum*, the causative agent of syphilis, recovered from an 18th century cemetery in Poland. We employ this genome in an in-depth comparison among historical and modern treponemal strains to assess changes in putatively functional genes and to evaluate selective pressures among treponemal subspecies. Furthermore, molecular clock analyses allow us to obtain better estimates for major divergence events in the pathogen's evolutionary history and reappraise the origin of Old World treponemal epidemics.

Mycobacterium tuberculosis diversity across the precolonial Andes

Nelson Elizabeth (1, 2) Lankapalli Aditya (1), Spyrou Maria (1, 3), Childebayeva Ainash (1), Vågene Åshild (4), Sabin Susanna (5), Guevara Evelyn (6), Rohrlach Adam Ben (1, 7), Fellows Yates James A. (1), Toyne J. Marla (8), Tung Tiffany A. (9), Krause Johannes (1), Herbig Alexander (1), Bos Kirsten I. (1)

1 - Department of Archaeogenetics, Max Planck Institute for the Science of Human History, (Germany), 2 - Department of Anthropology, University of Connecticut, (USA), 3 - Section for Evolutionary Genomics, The GLOBE Institute, Faculty of Health and Medical Sciences, University of Copenhagen, (Denmark), 4 - Center for Evolution and Medicine, Arizona State University, Tempe, (USA), 5 - Department of Forensic Medicine, University of Helsinki, (Finland), 6 - ARC Center of Excellence for Mathematical and Statistical Frontiers, School of Mathematical Sciences, The University of Adelaide, (Australia), 7 - Department of Anthropology, University of Central Florida, (USA), 8 - Department of Anthropology, Vanderbilt University, (USA) Ancient DNA can provide information on the evolution and strain level diversity of

clinically relevant pathogens, including the *Mycobacterium tuberculosis* complex (MTBC). Phylogenetic analyses of ancient MTBC genomes from coastal Peruvian contexts have revealed unexpected strains of MTBC in precolonial Andean human populations, where all forms identified to date are most closely related to those circulating in modern sea mammals (*M. pinnipedii*). Here we evaluate this diversity through consideration of two distinct contemporaneous cultural groups from different ecological zones of Andean Peru: the highlands and the subtropical forest. We report seven genomes from Late Intermediate Period (LIP, 1000 - 1400 CE) contexts of the central Andean highland site of Huari, which allowed us to evaluate MTBC diversity on a local scale. Additionally, we present one MTBC genome from the Chachapoya cliff side tomb of Diablo Huasi dating to the LIP (cal 1296-1396 CE), located in the high jungle on the eastern slopes of the northern Andean massif of Amazonas, Peru. Phylogenetic analysis of these genomes consistently revealed a close relationship to modern *M. pinnipedii* strains, with Diablo Huasi showing greatest homology to these modern forms. Our results provide insights into the evolution of MTBC in the Andes and expand the known geographic distribution of precolonial *M. pinnipedii* strains across all three major ecological zones of the Andes: the coast, the highlands, and the subtropical forest of the eastern slopes.

New ancient *Mycobacterium leprae* genomes reveal leprosy as a potential source of high strain diversity in medieval Europe

Schuenemann Verena (1), Pfrenkle Saskia (1), Neukamm Judith (1), Guellil Meriam (2), Keller Marcel (2), Molak Martyna (3), Avanzi Charlotte (4), Kushniarevich Lena (2), Montes Núria (5), Tukhbatova Rezeda (6), Matos Vítor (7), Ferreira Maria Teresa (7), Roberts Charlotte (8), Sheridan Alison (9), Krause Johannes (10), Scheib Christiana (2), Inskip Sarah (11)

1 - Institute of Evolutionary Medicine, University of Zurich, (Switzerland), 2 - Institute of Genomics, University of Tartu (Estonia), 3 - Centre of New Technologies, University of Warsaw (Poland), 4 - Mycobacteria Research Laboratories, Colorado State University (United States), 5 - Unitat d'Antropologia Biològica, Universitat Autònoma de Barcelona (Spain), 6 - Center of Excellence "Archaeometry", Kazan Federal University (Russia), 7 - Research Centre for Anthropology and Health (University of Coimbra Portugal), 8 - Department of Archaeology, Durham University (United Kingdom), 9 - Department of Scottish History and Archaeology, National Museums Scotland (United Kingdom), 10 - Max Planck Institute for Evolutionary Anthropology (Leipzig Germany), 11 - School of Archaeology and Ancient History, University of Leicester (United Kingdom)

Leprosy is one of the oldest recorded and most stigmatized diseases in human history, however its origins and past dissemination are still widely unknown. Using ancient DNA approaches on the major causative agent, *Mycobacterium leprae*, can greatly contribute to a better understanding of this disease's evolutionary history; previous studies have demonstrated. *M. leprae* shows a complex past distribution in some parts of Europe with at least four *M. leprae* lineages present since the early medieval period, and a high genomic continuity over the last 1,000 years. Here, we reconstructed 19 new medieval *M. leprae* genomes to further analyze the variation across Europe with a dedicated focus on previously un-studied regions (Scotland,

Iberia, Russia, Belarus) as well as the intra-regional strain diversity by adding data from multiple cases from the same locations (Cambridge, UK) and in leprosia in Portugal and Spain. Overall, we find a similar phylogeography across Europe including high diversity at leprosia and identify a new SNP type from Eastern Europe. The higher resolution of the phylogeny also allowed us to refine our understanding of the interspecies transfer between red squirrels and humans. Furthermore, with new estimates on the past population diversity of *M. leprae* we were able to obtain first insights into its global history in relation to major historic events. In summary, our results reveal that the high *M. leprae* diversity in medieval Europe may originate from the nature of leprosia, and highlight how studying ancient *M. leprae* strains improves understanding of the history of leprosy worldwide.

Metagenomic analysis of ancient dental calculus reveals unexplored diversity of oral archaeal *Methanobrevibacter*

Granehäll Lena (1, 2), Huang Kun D. (3, 4), Tett Adrian (3, 5), Manghi Paolo (3), O'sullivan Niall (1), Paladin Alice (1), Rota-Stabelli Omar (4), Segata Nicola (3), Zink Albert (1), Maixner Frank (1)

1 - Institute for Mummy Studies - EURAC Research (Italy), 2 - Ludwig-Maximilians-Universität München (Germany), 3 - Centre for Integrative Biology (CIBIO), University of Trento (Italy), 4 - Department of Sustainable Agro-Ecosystems and Bioresources, Fondazione Edmund Mach (Italy), 5 - CUBE - Division of Computational Systems Biology, Centre for Microbiology and Environmental Systems Science, University of Vienna (Austria)

Dental calculus (mineralized dental plaque) preserves many types of microfossils and biomolecules, including microbial and host DNA, and ancient calculus is thus an important source of information regarding our ancestral human oral microbiome. In this study, we taxonomically characterized the dental calculus microbiome from 20 ancient human skeletal remains originating from Trentino-South Tyrol, Italy, dating from the Neolithic (6000-3500 BCE) to the Early Middle Ages (400-1000 CE). We found a high abundance of the archaeal genus *Methanobrevibacter* in the calculus. However, only a fraction of the sequences showed high similarity to *Methanobrevibacter oralis*, the only described *Methanobrevibacter* species in the human oral microbiome so far. To further investigate the diversity of this genus, we used *de novo* metagenome assembly to reconstruct 11 *Methanobrevibacter* genomes from the ancient calculus samples. Besides the presence of *M. oralis* in one of the samples, our phylogenetic analysis revealed two hitherto uncharacterised and unnamed oral *Methanobrevibacter* species, that were prevalent in ancient calculus samples sampled from a broad range of geographical locations and time periods. Our study suggests that some members of the pre-industrial human oral microbiome such as the newly discovered archaeal species are now rare in the modern human oral microbiome.

Mass burial genomics reveals outbreak of enteric paratyphoid fever in the Late Medieval trade city Luebeck

Haller Magdalena (1), Callan Kimberly (1, 2), Susat Julian (1), Flux Anna Lena (3), Immel Alexander (1), Franke Andre (1), Herbig Alexander (4), Krause Johannes (4), Kupczok Anne (5, 6), Fouquet Gerhard (7), Hummel Susanne (3), Rieger Dirk (8), Nebel Almut (1), Krause-Kyora Ben (1)

1 - Institute of Clinical Molecular Biology, Kiel University, Kiel (Germany), 2 - Present address: Department of Genetics, Harvard Medical School, Boston (United States), 3 - Department of Historical Anthropology and Human Ecology, University of Göttingen, Göttingen (Germany), 4 - Max Planck Institute for the Science of Human History, Jena (Germany), 5 - Genomic Microbiology Group, Institute of General Microbiology, Kiel University, Kiel (Germany), 6 - Bioinformatics Group, Wageningen University & Research, Wageningen (Netherlands), 7 - Historic Seminar, Faculty of Arts and Humanities, Kiel University, Kiel (Germany), 8 - Department of Archaeology, Hanseatic City of Lübeck Historic Monuments Protection Authority, Lübeck (Germany)

*In the Middle Ages, European settlements of all sizes were repeatedly affected by outbreaks of infectious diseases. Some of the outbreaks reached epidemic proportions, the second plague pandemic being the most notorious of all. A Late Medieval mass-burial site next to the Heiligen-Geist-Hospital (HGH) in Lübeck, a city of the Hanseatic League, contained the skeletal remains of more than 800 individuals assumed to have died of an infectious disease, most probably of the plague. However, Lübeck was ravaged by at least six pestilences in the 14th century alone. We investigated ancient DNA extracts of 92 individuals from the HGH site to determine whether viral or bacterial pathogenic DNA was present that would help identify the cause of death. Metagenomic screening revealed evidence of an infection with *Salmonella enterica* subsp. *enterica* serovar *Paratyphi C*, suggesting an epidemic outbreak of enteric paratyphoid fever. A full reconstruction was possible for three *S. Paratyphi C* genomes that showed a close similarity to another strain from 1200 CE Norway. Based on radiocarbon dates, we determined the enteric paratyphoid fever outbreak in Lübeck to have occurred between 1360 and 1400 CE, historical records indicating the year 1367 CE as the most probable date. Our results also showed that the disease victims were people of northern and eastern European descent, confirming that Hanseatic Lübeck was an important trading centre for the Baltic region.*

Paleofeces reveal recent loss of diversity and microbial symbionts in the Western gut microbiome

Hübner Alexander (1, 2, 3), Hofman Courtney (4, 5), Velsko Irina (1), Fagernäs Zandra (1), Mann Allison E. (1, 6), Boenke Nicole (7), Bouwman Abigail (8), Jeong Choongwon (1, 9), Honap Tavni (4, 5), Hagan Richard (1, 10), Reinhard Karl (11), Aali Abolfazl (12), Lewis Cecil M. (4, 5), Stöllner Thomas (7, 13), Rühli Frank (8), Sankaranarayanan Krithivasan (5, 14), Warinner Christina (1, 3, 15)

1 - Max Planck Institute for the Science of Human History, Department of Archaeogenetics (Germany), 2 - Max Planck Institute for Evolutionary Anthropology, Department of Evolutionary Genetics (Germany), 3 - Friedrich-Schiller-Universität Jena, Faculty of Biological Sciences (Germany), 4 - University of Oklahoma, Department of Anthropology (United States), 5 - University of Oklahoma, Laboratories of Molecular Anthropology and Microbiome Research (United States), 6 - Clemson University, Department of Biological Sciences (United States), 7 - University of Bochum, Institute for Archaeological Studies (Germany), 8 - University of Zürich, Institute of Evolutionary Medicine (Switzerland), 9 - Seoul National University, School of Biological Sciences (South Korea), 10 - University of York - Department of Archaeology (United Kingdom), 11 - University of Nebraska, Institute of Agriculture and Natural Resources (United States), 12 - Archaeological Museum of Zanjan (Iran), 13 - German Mining Museum Bochum, Leibniz-Research Museum of Georesources, Research Department (Germany), 14 - University of Oklahoma, Department of Microbiology and Plant Biology (United States), 15 - Harvard University, Department of Anthropology (United States)

*The human gut microbiome plays a key role in human health by contributing to a wide range of essential biological and physiological functions. A decrease in gut microbiome diversity, mainly driven by diet and lifestyle changes, has been associated with both metabolic disorders and chronic inflammatory diseases. Western industrialized diets, in particular, are associated with greatly reduced gut microbiome diversity compared to more traditional subsistence strategies. To better understand the systematic decline of gut microbiome diversity in present-day industrialized societies, we studied 39 paleofeces samples from three different archaeological sites located in present-day Austria (Dürrenberg salt mine, Celtic Iron age: 500 BCE), Iran (Chehrabad salt mine, Achaemenid Empire: 500 BCE), and Mexico (La Cueva de los Muertos Chiquitos, Loma San Gabriel Culture: 700 CE). After confirming the exceptional preservation of these paleofeces, we found that the gut microbial communities of all three populations more closely resemble those found in traditional societies today than in Western industrialized populations. Furthermore, we identified several so-called «missing microbes» (e.g., *Treponema* spp.) in all three archaeological populations, indicating that although these taxa are today absent in modern industrialized populations, they were still present, including in European populations, as recently as 2,500 years ago. We conclude that the great reduction in microbial diversity observed in the modern Western industrialized gut microbiome appears to have occurred very recently - within the past 2,500 years - and may help explain the apparent greater susceptibility of industrialized populations to metabolic disorders and chronic inflammatory diseases.*

Investigating human-pathogen evolution using paired ancient genomics and proteomics

Scheib Christiana (1, 2), Guellil Meriam (2), Hagan Richard (3, 4), Hendy Jessica (5, 6), Keller Marcel (2), Inskip Sarah (7, 8), Cessford Craig (7, 9), Hui Ruoyun (7, 10), D'atanasio Eugenia (11), Dittmar Jenna (7, 12), Rose Alice (7), Mulder Bram (7), Mitchell Piers (7), O'connell Tamsin (7), Herbig Alexander (4), Krause Johannes (4), Warinner Christina (4, 13, 14), Kivisild Toomas (7, 15), Robb John E (7)

1 - St John's College, University of Cambridge (United Kingdom), 2 - Institute of Genomics, University of Tartu (Estonia), 3 - University of York - Department of Archaeology (United Kingdom), 4 - Max Planck Institute for the Science of Human History, Department of Archaeogenetics (Germany), 5 - Department of Archaeogenetics, Max Planck Institute for the Science of Human History, Jena (Germany), 6 - BioArCh, Department of Archaeology, University of York [York, UK] (United Kingdom), 7 - Department of Archaeology, University of Cambridge (United Kingdom), 8 - School of Archaeology and Ancient History, University of Leicester (United Kingdom), 9 - Cambridge Archaeological Unit (United Kingdom), 10 - The Alan Turing Institute (United Kingdom), 11 - Institute of Molecular Biology and Pathology, CNR (Italy), 12 - University of Aberdeen (United Kingdom), 13 - Faculty of Biological Sciences, Friedrich Schiller University Jena (Germany), 14 - Department of Anthropology, Harvard University, Cambridge (United States), 15 - Department of Human Genetics, KU Leuven (Belgium)

A number of factors influence a person's susceptibility to and manifestation of infectious disease including: inherited genes, diet, age, microbiome composition, and the virulence of the pathogen itself. Teasing apart these factors is especially difficult and in archaeogenomic studies, the focus is usually on the phylogeography of the people or the pathogen itself, though recently some studies have incorporated functional and/or immune-gene analysis. By layering multiple bioanthropological and geochemical techniques (e.g. aDNA, proteomics, isotopes, radiocarbon dating, histology, morphology, and osteology) per individual on a population scale, we can attempt to reconstruct a «health landscape» for that population through time and test the relationship between factors and manifestation of disease. I will present results from the After the Plague Project (Wellcome Trust), a multidisciplinary project incorporating all of the above mentioned techniques, wherein we optimised laboratory and bioinformatic methods to integrate the analysis of hundreds of individuals from the Neolithic to Post-medieval period in Cambridgeshire. On the 'omics' side, we used shotgun genomic data to combine human demography, kinship, phenotype prediction, oral microbiome diversity, and pathogen presence/genome analysis and developed a workflow for efficient co-extraction of proteins and DNA (endogenous and microbial/dietary) to study the diet, pathogen-specific proteins and immune response to infection, whether chronic (e.g. periodontal disease) or acute (e.g. plague). This framework has allowed us to detect unexpected bacterial and viral infections and to start to elucidate the complex interactions between diet, environment, hereditary traits and disease, which have and continue to shape our evolution as a species.

Molecular and microscopic analysis of human paleofeces from the salt mines in Hallstatt, Austria, provide precious insights into dietary habits and gut microbiome composition of Iron Age miners

Maixner Frank (1), Sarhan Mohamed S. (1), Huang Kun D. (2, 3), Tett Adrian (2, 4), Schoenaffinger Alexander (1, 5), Zingale Stefania (1), Cemper-Kiesslich Jan (6), Rota-Stabelli Omar (3), Rattei Thomas (4), Moritz Robert L. (7), Oegg Klaus (5), Segata Nicola (2), Zink Albert (1), Reschreiter Hans (8), Kowarik Kerstin (8)
1 - Institut for Mummy studies - EURAC Research (Italy), 2 - Centre for Integrative Biology (CIBIO), University of Trento (Italy), 3 - Department of Sustainable Agro-Ecosystems and Bioresources, Fondazione Edmund Mach (Italy), 4 - CUBE - Division of Computational Systems Biology, Centre for Microbiology and Environmental Systems Science, University of Vienna (Austria), 5 - Institute of Botany, University of Innsbruck (Austria), 6 - Interfaculty Department of Legal Medicine, University of Salzburg (Austria), 7 - Institute for Systems Biology [Seattle] (United States), 8 - Prehistoric Department, Museum of Natural History Vienna (Austria)

*The UNESCO World Heritage region Hallstatt-Dachstein/Salzkammergut represents one of Europe's oldest cultural and industrial landscapes with salt mines in the Hallstatt mountain dating back at least to the 14th century BC. The site gave name to the Hallstatt Period (800 to 400 BC) of the Early Iron Age in Europe. The high salt concentrations and the constant annual temperature at around 8°C inside the isolated mine workings perfectly preserved organic archaeological artefacts (e.g. clothing, mining tools) that provide unique insights into the daily life of a progressive community in Hallstatt. In order to reconstruct the diet of the former population and gain insights into the ancient gut microbiome composition, we subjected human paleofeces from the mining system to in-depth microscopic and metagenomic analysis. The microscopic survey identified bran and glumes of the Triticum/Hordeum type as one of the most prevalent dietary plant fragments. This highly fibrous, carbohydrate-rich diet was supplemented with proteins from broad beans and occasionally with fruits and nuts. The metagenomic analyses supported and further extended the microscopic results by identifying additional plant (walnut) and animal (bovine) diets. Furthermore, the metagenomic analysis allowed the reconstruction of ancient intestinal microbiomes and comparative analysis with modern gut microbiomes provided insights into the genetic diversity of selected gut bacteria (e.g. *Prevotella copri*). The observed decline in microbial diversity within millennia could be possibly due to dietary changes. Our observations will allow to establish hypotheses on the rise of modern diseases such as obesity that we can investigate in modern clinical studies.*

Tracing human evolution through oral microbiome gene content

Velsko Irina (1), Fellows Yates James (1), Kühnert Denise (1), Herbig Alexander (1), Warinner Christina (1, 2)

1 - Max Planck Institute for the Science of Human History (Germany), 2 - Harvard University (United States)

Bacterial genetic evolutionary changes may arise and become fixed in a population much faster than occurs in humans. This offers a possibility of tracing human

behavioral change through the genetic evolution of host-associated bacteria of the microbiome. Human salivary amylase is a multi-copy gene that is thought to have undergone copy number expansion in response to increasing dietary starch through human evolution. Several oral Streptococcus species produce proteins that bind salivary amylase. Dating the introduction of amylase-binding protein (ABP) genes to the oral microbiome, or dating population expansion of the genes, may help us understand more precisely when humans started to consume starchier diets. We investigated the presence of streptococcal ABP genes in ancient dental calculus, as a proxy for timing human dietary changes. Ancient dental calculus from anatomically modern humans(n=24), Neanderthals(n=11), chimpanzees(n=20), gorillas(n=29), and howler monkeys(n=5), as well as modern human calculus (n=18), was mapped against ABP genes from 15 Streptococcus strains. Calculus from anatomically modern humans had ABP genes covered at least 40% at 1X, coverage fell below this cut-off in Neanderthal calculus, and calculus from chimpanzees, gorillas, and howler monkeys had no mapped reads. Ancient samples had fewer than 100 mapped reads for each gene, but the alignments showed A/T mismatches at molecule ends indicative of ancient DNA damage. Preliminary BEAST2 dating analyses suggest gene expansion began 8000 years ago. We developed a capture for these genes and expect to pick up the gene in more samples and at higher coverage, allowing more accurate dating with BEAST2.

Insights into the genomic evolution of *Yersinia pestis* through comparative pangenomics and de novo assembly

Andrades Valtueña Aida (1), Spyrou Maria A. (1), Nelson Elizabeth A. (1), Carty Niamh (2), Hartle Robert (2), Henderson Michael (2), Knox Elizabeth L. (2), Walker Don (2), Bos Kirsten I. (1), Herbig Alexander (1)

1 - Max Planck Institute for the Science of Human History (Germany), 2 - Museum of London Archaeology (MOLA) (United Kingdom)

*Ancient DNA (aDNA) pathogen studies have been focused on reconstructing genomes with a reference-based approach. This consists of mapping the obtained reads for a given sample to a single reference genome. While this has provided insights into both phylogenetic placement and divergence time estimation, the field has only started to scrape the surface of gene content variation in ancient pathogens. Some studies have begun to address this question by looking at specific virulence genes present in the reference genome, however the change of the overall gene content (the pangenome) over time remains largely unexplored. Here we will showcase the utilisation of pangenome analysis in *Yersinia pestis*, the causative agent of plague. *Y. pestis* is one of the best studied pathogens in the aDNA field, from which information has been extracted from the first and second historically-known pandemics but also from its early evolution during the Stone Age. Insights into the evolution of the genomic structure of *Y. pestis* will be explored from studying presence/absence of genes, as well as gene pseudogenisation across the phylogeny. Furthermore, we present a de novo assembled genome dating to the 17th century from the New Churchyard burial ground (London). This assembly provides information into the structural variation in *Y. pestis* genomes towards the end of the second pandemic, including loss of genes and genomic rearrangements. Ancient*

DNA in combination with comparative pangenomics allows for the exploration of temporal and spatial transects to understand *Y. pestis* genomic evolution in more detail than previously possible.

Towards an understanding of the early history of the Second Plague Pandemic
Spyrou Maria (1, 2), Musralina Lyazzat (3, 4, 5), Gneccchi-Rusccone Guido A. (1), Kocher Arthur (6), Kühnert Denise (6), Bos Kirsten I. (1), Djansugurova Leyla (3, 4), Haak Wolfgang (1), Slavin Philip (7), Krause Johannes (1, 8)

1 - Department of Archaeogenetics, Max Planck Institute for the Science of Human History, Jena (Germany), 2 - Institute for Archaeological Sciences (INA), University of Tuebingen (Germany), 3 - Institute of General Genetics and Cytology, Almaty (Kazakhstan), 4 - Kazakh National University by al-Farabi, Almaty (Kazakhstan), 5 - Department of Archaeogenetics, Max Planck Institute for the Science of Human History, Jena Max Planck Institute for the Science of Human History, Jena (Germany), 6 - Transmission, Infection, Diversification & Evolution Group, Max-Planck Institute for the Science of Human History, Jena (Germany), 7 - University of Stirling, Stirling FK9 4LA (United Kingdom), 8 - Department of Archaeogenetics, Max Planck Institute for Evolutionary Anthropology, Leipzig (Germany)

The Black Death (1346-1353 CE) was the initial wave of a pandemic that lasted until the 18th century, affected Europe, Asia and Africa, and is known as the Second Plague Pandemic. Although the pandemic has been characterised as one of the largest infectious disease catastrophes of the pre-modern era, its geographic origins remain unclear. Ancient DNA analyses of epidemic victims from the 14th-18th centuries have revealed the bacterium *Yersinia pestis*, as the primary infectious agent responsible for this event. Such studies have proposed that the initial wave entered Europe through the western region of modern-day Russia and have shown the absence of bacterial genetic diversity in western Europe during the Black Death. Moreover, they have revealed a link between post-Black Death epidemics and modern *Y. pestis* diversity, and have characterized a distinct lineage that persisted in western Eurasia between the 14th and 18th centuries CE. To date, medieval plague research has maintained a somewhat geographically narrow-focused view of the Second Plague Pandemic, a fact that has challenged efforts on the identification of its primary source. Here, we present newly generated and previously published *Y. pestis* genomic data from regions outside of Europe. These data are interpreted within a framework of historical and archaeological evidence to elucidate new possibilities in characterising the early history of the Second Plague Pandemic.

Genetic history of the oldest Upper Palaeolithic modern humans in Europe

Hajdinjak Mateja (1, 2), Mafessoni Fabrizio (1), Skov Laurits (1), Vernot Benjamin (1), Hübner Alexander (1, 3), Fu Qiaomei (4), Essel Elena (1), Nagel Sarah (1), Nickel Birgit (1), Richter Julia (1), Moldovan Oana Teodora (5, 6), Constantin Silviu (7, 8), Endarova Elena (9), Zahariev Nikolay (10), Spasov Rosen (10), Welker Frido (1, 11), Smith Geoff M. (1), Sinet-Mathiot Virginie (1), Paskulin Lindsey (12), Fewlass Helen (1), Talamo Sahra (1, 13), Rezek Zeljko (1, 14), Sirakova Svoboda (15), Sirakov Nikolay (15), Mcpherron Shannon P. (1), Tsanova Tsenka (1), Hublin Jean-Jacques (1, 16), Peter Benjamin M. (1), Meyer Matthias (1), Skoglund Pontus (2), Kelso Janet (1), Pääbo Svante (1)

1 - Max Planck Institute for Evolutionary Anthropology (Germany), 2 - The Francis Crick Institute [London] (United Kingdom), 3 - Max Planck Institute for the Science of Human History (Germany), 4 - Key Laboratory of Vertebrate Evolution and Human Origins of Chinese Academy of Sciences, IVPP, Center for Excellence in Life and Paleoenvironment (China), 5 - Emil Racovita Institute of Speleology, Cluj Department (Romania), 6 - Romanian Institute of Science and Technology (Romania), 7 - Emil Racovita Institute of Speleology, Department of Geospeleology and Paleontology (Romania), 8 - Centro Nacional de Investigación sobre la Evolución Humana (CENIEH) (Spain), 9 - National History Museum (Bulgaria), 10 - Archaeology Department, New Bulgarian University (Bulgaria), 11 - Section for Evolutionary Genomics, GLOBE Institute, University of Copenhagen (Denmark), 12 - Department of Archaeology, University of Aberdeen (United Kingdom), 13 - Department of Chemistry 'G. Ciamician', University of Bologna (Italy), 14 - University of Pennsylvania Museum of Archaeology and Anthropology (United States), 15 - National Institute of Archaeology with Museum, Bulgarian Academy of Sciences (Bulgaria), 16 - Chaire de Paléoanthropologie, Collège de France (France)

Modern humans started spreading across Eurasia by at least 45,000 years before present (BP). However, the genetic history of these early dispersals, the extent to which they interacted with resident archaic populations, and the extent to which they contributed to later populations are poorly understood. Modern human remains from this time period are scarce and there is even smaller number of individuals from whom genome-wide data are available. Among the oldest modern humans from Eurasia are a ~40,000-year-old Oase1 and ~45,000-year-old Ust'-Ishim, both of whom did not contribute considerably to later populations. Here we present genome-wide data from human remains found in direct association with an Initial Upper Palaeolithic (IUP) assemblage at Bacho Kiro Cave, Bulgaria. Directly dated to between 46,790 and 42,810 cal. BP, these individuals represent the oldest Upper Palaeolithic modern humans in Europe recovered to date. Counter to what would be expected for ancient individuals from Europe, the IUP Bacho Kiro Cave individuals are more related to present-day and ancient populations with East Asian ancestry than to later West Eurasians. These results show that multiple differentially related human groups were present in Eurasia during the early Upper Palaeolithic. Moreover, based on the segments of Neandertal ancestry detected in their genomes, we find that the IUP Bacho Kiro Cave individuals had Neandertal ancestors only a few

generations back in their family history, similar to Oase1, and suggesting that Neandertals and modern humans mixed frequently when they met.

Coevolution and Cariogenicity: Analysis of 80 Ancient *Streptococcus mutans* Genomes Spanning the Neolithic Transition

Michel Megan (1, 2, 3), Valtueña Aida Andrades (3), Barquera Rodrigo (3), Ferraz Da Silva Tiago (3, 4), Guevara Evelyn (5), Mittnik Alissa (6), Nägele Kathrin (3), Nelson Elizabeth A. (3), Neumann Gunnar (1, 3), Spyrou Maria (3), Villalba Vanessa (3), Bos Kirsten (3), Haak Wolfgang (3), Posth Cosimo (3), Stockhammer Philipp (1, 3, 7), Herbig Alexander (3), Krause Johannes (1, 3)
1 - Max Planck-Harvard Research Center for the Archaeoscience of the Ancient Mediterranean (Germany), 2 - Department of Human Evolutionary Biology, Harvard University (United States), 3 - Archaeogenetics Department, Max Planck Institute for the Science of Human History (Germany), 4 - Institute of Bioscience, Department of Genetics and Evolutionary Biology, University of São Paulo (Brazil), 5 - Department of Forensic Medicine, University of Helsinki (Finland), 6 - Department of Genetics, Harvard Medical School (United States), 7 - Institute for Pre- and Protohistoric Archaeology and Archaeology of the Roman Provinces, Ludwig Maximilian University (Germany)

*The increased prevalence of dental cavities in the Neolithic period is a commonly-cited example of an evolutionary mismatch, in which bacterial fermentation of carbohydrate-rich agricultural diets generated more cariogenic plaque microenvironments. The gram-positive opportunistic pathogen *Streptococcus mutans* plays a key role in caries pathogenesis in modern populations, given its ability to form biofilms, metabolize diverse carbohydrates, and withstand acidic metabolic byproducts. However, whether these virulent traits pre-date the spread of agriculture or represent coevolutionary adaptations to this new human dietary niche remains to be established. To address this question, we generate the first ancient *S. mutans* genome-wide data using a novel in-solution DNA capture designed to encompass modern pan-genomic diversity. From a global set of 80 individuals spanning the Mesolithic through the Medieval period, 60 samples exceeded 3X-fold average genomic coverage and display fragment lengths and damage patterns characteristic of authentic ancient DNA. Based on analysis of heterozygous SNP calls, we conclude that the majority of samples harbor single *S. mutans* isolates, while others consist of major and minor strain mixtures. We identify several loci that are differentially present in European Neolithic individuals compared to a group of six ancient hunter-gathers from Europe, Africa, and South America. Among these is the ComCDE region, part of a pathway regulating virulence-associated phenotypes including bacteriocin production, competence for natural genetic transformation, and biofilm formation. Based on these results, we highlight ancient pan-genome analysis as a promising approach for further exploration of virulence acquisition in the cariogenic oral microbe *Streptococcus mutans*.*

Tracking the origins and spread of donkey domestication using ancient genomes

Todd Evelyn (1), Chauvey Lorelei (1), Schiavinato Stéphanie (1), Tonasso Laure (1), The Erc Pegasus Consortium ., Arbuckle Ben (2), Beja-Pereira Albano (3), Bradley Daniel (4), Clavel Benoit (5), Fages Antoine (1, 6, 7), Gardeisen Armelle (8), Gaunitz Charleen (1, 6), Lepetz Sébastien (5), Mashkour Marjan (5), Outram Alan (9), Vedat Onar (10), Orlando Ludovic (1)

1 - Centre for Anthropobiology and Genomics of Toulouse (CAGT) - UMR5288 (France), 2 - Department of Anthropology, University of North Carolina at Chapel Hill (United States), 3 - Research Center in Biodiversity and Genetic Resources (CIBIO), University of Porto (Portugal), 4 - Smurfit Institute of Genetics, Trinity College Dublin (Ireland), 5 - Archéozoologie - Archéobotanique. Sociétés, Pratiques et Environnements - UMR 7209 (France), 6 - Centre for GeoGenetics (Denmark), 7 - Department of Environmental Sciences, University of Basel (Switzerland), 8 - Archéologie des Sociétés Méditerranéennes - UMR5140 (France), 9 - Department of Archaeology, University of Exeter (United Kingdom), 10 - Anatomy Department, Istanbul University Faculty of Veterinary Medicine (Turkey)

*The domestication of the donkey (*Equus asinus*) has allowed human societies to transport heavy goods over long distances in arid conditions many thousands of years before the invention of machinery. Since their domestication, donkeys have also been used by humans for the production of food, to guard livestock and in traditional medicines. However, unlike their close relative the domestic horse (*Equus caballus*), little is known about the process of donkey domestication. There is early archaeological evidence of domestic donkeys in the Nile Valley, the Horn of Africa and Persia. It is, however, currently not known whether all domestic donkeys arose from a unique domestication or if they were domesticated multiple times. Additionally, the process in which donkeys subsequently spread throughout the world after their domestication is also not known. In this study, we generated and analyzed an extensive dataset of modern donkey genomes to understand the genetic makeup and relationships between different subpopulations throughout the world. We also created a genomic time series using ancient donkey remains spread across Europe and South West Asia to address changes in the genetic makeup of donkeys throughout history. These findings revisit current scenarios on the domestication process and the subsequent spread of the animal throughout the world.*

100,000 years of wolf population dynamics

Bergström Anders (1), Stanton David (2), Talon Ulrike H. (3), Frantz Laurent (4), Sinding Mikkel-Holger S. (5), Ersmark Erik (2), Schuenemann Verena (6), Cassatt-Johnstone Molly (7), Davy Tom (1), Girdland-Flink Linus (8), Gopalakrishnan Shyam (5), Madrigal Jazmín Ramos (5), Feuerborn Tatiana R. (5), Münzel Susanne C. (9), Pfrengle Saskia (9), Conard Nicholas (9), Carøe Christian (5), Haile James (10), Linderholm Anna (11), Androsov Semyon (12), Benecke Norbert (13), Bocherens Hervé (9), Carden Ruth F. (14), Fedorov Sergey (15), Lázníčková-Galetová Martina (16), Gasparik Mihaly (17), Germonpré Mietje (18), Grigoriev Semyon (15), Groves Pam (19), Ivanova

Varvara V. (20), Janssens Luc (21), Jennings Richard P. (22), Kasparov Aleksei K. (23), Kirrilova Irina V. (24), Kuzmin Yaroslav (25), Nikolskiy Pavel (26), Pavlova Elena Y. (27), Pilot Małgorzata (28), Pitulko Vladimir V. (23), Plotnikov Valeri (29), Protopopov Albert (29), Sablin Mikhail (30), Schidlovskiy Fedor K. (24), Storå Jan (31), Leonard Jennifer A. (32), Pinhasi Ron (33), Shapiro Beth (7), Götherström Anders (31), Hofreiter Michael (3), Gilbert M. Thomas P. (5), Larson Greger (10), Krause Johannes (34), Dalén Love (2), Skoglund Pontus (1)

1 - Francis Crick Institute (United Kingdom), 2 - Swedish Museum of Natural History (Sweden), 3 - University of Potsdam (Germany), 4 - Ludwig-Maximilian-University (Germany), 5 - University of Copenhagen (Denmark), 6 - University of Zurich (Switzerland), 7 - University of California, Santa Cruz (United States), 8 - University of Aberdeen (United Kingdom), 9 - University of Tübingen (Germany), 10 - University of Oxford (United Kingdom), 11 - Texas A&M University (United States), 12 - Museum 'Severnyi Mir' (Russia), 13 - German Archaeological Institute (Germany), 14 - University College Dublin (Ireland), 15 - North-Eastern Federal University (Russia), 16 - Moravian Museum (Czech Republic), 17 - Hungarian Natural History Museum (Hungary), 18 - Royal Belgian Institute of Natural Sciences (Belgium), 19 - University of Alaska (United States), 20 - VNIIOkeangeologiya (Russia), 21 - University of Leiden (Netherlands), 22 - Liverpool John Moores University (United Kingdom), 23 - Institute for the History of Material Culture, Russian Academy of Sciences (Russia), 24 - Ice Age Museum, Shidlovskiy National Alliance Ice Age (Russia), 25 - Institute of Geology and Mineralogy (Russia), 26 - Geological Institute, Russian Academy of Sciences (Russia), 27 - Arctic & Antarctic Research Institute (Russia), 28 - Museum & Institute of Zoology, Polish Academy of Sciences (Poland), 29 - Academy of Sciences of Sakha Republic (Russia), 30 - Zoological Institute of the Russian Academy of Sciences (Russia), 31 - Stockholm University (Sweden), 32 - Estación Biológica de Doñana (Spain), 33 - University of Vienna (Austria), 34 - Max Planck Institute for Evolutionary Anthropology (Germany)

The highly widespread grey wolf survived the environmental changes of the late Pleistocene, but present-day population structure in the species appears to have formed mostly after the last glacial maximum (LGM), i.e. in the last 25,000 years. To understand the history of this carnivore in detail, we have compiled a time series of 73 ancient wolf genomes with a median coverage of 1x from Europe, Siberia and North America, spanning the last 100,000 years. We find that the turnover of wolf ancestry after the LGM was incomplete, with European wolves retaining some deep local ancestry. We also find that a similar ancestry turnover had occurred earlier, suggesting an ongoing homogenization process unfolding over tens of thousands of years, driven by gene flow from Siberia into the rest of the world. Strong population structure never seemed to build up, as genetic differentiation was low and divergence times shallow throughout the time series. Despite this, the retention of minority fractions of deeper ancestry in most present-day worldwide populations suggests that wolves did not experience widespread local extinction in the late Pleistocene. We also identify positively selected variants by directly testing for allele frequency change over time, and investigate what the understanding of the deep history of wolves can reveal about the origin of dogs.

A whole-town approach to understanding mobility: an isotopic investigation of Medieval Cambridge, UK

Rose Alice (1), Bradbury Harold (2), Cessford Craig (1, 3), Dittmar Jenna (4), Hui Ruoyun (5), Inskip Sarah (6), Kivisild Toomas (7), Mitchell Piers (1), Mulder Bram (1), O'connell Tamsin (1), Scheib Christiana (8, 9), Robb John (1)

1 - Department of Archaeology, University of Cambridge (United Kingdom), 2 - Department of Earth Sciences, University of Cambridge (United Kingdom), 3 - Cambridge Archaeological Unit (United Kingdom), 4 - Department of Archaeology, University of Aberdeen (United Kingdom), 5 - The Alan Turing Institute (United Kingdom), 6 - School of Archaeology and Ancient History, University of Leicester (United Kingdom), 7 - Department of Human Genetics, KU Leuven (Belgium), 8 - Institute of Genomics, University of Tartu (Estonia), 9 - St John's College, University of Cambridge (United Kingdom)

In Medieval England, people were mobile for a variety of reasons. In particular, the social and economic consequences of the plague pandemic in the mid-14th century, known as the Black Death, is assumed to have influenced patterns of mobility. This paper integrates $87\text{Sr}/86\text{Sr}$ and $\delta^{18}\text{O}$ data with high-resolution contextual information generated as part of the 'After the Plague: Health and History in Medieval Cambridge' project to explore mobility within the Medieval town of Cambridge, to investigate whether social and temporal changes are evident, and to assess whether the impact of the Black Death can be detected. 113 $87\text{Sr}/86\text{Sr}$ results and 169 carbonate $\delta^{18}\text{O}$ results were obtained from tooth enamel samples from individuals excavated from six sites (c.10th-16th centuries) located in Cambridge or its rural hinterlands. The sample was demographically proportional, and represented a mixture of social groups, from 'ordinary' parish individuals, to Augustinian Friars, to those who may have received care in the Medieval Hospital of St John the Evangelist. This strategy of large-scale representative sampling across various cemeteries and social groups within a geographically constrained area has not been previously attempted in isotopic studies of mobility in Medieval England and allowed for a unique whole-town approach to assessing mobility in the period. From this, several individuals were identified as having spent their childhoods outside of Cambridge, subtle variations in mobility were seen between males and females in the parish cemeteries, but overall changes in $87\text{Sr}/86\text{Sr}$ and $\delta^{18}\text{O}$ values through time were not detected.

Evolutionary insights into *Tannerella forsythia* in the Americas through paleogenomics

Bravo-Lopez Miriam (1), Cruz Dávalos Diana I. (2, 3), Villa-Islas Viridiana (1), Reis Silvia (4), Bastos Murilo (5), Neuenschwander Samuel (6, 7), Stenderup Jesper (8), Rodrigues-Carvalho Claudia (4), Allentoft Morten E. (8), Malaspinas Anna-Sapfo (3) (7), Ávila-Arcos María C. (1)

1 - International Laboratory for Human Genome Research (LIIGH-UNAM) (Mexico), 2 - University of Lausanne (Switzerland), 3 - Swiss Institute of Bioinformatics (Switzerland), 4 - Universidade Federal Rural do Rio de Janeiro (Brazil), 5 - Federal University of Rio de Janeiro (Brazil), 6 - Vital-IT, Swiss Institute of Bioinformatics (Switzerland), 7 - Department of Computational Biology (University of Lausanne Switzerland), 8 - University of Copenhagen (Denmark)

Tannerella forsythia is one of the main bacterial contributors to the development of

periodontitis, a common condition in ancient and modern populations. The evidence of T. forsythia DNA in archaic hominins suggests a long-standing relationship with its human host. There is limited knowledge of the evolutionary history of this oral pathogen in a spatio-temporal framework across the Americas. Here, we report the recovery of ancient DNA (aDNA) of this pathogen from the so-called «Botocudo» hunter-gatherers from Central-Eastern Brazil. We extracted aDNA, built sequencing libraries, and generated shotgun sequencing data from teeth. We used Kraken2 to taxonomically classify the reads and to screen for the presence of pathogens. We identified numerous reads assigned to T. forsythia in two individuals from European Pre- and Post-Contact periods, respectively. The identification of the characteristic damage patterns expected for aDNA, supported its ancient nature. We reconstructed one T. forsythia genome from the Post-Contact individual at 23X depth. We placed the reconstructed genome in a phylogenetic tree including available ancient T. forsythia genomic sequences from Chile, Mexico, Europe, as well as modern strains. Overall, the phylogenetic placement of T. forsythia genomes from the Americas followed a temporal trend; the Pre-contact strains formed a monophyletic clade, as previously observed; while the Post-Contact strains clustered with a modern genome and with ancient European strains. The genetic characterization of T. forsythia in ancient individuals from Brazil provides additional insights on the transmission dynamics of the pathogens introduced after European colonization.

Genetic ancestry changes in Stone to Bronze Age transition in the East European plain

Saag Lethi (1), Vasilyev Sergey (2), Varul Liivi (3), Kosorukova Natalia (4), Gerasimov Dmitriy (2), Oshibkina Svetlana (2), Griffith Samuel (1), Solnik Anu (1), Saag Lauri (1), D'atanasio Eugenia (5), Metspalu Ene (1), Reidla Maere (1), Rootsi Siiri (1), Kivisild Toomas (1, 6), Scheib Christiana Lyn (1, 7), Tambets Kristiina (1), Kriiska Aivar (1), Metspalu Mait (1)

1 - University of Tartu (Estonia), 2 - Russian Academy of Sciences (Russia), 3 - Tallinn University (Estonia), 4 - Cherepovets State University (Russia), 5 - National Research Council (Italy), 6 - KULeuven (Belgium), 7 - University of Cambridge (United Kingdom)

The transition from the Stone to the Bronze Age in Central and Western Europe was a period of major population movements originating from the Ponto-Caspian Steppe. Here, we report new genome-wide sequence data from 30 individuals from the territory north of this area - from the under-studied Western part of present-day Russia. We present genetic data from three Stone Age hunter-gatherers (10,800-4,250 cal BCE), including an on average 5x covered shotgun sequenced genome. Furthermore, we have sequenced the genomes of 26 Bronze Age farmers associated with the Fatyanovo Culture (2,900-2,050 cal BCE). Fatyanovo Culture is the easternmost extension of the Corded Ware cultural complex that was spread over a large area in Western Russia and introduced animal husbandry and probably crop cultivation into the forest belt. We show that Eastern hunter-gatherer ancestry was present in Northwestern Russia already from around 10,000 BCE. Hence, we present the highest coverage whole genome and oldest individual with this ancestry

published so far. Furthermore, we see a change in ancestry with the arrival of farming - Fatyanovo Culture individuals were genetically similar to other Corded Ware cultures, carrying a mixture of Steppe and European early farmer ancestry. We propose that the possible origin of the migration leading to the formation of the Fatyanovo Culture and of the Corded Ware cultures in general could be modern-day Ukraine, which is the closest area where these ancestries coexisted from around 3,000 BCE.

Ancient DNA reveals admixed genetic ancestry and no differences between socioeconomically stratified burial groups at the Christian Period Nubian site of Kulubnarti

Sirak Kendra (1, 2), Van Gerven Dennis (3), Thompson Jessica (4), Pinhasi Ron (5), Reich David (1, 2, 6, 7)

1 - Harvard University (United States), 2 - Harvard Medical School (United States), 3 - University of Colorado at Boulder (United States), 4 - Yale University (United States), 5 - University of Vienna (Austria), 6 - Broad Institute of MIT and Harvard (United States), 7 - Howard Hughes Medical Institute (United States)

Nubia has been a corridor for demographic and cultural exchange between sub-Saharan Africa, Egypt, and West Eurasia since prehistory, but little is known about the genetic landscape of the region before the Islamic migrations of the late 1st-2nd millennia CE. Ancient DNA analysis of people from Christian Period (~650-1000 CE) Kulubnarti can elucidate the genetic ancestry of a group from pre-Islamic Nubia and address questions about the relationship among people buried in two cemeteries that appear to be socially stratified. Analyzing genome-wide data for 66 individuals, we identify an average of ~43% Nilotic-related ancestry, with the remaining ancestry reflecting a West Eurasian-related gene pool likely introduced into Nubia through Egypt, but ultimately deriving from an ancestry pool like that found in the Bronze and Iron Age Levant. The Kulubnarti gene pool formed over the course of at least a millennium, with disproportionately female-associated West Eurasian ancestry. The frequency of 8-20cM ROH indicates limited population-level relatedness and implies connections with a broader population. It is possible that these connections were primarily female-mediated, and that Kulubnarti was a patrilineal and patrilocal society that followed a system of patrilineal primogeniture. We identify seven pairs of inter-cemetery relatives consistent with a scenario of fluidity between groups and the absence of a caste-like system of social division at Kulubnarti. Ancient DNA provides a new line of evidence supporting the hypothesis that the burial of people in two cemeteries at Kulubnarti was not strongly rooted in genetic differences.

Identification of hominin and faunal turnovers at Denisova Cave from sediment DNA

Zavala Elena (1), Jacobs Zenobia (2, 3), Vernot Benjamin (1), Essel Elena (1), De Filippo Cesare (1), Nagel Sarah (1), Richter Julia (1), Romagné Frédéric (1), Schmidt Anna (1), O'gorman Kieran (2), Li Bo (2, 3), Kozlikin Maxim B. (4), Shunkov Michael V. (4), Derevianko Anatoly P. (4), Slon Viviane (1, 5, 6), Kelso

Janet (1), Pääbo Svante (1), Roberts Richard G. (2, 3), Meyer Matthias (1)

1 - Max Planck Institute for Evolutionary Anthropology (Germany), 2 - Centre for Archaeological Science, School of Earth, Atmospheric and Life Sciences, University of Wollongong (Australia), 3 - Australian Research Council (ARC) Centre of Excellence for Australian Biodiversity and Heritage, University of Wollongong (Australia), 4 - Institute of Archaeology and Ethnography, Russian Academy of Sciences (Russia), 5 - Department of Anatomy and Anthropology and Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University (Israel), 6 - The Dan David Center for Human Evolution and Biohistory Research, Tel Aviv University (Israel)

The integration of ancient DNA with paleo-ecological studies allows us to investigate both the evolutionary history of humans and the environments they lived in. Currently, these studies are hampered by the scarcity of hominin remains and the time and resources required for morphological identification of faunal remains. The discovery that DNA from Pleistocene mammals, including hominins, can be retrieved from cave sediments opens up the possibility of examining the relationship between faunal composition and hominin occupation over time at archaeological sites. We explore this possibility at Denisova Cave, a site in Russia's Altai Mountains, which is thought to be a contact zone for different faunal and hominin groups. We tested 728 sediment samples from the cave's Pleistocene layers for faunal and hominin mitochondrial (mt) DNA, using a fully automated workflow for DNA extraction, library preparation and hybridization capture. Ancient mammalian mtDNA was identified in 685 (94%) samples, enabling us to explore the extent to which mammalian mtDNA preservation correlates with the age and properties of the sediments it was recovered from. Ancient hominin mtDNA was identified in 175 (24%) samples. We identified changes in the relative proportions of DNA from various mammalian families, shifts in the presence of mtDNA from various mammals and different hominin groups, as well as turnovers of mitochondrial lineages, some of which appear to coincide with past climatic changes. Our study demonstrates the potential of using sediment DNA for increasing our understanding of past faunal diversity, paleoecology, and hominin occupations at archaeological sites.

A Window into Late Bronze Age Central Europe: Multiproxy investigations at Kuckenburg

Orfanou Eleftheria (1), Himmel Marie (1), Paust Enrico (2), Roberts Patrick (3), Spengler Robert (3), Ettl Peter (2), Haak Wolfgang (1)

1 - Max Planck Institute for the Science of Human History, Department of Archaeogenetics (Germany), 2 - Lehrstuhl für Ur- und Frühgeschichte mit Sammlung UFG, Jena (Germany), 3 - Max Planck Institute for the Science of Human History, Department of Archaeology (Germany)

The coexistence of different mortuary practices within a given site offers the possibility of studying the intersection of demographic, social, and cultural variability. This study focuses on a multidisciplinary analysis of the human skeletal remains from the Late Bronze Age (LBA) site of Kuckenburg in central Germany. The site constitutes a unique case-study of parallel practice of both inhumations and cremations during the Urnfield period (1300 - c.800 BC), when, elsewhere in Europe, cremations appear to have been the norm of mortuary practice. By combining ancient DNA, stable isotope, and archaeological analyses, we aim to better understand the Urnfield period related groups in the LBA of Central Europe. Analysis of genome-wide data from 26 inhumated individuals suggests a shared genetic profile with individuals from the preceding Early Bronze Age period, indicating genetic continuity over time. In addition, stable isotope analyses suggest a similar diet to the earlier time period, but with some subtle variation. We also demonstrate that all LBA individuals from Kuckenburg were unrelated, with the exception of a mother buried with her two daughters. This triple burial, like most burials at Kuckenburg, points to a multi-phase burial rite. Our findings indicate that different mortuary practices at Kuckenburg were not the result of a new genetic group coming in but rather increasing cultural variation within a local population. Our study demonstrates that a combination of multiproxy evidence can allow for the reconstruction of a more complete picture of the past.

Analysing animal mobility at a large scale: challenges and possibilities

Valenzuela-Lamas Silvia (1), Nieto-Espinet Ariadna (1), Trentacoste Angela (2), Guimarães Sílvia (1), Bosch Delphine (3)

1 - Institució Milà i Fontanals (IMF-CSIC) (Spain), 2 - School of Archaeology, University of Oxford (United Kingdom), 3 - Géosciences Montpellier (France)

The analysis of strontium isotopes ($^{87}\text{Sr}/^{86}\text{Sr}$) from animal enamel is increasingly used to assess animal mobility. Combined with oxygen isotopes ($\delta^{18}\text{O}$), these analyses open new perspectives for characterising source areas, mobility patterns and changes through time; however, these approaches also present challenges due to their financial costs, their destructive nature and the baselines needed to interpret results. The present contribution will provide a discussion of the sampling strategy and the results obtained in the frame of the ERC project 'ZoomWest- Zooarchaeology and Mobility in the Western Mediterranean: from the Late Bronze Age to Late Antiquity'. The project combines the study of animal mobility and animal husbandry, and both isotopic (strontium and oxygen) and zooarchaeological data are analysed at a large chronological and spatial scale. The discussion will include some of the

results obtained from these analyses, which suggest different mobility patterns depending on the species and the time frame considered, thus suggesting that mobility depends of a variety of factors including the social role of animals and the socio-political system.

CASCADE: A Custom-Made Archiving System for the Conservation of Ancient DNA Experimental Data

Mata Xavier (1), Dolle Dirk, Fages Antoine (1, 2), Schiavinato Stéphanie (1), Tonasso Laure (1), Chauvey Lorelei (1), Wagner Stefanie (1,3), Der Sarkissian Clio (1), Fromentier Aurore (1), Seguin-Orlando Andaine (1), Orlando Ludovic (1)

1 - Centre d'Anthropobiologie et de Genomique de Toulouse (CAGT) (France), 2 - Centre for GeoGenetics (Natural History Museum of Denmark, Copenhagen, Denmark), 3- BIOGECO, INRA, Univ. Bordeaux (Cestas, France)

The field of ancient genomics has undergone a true revolution during the last decade. The experimental procedures underlying ancient DNA characterization are now well integrated in the laboratory and even subject to automation. These procedures have dramatically improved the efficiency of data generation. Together with the growing diversity of archaeological samples that are amenable to DNA sequencing, this has resulted in considerably enhancing the processing capacity of individual laboratories. However, this increase in the volume of processed samples has come with important logistical challenges pertaining to the traceability and long-term storage of the whole metadata generated during the experimental process. Here we present CASCADE, a laboratory information management system (LIMS) dealing with the specificities of ancient DNA sample processing and tracking, applicable by all types of laboratories, and scalable to large projects involving the analysis of thousands of samples. By providing live tracking of experimental progress, CASCADE not only provides a constructive collaborative experience, particularly in sharing information in real time with third parties, but also improves the efficiency of data generation and lab traceability. CASCADE is fully available for free upon request and can be installed on any computational platforms through a virtual environment.

First insights into the population genetics of the extinct Darwin's ground sloth (*Mylodon darwini*) from Cueva del Milodon, Chile.

Zicos Maria (1, 2) Frantz Laurent (3, 4), Brace Selina (1), Turvey Samuel (5)

1 - Department of Earth Sciences, The Natural History Museum, London (United Kingdom), 2 - School of Biological and Chemical Sciences, Queen Mary University of London (United Kingdom), 3 - School of Biological and Chemical Sciences, Queen Mary University of London (United Kingdom), 4 - Palaeogenomics Group, Department of Veterinary Sciences, Ludwig Maximilian University, Munich (Germany), 5 - Institute of Zoology, Zoological Society of London (United Kingdom)

Sloths (*Xenarthra*, *Folivora*) were one of the dominant mammalian groups in Southern and Central America until the early Holocene. *Folivora* used to have a large range of body sizes, locomotion and ecology, while extant sloths are morphologically and ecologically similar. Research into the molecular evolution and biology of sloths, previously limited to extant species, has started to leverage information contained in the large recent fossil record of this group through ancient DNA methods. Cueva del Milodon (Ultima Esperanza, Chile) is renowned for its exceptionally preserved faunal

record. Remains of Darwin's ground sloth (*Myodon darwini*) are found there from the end of the last Ice Age to their extinction in the early Holocene. Here we present novel findings from NGS generated genomic data from multiple *M. darwini* individuals, exploring genetic diversity in this site. Using ancient DNA methods, twelve new mitochondrial genomes and two nuclear genomes were recovered from bone, skin and coprolites from Cueva del Milodón in British and Swiss museum collections. The mitochondrial diversity was low, yet higher than that found in another single-locality megaherbivore population, sampled near its extinction: Holocene woolly mammoths (*Mammuthus primigenius*) from Wrangel Island. Nuclear diversity in the two individuals sequenced to higher coverage will reveal whether low diversity is also found in the nuclear genome. These findings, combined with direct radiocarbon dates, will be used to reconstruct the demographic history of the species, and to test models for their extinction, hoping to better understand why these sloths became extinct, while extant sloths persisted.

Identifying biological and behavioural diversity among European Neolithic farmers

Goude Gwenaëlle (1), Rivollat Maité (2, 3), Schmitt Aurore (4), Salazar-García Domingo (5, 6), Villotte Sébastien (7), Lambert Aurore (8, 9), Sanz Antoine, Brige Pauline (10, 11), Pemonge Marie-Hélène (12), Haak Wolfgang (13), Deguilloux Marie-France (14)

1 - Laboratoire Méditerranéen de Préhistoire Europe Afrique (France), 2 - de la Préhistoire à l'Actuel, Cultures, Environnement, Anthropologie (France), 3 - Department of Archaeogenetics, Max Planck Institute for the Science of Human History, (Germany), 4 - Anthropologie bio-culturelle, Droit, Ethique et Santé (France), 5 - Department of Geological Sciences. University of Cape Town (South Africa), 6 - Departamento de Geografía, Prehistoria y Arqueología. Universidad del País Vasco-Euskal Herriko Unibertsitatea. (Spain), 7 - De la Préhistoire à l'Actuel : Culture, Environnement et Anthropologie (France), 8 - Anthropologie bio-culturelle, Droit, Ethique et Santé (France), 9 - EVEHA, LIMOGES (France), 10 - Aix Marseille Univ, LIIE, Marseille (France), 11 - Aix Marseille Univ, CERIMED, Marseille (France), 12 - PACEA (UMR 5199, France), 13 - Max Planck Institute for the Science of Human History, Department of Archaeogenetics (Germany), 14 - De la Préhistoire à l'Actuel : Culture, Environnement et Anthropologie (PESSAC, France)

Applying anthropological and biomolecular methods, we analysed Middle Neolithic human remains from the site of Pontcharaud 2 (ca. 4300-3900 BCE; Auvergne, France) to reconstruct behavioural patterns, life history and social organisation of early farmers in Western Europe. Data from bioanthropological investigations (n=61 individuals), CT-scans (femur, humerus; n=47 individuals), new stable isotope measurements (bone collagen $\delta^{34}\text{S}$ on 56 individuals; $^{87}\text{Sr}/^{86}\text{Sr}$ on teeth enamel for 17 individuals) and ancient DNA analyses (n=12 individuals) were collated and integrated with contextual data from funerary treatments and previous published isotopic data ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$). Our results highlight two main observations: i) the role of females in the community (diet, mobility, activities), and ii) the link between mortuary treatment and possible social status and kinship of certain individuals. New stable isotope data confirms previous findings, which showed that females were exposed to more variable environments compared to male. Strontium and sulphur isotope ratios also indicate non-local signals and thus a different mobility pattern for two females, one of which was buried in an alleged founder grave. Cross-sectional geometry

analysis of femora also exhibit female/male differences and suggest gender/sex specific activities. The seven adult/immature male individuals of the only multiple grave provided different stable isotope results (C, N, S), indicating different origin and/or animal protein intake compared to the individuals buried in single/double graves. Our multidisciplinary data suggests variabilities in diet, mobility, social status and organisation among this human group.

Ancient DNA analysis of feathers from funerary bundles at the pre-Hispanic religious center of Pachacamac (Peru)

Bover Pere (1, 2), Llamas Bastien (3), Heiniger Holly (3), Olah George (4, 5), Segura Rafael (6), Shimada Izumi (6)

1 - Aragonese Foundation for Research and Development (ARAID) (Spain), 2 - IUCA - Universidad de Zaragoza (Spain), 3 - Australian Centre for Ancient DNA - University of Adelaide (Australia), 4 - Fenner School of Environment and Society - Australian National University (Australia), 5 - Wildlife Messengers - Richmond, Virginia (United States), 6 - Department of Anthropology - Southern Illinois University (United States)

Pachacamac is a famed pre-Hispanic religious center on the central coast of Peru, which cultural chronology has been divided into four periods: Lima (ca. A.D. 350-650), Pachacamac (ca. A.D. 650-1000), Ychsma (ca. A.D. 1000-1470), and Inca ca. A.D. 1470-1533). We analyzed feathers derived from the headdresses atop the false (artificial) heads that were constructed atop the globular funerary bundles that were tightly packed inside a large stone-lined chamber tomb immediately in front of the highly sacred Painted Temple. Each of the excavated bundles encased in most cases a tightly flexed human skeleton. They date to a period spanning ca. 1000 to 1400 CE. Feather DNA was extracted using a silicicon dioxide suspension method and genetic libraries were enriched for mitochondrial genomes using home-made RNA probes. From the 25 feathers used, nine provided enough endogenous DNA to identify the bird, including three Scarlet macaws (*Ara macao macao*), three Blue-and-yellow macaws (*Ara ararauna*), a Red-and-green macaw (*Ara chloropterus*), a Southern mealy amazon (*Amazona farinosa*), and a Sabine's gull (*Xema sabini*). The presence of the *Psittaciformes* identified in this study in a coastal region far from the current geographic distribution of these species, agree with the exploitation of colourful big feathers from birds living in the highland areas in the forest to be used as decoration of the funerary bundles of the social elites buried at Pachacamac.

Genetic continuity in Central Asia since Iron Age

Guarino-Vignon Perle (1), Marchi Nina (1, 2), Monnereau Aurore (1, 3), Chimènes Amélie (1), Bendezu Sarmiento Julio (1, 4), Lhuillier Johanna (5), Mashkour Marjan (6), Heyer Evelyne (1), Bon Céline (1)

1 - UMR7206 Éco-Anthropologie (France), 2 - CMPG group, Institute of Ecology and Evolution (Bern, Switzerland), 3 - BioArCh, Environment, Department of Archaeology University of York (United Kingdom), 4 - Délégation archéologique française en Afghanistan (Afghanistan), 5 - ARCHEORIENT - Environnements et sociétés de l'Orient ancien (France), 6 - Centre National de Recherche Scientifique, CNRS (France)

Since prehistoric times, South Central Asia has been a region at the crossroads of the movement of people, cultures, and goods. Today, Central Asia is populated by populations divided into two cultural and linguistic groups: the Indo-Iranian group and the Turko-Mongolian group. Genetics unveiled that migrations from East Asia contributed to the spread of Turko-Mongolian populations in Central Asia and to the partial replacement of Indo-Iranian population. However the origin of the latter is still little known. To shed light on this, we compare the genetic data on two current-day populations obtained in our laboratory - Yagnobis and Tajiks - with the increasing number of published ancient genomes and four new genomes from the Bronze Age to the Middle Age from Sialk (Iran) and Ulug-Depe (Turkmenistan). Using PCA, f_3 and D -statistics, and qpAdm we show that the present Indo-Iranian populations from Central Asia show a strong genetic continuity with the Iron Age samples from Turkmenistan and Tajikistan. With qpAdm, we model Yagnobis as a mixture of 93% Iron Age individuals from Turkmenistan and 7% from East Asia. For the Tajiks, we observe a more important East Asian ancestry and an additional admixture event with a South Asian population. Our results therefore suggest that beside complex history and settlement, Central Asia shows a remarkable genetic continuity since the Iron Age.

Identifying the geospatial origins of Altai Neolithic and Bronze Age communities in bone collagen δ^{2H} and δ^{18O}

Makarewicz Cheryl (1) Reynard Linda (2), Grushin Sergey (3), Kiryushin Kirill (3), Tishkin Alexey (3)

1 - University of Kiel (Germany), 2 - Boise State University (United States), 3 - Altai State University (Russia)

The hydrogen and oxygen isotopic composition of precipitation varies predictably at continental, regional, and local scales, influenced by amount effects, temperature, altitude, and aridity. Here, we explore investigate geospatial variation in the bone collagen δ^{2H} and δ^{18O} values in obligate drinking cattle, and semi-obligate drinking caprines, and humans from Neolithic and Bronze Age communities located the Altai, a region that served as a conduit for the spread of mobile pastoralism into Inner Asia and the translocation livestock domesticates. We juxtapose these results against δ^{18O} values measured from biopapatite carbonates in order to further establish the biological basis of hydrogen and oxygen isotopes from collagenous proteins. In doing so, we seek to better establish the utility of bone collagen δ^{2H} and δ^{18O} as a biomarker for human and animal geospatial origins in a landscape that supports a complex local hydrological regime that includes numerous snowmelt-fed rivers and lakes.

Organic networks and foodstuffs in Bronze Age south-eastern Arabia: preliminary results of lipid residue analysis of local and imported vessels

Suryanarayan Akshyeta (1), Degli Esposti Michele (2), Méry Sophie (3), Mazuy Arnaud (1), Regert Martine (1)

1 - Culture et Environnements, Préhistoire, Antiquité, Moyen-Age (CEPAM) (France), 2 - Centre de Recherche en Archéologie, Archéosciences, Histoire (France), 3 - Archéologies et Sciences de l'Antiquité (France)

A range of raw materials and finished products, including animals, textiles, copper, tin, semi-precious stones, as well as softstone and ceramic vessels, which may have contained organic products, were moved through exchange networks between the Arabian Peninsula, Iraq, Iran and South Asia during the Bronze Age. However, as organic remains constitute the perishable 'missing majority' of evidence in the archaeological record, we have little understanding of what foodstuffs or organic products were a part of these exchange networks, as well as what was cooked or prepared in ceramic vessels as part of quotidian activities. This paper will present the preliminary results of lipid residue analysis from local and imported vessels from the sites of Salut-ST1 in central Oman (Frenez et al., 2016; Méry et al., 2017) and Hili 8 and Hili North Tomb A in al-Ain, United Arab Emirates (Méry, 2000). In this study, absorbed lipids were extracted and analysed via Gas Chromatography-Mass Spectrometry (GC-MS) from a range of vessels, including local, regional, Indus, Mesopotamian and Iranian wares. A majority of the lipid profiles are indicative of degraded animal fats, which will further be analysed by Gas Chromatography-combustion-Isotopic Ratio Mass Spectrometry (GC-c-IRMS) to distinguish between terrestrial and marine sources and dairy and carcass fats. The preliminary results raise important questions about food production, storage and the exchange and use/re-use of pottery at these sites with broader implications for our understanding of subsistence and exchange networks in the third millennium BC in the region.

Inbreeding in ancient Malta

Ariano Bruno (1), Cassidy Lara (1), Mclaughlin Thomas Rowan (2), Power Ronika K. (3), Mercieca-Spiteri Bernardette (4), Stock Jay (5), Stoddart Simon (5), Malone Caroline (2), Mattiangeli Valeria (1), Bradley Dan (1)

1 - Trinity College Dublin, Smurfit Institute of Genetics (Ireland), 2 - Queen's University Belfast (United Kingdom), 3 - Macquarie University (Australia), 4 - Superintendence of Cultural Heritage (Malta), 5 - Cambridge University (United Kingdom)

Genomes from the Late Neolithic Xagħra Brochtorff Circle burial cave in Gozo (Malta) show unusually strong signals of restricted population size. These are examined, firstly using genomic runs of homozygosity, which inform on inbreeding loops in both recent and remote ancestry; all show excess short runs and one individual also has pronounced long runs of autozygosity. Secondly, levels of identity by descent indicate a degree of close shared ancestry among the individuals studied. These patterns are contextualised using similar analyses of published shotgun-sequenced genomes from neighbouring Neolithic European contexts, and discussed with reference to the unique archaeological setting of this island site.

A minimally destructive protocol for DNA extraction from ancient teeth

Harney Éadaoin (1, 2, 3, 4), Cheronet Olivia (5), Fernandes Daniel (5, 6), Sirak Kendra (2, 4), Rohland Nadin (4, 7), Reich David (2, 4, 7, 8), Pinhasi Ron (5)

1 - Department of Organismic and Evolutionary Biology, Cambridge, (United States), 2 - Department of Human Evolutionary Biology, Harvard University, Cambridge (United States), 3 - The Max Planck-Harvard Research Center for the Archaeoscience of the Ancient Mediterranean, Cambridge, Massachusetts (United States), 4 - Department of Genetics, Harvard Medical School (Boston, United States), 5 - Department of Evolutionary Anthropology, University of Vienna (Austria), 6 -

CIAS, Department of Life Sciences, University of Coimbra (Portugal), 7 - Howard Hughes Medical Institute, Harvard Medical School, Boston (United States), 8 - Broad Institute of MIT and Harvard, Cambridge (United States)

As the field of ancient DNA (aDNA) has grown, the destructive nature of sampling from skeletal remains has become an area of ethical concern. aDNA sampling methods - although optimized for efficient DNA extraction - are typically destructive, relying on drilling, cutting or grinding-based approaches to produce powder from bones and teeth. There are concerns regarding the physical impact of invasive sampling on ancient remains, particularly on key skeletal collections. Here we describe a minimally destructive protocol for extracting DNA from ancient teeth. The outermost layer of tooth roots, known as cementum, has been identified as an optimal target for aDNA sampling due to its high concentration of endogenous DNA. However, the extraction of aDNA from the cementum using destructive sampling methods often results in the loss of one or more entire tooth roots. Here, we present a minimally destructive method for extracting aDNA from dental cementum that does not require drilling or grinding. Following extraction, the tooth remains intact and is safe to handle, enabling future morphological studies, in addition to biochemical analyses, such as radiocarbon dating. We applied this minimally destructive method to 30 tooth roots and found that it produces aDNA of comparable quality to extracts produced using more traditional destructive sampling processes. We also find that the application of a decontamination protocol involving diluted bleach and UV light irradiation is sufficient to minimize surface-level contamination. This protocol serves as a promising alternative to more destructive sampling methods when preservation of skeletal morphology is of the utmost importance.

Thousands of years of human-dog relationships: uncovering adaptation to dietary changes of dogs, resulting from the transition to farming

Ollivier Morgane (1), Hitte Christophe (1), Hubler-Barloy Frederique (1), Debruyne Regis (1), Brassard Colline (1), Balasse Marie (1), Radu Valentin (1), Boroneant Adina (1), Diquelou Deborah (1), Herrel Anthony (1), Brehard Stephanie (1), Balasescu Adrian (1)

1 - Ecosystèmes, biodiversité, évolution, Rennes (France)

Following its domestication, the dog experienced the growing impact of humans on their environment and has witnessed the gradual evolution of their lifestyle. Dog is therefore a unique model that allows to study the biological changes resulting from the adaptation to cultural changes. A change in diet was a major shift during the Neolithic transition. By living so close to humans, we hypothesize that dogs were also impacted by this dietary transition. Here, we investigate the effect of dietary changes resulting from the transition to farming on the evolution of dogs at the genomic, morphological and functional levels. We targeted 86 dogs and 9 wild-canid ancient samples (mandibles and coprolithes) coming from Paleolithic to late chalcolithic Romanian sites. The morphological study of the mandibles suggests that archaeological dogs may have had a jaw system optimised for hard biting at shallow gape angles. First isotopic analysis suggest that Chalcolithic dogs are also at a lower trophic level compared to the Mesolithic canids. High-throughput sequencing of the dental pulp and the coprolithes allowed us to study past microbiomes (under analysis). Finally, mitochondrial captures were performed on all samples together

with nuclear capture (targeting 5000 functional and SNPs) on the best 50 samples. This study calls for very complementary disciplines and methodologies and will provide for each individual, a hitherto unparalleled and complete view of its diet and adaptations in relation to the chrono-cultural context and phylogeographic origin of each dog population.

Interpreting Life Events using Multi-Tissue Stable Isotope Analysis, Viru Valley Peru

Hyland Corrie (1, 2), Millaire Jean-François (3), Szpak Paul (2)

1 - School of Archaeology, University of Oxford (United Kingdom), 2 - Trent University (Canada), 3 - Université du Québec à Trois-Rivières (Canada)

Stable isotope analysis can provide incredible insight into the life histories of individuals especially when multiple tissues are available and a wide range of analyses are employed. Multi-tissue (bone collagen, hair, nail, skin, and tendon) stable isotope analysis (carbon, nitrogen, sulfur, and strontium) on thirteen individuals from the lower Virú Valley identified non-seasonal changes in a predominantly C4-based terrestrial diet. Closer intra-individual examination revealed a potential past pregnancy for Burial 7 and further support for the identification of Burial 8 as a warrior. Burial 5, however, had a stable isotope signature unlike any previously found on the north coast of Peru, indicating both a large contribution of C3-terrestrial resources to their diet and an $87\text{Sr}/86\text{Sr}$ ratio suggestive of highland residence during childhood. This research provides the first strong stable isotope evidence of a highland individual within a coastal burial in northern Peru, provides new insight into the life histories of these individuals, and stands as an example of the wealth of information that can be gained from the combination of multiple stable isotope techniques.

Ancient DNA analyses of Neolithic Atlantic Bluefin Tuna from Scandinavia

Eriksen Emma Falkeid (1), Atmore Lane (1), Nielsen Svein Vatsvåg (2), Persson Per (2), Star Bastiaan (1)

1 - Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo (Norway), 2 - Department of archaeology, Museum of Cultural History, University of Oslo (Norway)

Atlantic bluefin tuna is a commercially important marine apex predator distributed across the Atlantic Ocean, from the Gulf of Mexico to the Mediterranean and the Norwegian Sea. In recent history, Atlantic bluefin tuna has experienced severe overfishing, resulting in population collapse and a listing on the IUCN red list. Humans are known to have harvested Atlantic bluefin for millennia - trap fisheries around the Mediterranean have been documented by historical sources for the past 2600 years, and archeological findings along the Skagerrak coast indicate Atlantic bluefin exploitation in Scandinavian Mesolithic and Neolithic periods. While it is known that tuna fishing has occurred for thousands of years, the impact human exploitation has had on tuna evolution and ecology remains poorly understood. Recent excavations at Jortveit, southern Norway, have produced an abundance of tuna bones dated to the Early and Middle Neolithic period (3900-2350 BCE). Producing more tuna bones than any other known location in Scandinavia, the

Jortveit excavations provide a unique opportunity for a population-scale study of ancient tuna and will allow comparative analyses between ancient and modern populations. Analyses of numerous specimens revealed remarkable aDNA preservation with 100% library success and samples yielding, on average, 30% endogenous DNA. We compare whole-genome shotgun data from this ancient set of specimens to modern conspecifics, providing the oldest population scale baseline comparison of Atlantic bluefin tuna.

Thinking Outside the Box with Human Mobility Studies: a case study from early medieval Europe

Leggett Samantha (1)

1 - Department of Archaeology, University of Cambridge (United Kingdom)

Early medieval migrations are hotly contested with a pendulum of debate which, with new evidence and thinking, swings between elite replacement models of top-down cultural change and large-scale migration. Settlement archaeology, grave goods, and ancient and modern DNA have all been used to tackle this, but are still hampered by theoretical and methodological constraints, and reliance on prevailing narratives. Isotopic analysis is uniquely placed to add to this debate but until now has lacked a large enough dataset to look at these events on a satisfactory scale and methods for determining migrants in isotopic studies still leave much to be desired, relying heavily on outlier determination, and origin exclusion, with pinpointing origins (rightly so) being actively discouraged. In this paper I propose a new data analysis strategy for human mobility studies. This paper follows on from Lightfoot and O'Connell's (2016) article where statistical methods and critical thinking for identifying human migrants were suggested and moves beyond purely frequentist «outlier» approaches and comparisons to isoscapes. It advocates a «New Statistics» workflow based on Exploratory Data Analysis (EDA) and Bayesian thinking to investigate human mobility in the early medieval period in Europe. This methodology is flexible and more appropriate for isotopic provenancing studies, especially in archaeology and ecology, considering uncertainty and propagated error (especially in oxygen), taking a relatedness/similarity approach. Not only does this paper demonstrate a new workflow for analysing human bioapatite data but through this approach a more definitive answer to the debate over these migrations has been reached.

Methodological advancements in molecular technologies for uncovering the presence of concurrent diseases in past populations

Giffin Karen (1), Spyrou Maria (1), Van De Vijver Katrien (2), Kozakaitė Justina (3), Hübler Ron (1), Miliauskienė Žydrūnė (3), Jankauskas Rimantas (3), Herbig Alexander (1), Bos Kirsten (1)

1 - Max-Planck-Institut für Menschheitsgeschichte (Germany), 2 - Royal Belgian Institute of Natural Sciences (Belgium), 3 - Department of Anthropology University of Vilnius (Lithuania)

Analysis of infectious disease in past populations can be confounded by the absence of historical documentation or skeletal changes that can indicate infection. These ambiguities hinder understanding of past epidemiological events. Molecular methods for pathogen detection are valuable tools that can further inform our interpretation of historic and prehistoric outbreaks. Large-scale DNA sequencing permits a thorough analysis of genetic information, and the high resolution that can be achieved

increases the likelihood of identifying pathogens in archaeological specimens. New methodologies permit broad and simultaneous screening for multiple agents of disease within a population, burial or individual, allowing identification of candidate pathogens for targeted genomic analysis. Here we apply this approach to the analysis of teeth from 176 individuals from two contexts: attritional burials from a parish cemetery in Mechelen, Belgium used between the 10th and 18th centuries, and a purported plague catastrophe burial ground from 15th - 16th century Vilnius, Lithuania. Historical information regarding either cemetery was minimal and any existing skeletal pathology was not pathognomonic. Shotgun sequencing combined with an advanced computational pathogen screening pipeline revealed the presence of several pathogens, including Salmonella enterica subsp. enterica (paratyphoid fever) and Yersinia pestis (plague). Employment of enrichment methods following detection allowed for the generation of several genomes for comprehensive analyses. This research demonstrates the ability of these 8 to reveal complexity in the disease landscape of past populations that may otherwise not be brought to light.

Ancient genomes reveal long range influence of the site and culture of Tiwanaku

Popović Danijela (1), Molak Martyna (1, 2), Ziołkowski Mariusz (3), Vranich Alexei (4), Sobczyk Maciej (3), Vidaurre Delfor Ulloa (5), Agresti Guido (3), Skrzypczak Magdalena (1), Ginalski Krzysztof (1), Lamnidis Theiseas Christos (6), Nakatsuka Nathan (7, 8), Mallick Swapan (7, 9, 10), Baca Mateusz (1)
1 - Centre of New technologies, University of Warsaw, Warsaw (Poland), 2 - Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw (Poland), 3 - Centre for Andean Studies, University of Warsaw (Poland), 4 - Department of Anthropology, University of Texas, San Antonio (United States), 5 - Unit of Archeology and Museums - Vice-Ministry of Interculturality (Bolivia), 6 - Department of Archaeogenetics, Max Planck Institute for the Science of Human History, Jena (Germany), 7 - Department of Genetics, Harvard Medical School, Boston (United States), 8 - Harvard-MIT Division of Health Sciences and Technology, Boston (United States), 9 - Howard Hughes Medical Institute, Harvard Medical School, Boston (United States), 10 - Broad Institute of Harvard and MIT, Cambridge (United States)

Tiwanaku was a civilization that flourished in the Lake Titicaca Basin (present-day Bolivia) between 500 and 1000 CE. At its apogee, Tiwanaku controlled the lake's southern shores and influenced certain areas of the Southern Andes. There is a considerable amount of archaeological and anthropological data concerning the Tiwanaku culture; however, our understanding of the population of the site of Tiwanaku is limited. To understand the population dynamics at different stages of the Tiwanaku cultural development, we analyzed 17 low-coverage genomes from individuals dated between 300 and 1500 CE. We found that the population from the Lake Titicaca Basin remained genetically unchanged throughout more than 1200 years, indicating that significant cultural and political changes were not associated with large scale population movements. In contrast, individuals excavated from Tiwanaku's ritual core were highly heterogeneous, some with genetic ancestry from as far away as the Amazon, supporting the proposition of foreign presence at the site. However, mixed-ancestry individuals' presence suggests they were local descendants of incomers from afar rather than captives or visiting pilgrims. A number of human offerings from the Akapana Platform dating to ca. 950 CE mark the end of

active construction and maintenance of the monumental core and the wane of Tiwanaku culture.

Insights from amelogenin peptide analysis on sex, gender, and childhood in prehistoric societies

Bas Marlon (1, 2), Kanz Fabian (2), Janker Lukas (3), Rebay-Salisbury Katharina (1)

1 - Austrian Academy of Sciences - Austrian Archaeological Institute (Austria), 2 - Medical University of Vienna - Unit of Forensic Anthropology (Austria), 3 - University of Vienna - Department of Analytical Chemistry (Austria)

The investigation of sex-b(i)ased differences in childhood treatment was hindered, until recently, by the difficulty of assessing the sex of children's skeletal remains. This has changed with the discovery that peptides in human dental enamel are sex-specific and can be cost-effectively and almost non-destructively analysed by nanoLC-MS/MS. Sex-specific differences in infant mortality, indicators of health, nutrition and trauma, as well as sex-specific selection for burial can now be investigated. In this paper, we present the first case study combining dental imaging and peptide analysis for a statistically significant number of children that have been sex-identified - 75 children from the Early Bronze-Age population of Franzhausen I in present-day Austria. We compare burial practices, as well as paleodietary dental microwear and macrowear data between the two sexes and discuss the findings in the cultural context of our case study.

Potential of archaeogenetic studies of dental calculus to shed light on past human migrations in the Pacific

Fagnäs Zandra (1), Tromp Monica (2, 3), Bedford Stuart (4, 5), Buckley Hallie (2), Chevalier Alexandre (6), Clark Geoffrey (5), Cruz Berrocal María (7), Dudgeon John (8), Flexner James (9), Leal-Tamarii Anatauarii (10), Lewis Cecil (11), Nägele Kathrin (1, 12), Ozga Andrew (13), Posth Cosimo (1, 14), Shing Richard (15), Spriggs Matthew (15, 16), Tsang Cheng-Hwa (17), Valentin Frédérique (18), Velsko Irina (1), Warinner Christina (1, 19, 20)

1 - Department of Archaeogenetics, Max Planck Institute for the Science of Human History (Germany), 2 - Department of Anatomy, School of Biomedical Sciences, University of Otago (New Zealand), 3 - Southern Pacific Archaeological Research, Archaeology Programme, University of Otago (New Zealand), 4 - Department of Linguistic and Cultural Evolution, Max Planck Institute for the Science of Human History (Germany), 5 - Department of Archaeology and Natural History, College of Asia and the Pacific, The Australian National University (Australia), 6 - Quaternary Environments and Humans Program, Royal Belgian Institute of Natural Sciences (Belgium), 7 - Department of History and Sociology, University of Konstanz (Germany), 8 - Department of Anthropology, Idaho State University (United States), 9 - School of Philosophical and Historical Inquiry, University of Sydney (Australia), 10 - Direction de la Culture et du Patrimoine (French Polynesia), 11 - Department of Anthropology, University of Oklahoma (United States), 12 - Department of Archaeogenetics, Max Planck Institute for Evolutionary Anthropology (Germany), 13 - Halmos College of Arts and Sciences, Nova Southeastern University (United States), 14 - Department of Archaeo- and Palaeogenetics, Eberhard Karls Universität Tübingen (Germany), 15 - Vanuatu Cultural Centre (Vanuatu), 16 - School of

Archaeology and Anthropology, College of Arts & Social Sciences, The Australian National University (Australia), 17 - Institute of Anthropology, National Tsinghua University (Taiwan), 18 - CNRS UMR 7041 (France), 19 - Department of Anthropology, Harvard University (United States), 20 - Faculty of Biological Sciences, Friedrich Schiller University (Germany)

Past human migrations are commonly traced by changes in the genetic composition of archaeological individuals. However, the microbiome accompanying humans, which may also experience evolutionary changes related to altered living conditions and diet, offers another source to understand human migrations. Short generation times allow microbial evolutionary changes to become fixed in populations over short periods, during which changes in the human genome are not easily detected by current population genetic 8. The colonization of Remote Oceania took place over only a few centuries, making it a promising case study for investigating human migration via the microbiome. Here, we present preliminary results from a metagenomic study of 106 dental calculus samples from 13 different islands, stretching from Taiwan to Rapa Nui and spanning a time range of approximately 3,000-200 BP. Sample preservation is highly variable, with approximately half having a well-enough preserved oral microbiome to allow for subsequent analyses. There are no clear spatial or temporal patterns in preservation, indicating the potential to recover well-preserved dental calculus microbiomes across various tropical environments. We find minor spatial and temporal patterns in microbial community composition, which may be more pronounced with larger sample sizes. However, the main driver of variation is the sample processing laboratory, indicating that differences in laboratory 8 may obscure subtle microbiome changes. Nevertheless, despite preservational variation and laboratory processing biases, ancient dental calculus holds the potential to trace fine-scale migrations and dietary adaptations, offering a deeper understanding of human activity and migration patterns in the Pacific.

Dredging up genomes: leveraging submerged human remains to understand life and death around the River Thames

Green Eleanor (1, 2) Arthur Nichola (2), Bonney Heather (2), Speller Camilla (3), Barnes Ian (2), Wales Nathan (1)

1 - University of York [York, UK] (United Kingdom), 2 - Natural History Museum [London, UK] (United Kingdom), 3 - University of British Columbia (Canada)

In addition to the vast catalogs museums make available to archaeologists and other researchers, many museums house specimens with limited contextual information. We explore the extent to which these materials can contribute to our understanding about the past by focusing on a unique collection of human remains which circuitously found their way to the Natural History Museum, London. These remains belong to a large collection of disarticulated human crania, some showing signs of trauma, recovered from the River Thames (South of England, UK). To assess the survival of DNA in a riverine environment, we analysed genome-wide data extracted from the petrous bone of 30 individuals recovered across ~75 km of the Thames. Radiocarbon dating indicates that the 30 skulls selected for analysis span the Neolithic to the post-medieval periods. In many cases, the samples show a high endogenous DNA content and a long fragment length, and alongside deamination patterns, these findings suggest that riverine environments are under-appreciated contexts for ancient DNA preservation. Low-depth shotgun data combined with the

radiocarbon dates revealed genomic ancestry patterns that are consistent with contemporaneous British individuals. Genomic sex ID demonstrates a high proportion of male individuals, suggesting non-random patterns of deposition into the river. DNA damage patterns and metagenomic data will be presented to determine whether it is possible to discriminate between individuals who entered the river perimortem and were never recovered, compared to those who were interned close to the riverbank and over time were subsequently washed into the Thames.

Adsorption behaviour of single and double stranded DNA on mineral surfaces: implications for DNA survival in sedimentary archives

Kontopoulos Ioannis (1), Quesada Sanz Jaime (1), Krarup Sand Karina (1)

1 - Section for GeoGenetics, GLOBE Institute, University of Copenhagen (Denmark)

The recovery and sequencing of sedimentary ancient DNA (sedaDNA) can provide valuable information on past biodiversity. Its long-term survival however depends on its adsorption to mineral surfaces. The DNA adsorption capacity of a mineral is highly dependent on mineral surface charge, and the composition and ionic strength of the background electrolytes. The charge density at the adsorption sites further influences the conformation of the DNA. Double stranded (ds) DNA is negatively charged and can bind directly to positively charged sites at the mineral surface or through cationic bridges to negatively charged sites. The positively charged amines are considered to have some contribution to the binding process but their effect is less clear. We here wish to highlight that the local deposition environment can significantly influence the degree of DNA adsorption and survival in the sedimentary archives. We have used atomic force microscopy (AFM) to visualize the adsorption behaviour of single stranded (ss) DNA and double stranded (ds) DNA on positively (calcite, hematite) and negatively (mica) charged mineral surfaces. The AFM images provide an insight at the nanoscale of DNA-mineral interactions. We have studied the DNA adsorption behaviour in a range of electrolyte conditions (MgCl₂, NaCl, seawater) to the minerals which all have different surface charges and charge densities. Our data provide an in-depth insight into the interdependence of solution composition and mineral characteristics (surface morphology and charge) for DNA adsorption and conformation.

Using museum specimens to assess the genomic impact of recent population decline on Andean bears

Chacón-Duque J. Camilo (1) Pulido-Santacruz Paola (2), Ciezarek Adam (3), González Mailyn (2), Haerty Wilfried (3), Di-Palma Federica (4), Brace Selina (5), Barnes Ian (5)

1 - Natural History Museum [London] (United Kingdom), 2 - Instituto de investigación de recursos biológicos Alexander von Humboldt (Colombia), 3 - Earlham Institute (United Kingdom), 4 - University of East Anglia (United Kingdom), 5 - Natural History Museum, London (United Kingdom)

We are currently carrying out a conservation genomics project on the Andean bear (*Tremarctos ornatus*) - the only species of bear in South America and threatened with extinction, where we will assess population viability and genomic diversity across the species' fragmented range in Colombia to inform conservation. The present research aims to characterise the genomic impact of recent population size changes in Colombian Andean bear populations by coupling genomic data from present-day

populations with that of museum specimens. Specifically, we will assess temporal changes in genetic parameters including heterozygosity, inbreeding and genetic load. We sampled dry tissues (skin and teeth) of 34 museum specimens collected throughout the distribution range of the bear in Colombia, with the oldest individual collected in 1913 (mean=1973, Q1=1952, Q3=1993). Of those, 32 samples were sequenced at low coverage to assess levels of endogenous DNA content, with 23 yielding >10%. A total of 15 samples above 40% endogenous DNA content were sequenced at higher coverages (mean=5.2X, Q1=3.3X, Q3=6.8X). These sequences are being used for SNP discovery - in order to define strategies to sequence the remaining museum samples as well as present-day samples collected using non-invasive approaches - and population genetic analyses. This research will enable us to resolve whether populations have suffered recent dramatic population declines due to anthropic effects, or whether they have remained constant at low numbers for longer periods. This is something that cannot be done solely based on present-day genomic data but is crucial for species conservation.

Conformational analysis and water dynamics: a study on the survival of beta-lactoglobulin peptides.

Fonseca Beatriz (1), Matthew Collins (1), Colin Freeman (1)

1 - Globe Institute (Denmark)

One of the greatest successes of the application of Palaeoproteomics to Archaeology has been the ability to track evidence of dairying practice, both in terms of its origin and the selection of animal species. To this end, the whey protein β -lactoglobulin entrapped in pottery and dental calculus has been often targeted. Why is β -lactoglobulin so commonly recovered? Is it because this protein is particularly robust or is it something to do with its association with either calcium phosphate (dental calculus) or calcium carbonate (limescale)? Hydrolysis plays a big part in the breakdown of proteins. Therefore, it is essential to explore the role of water in degradation to uncover some of the patterns linked to protein survival. What is the role of the mineral surface in limiting hydrolysis? One approach to explain these questions is to examine the molecular behaviour of this protein and in particular of the peptide most commonly recovered, which fortunately has one amino acid substitution in its sequence that allows for discrimination between cow, sheep and goat. In this study, I will use a combination of Molecular Dynamics and Quantum Mechanics methods to explore the behaviour of these three peptides both in water and when attached to a mineral surface, in order to better understand their survival in the Archaeological record. Using the Amber14SB forcefield and the SPC/E water model in Gromacs, molecular dynamics simulations of the three peptides in bulk water are then first performed to explore their conformational space and their distinct interactions with water.

Pleistocene population expansion of the extinct eastern moa (*Emeus crassus*) revealed by ancient mitochondrial genomes

Verry Alexander (1), Mitchell Kieren (2), Wallis Graham (1), Waters Jonathan (1), Rawlence Nicolas (1)

1 - Otago Palaeogenetics Laboratory, Department of Zoology, University of Otago, Dunedin (New Zealand), 2 - Centre for Australian Biodiversity and Heritage (CABAH), School of Biological Sciences, University of Adelaide (Australia)

*New Zealand provides a unique opportunity to investigate the response of extinct megafauna to major climatic and environmental changes in the absence of anthropogenic effects. Before human arrival, New Zealand contained a diverse avifaunal assemblage, dominated by giant, flightless paleognathus birds, the moa. Nine species of moa are currently recognised, including the eastern moa (*Emeus crassus*), which inhabited lowland forest and wetland habitats of the eastern South Island. Previous ancient DNA analysis of eastern moa suggest low mitochondrial DNA diversity and a lack of phylogeographic structure. This is hypothesised to be related to a reduction in favourable habitat during the Last Glacial Maximum (29-19 Kya), followed by rapid population expansion as areas of more favourable forested habitat increased throughout the Holocene. We sequenced mitochondrial genomes of eastern moa from throughout their geographical range, covering the last 14,000 calendar years BP, to investigate the impacts of Late Pleistocene-associated climate/habitat change on eastern moa populations. We find that the panmictic eastern moa exhibited low levels of genetic diversity throughout the Holocene. Eastern moa from the Late Pleistocene display greater genetic diversity, with novel haplotypes unique to this period. Demographic analyses suggest that eastern moa underwent a population expansion coinciding with the end of the Last Glacial Maximum and a warming climate. Our results demonstrate the impact of Pleistocene climate change on megafaunal species in the absence of humans.*

A novel study of the history of domesticated sheep in the Bronze and Iron Age Central Asia

Elias Rodrigues Soares André (1) Schröder Oskar (2), Boroffka Nikolaus (3), Benecke Norbert (4), Kaniuth Kai (5), Sverchkov Leonid (6), Günther Torsten (1)
1 - Human Evolution Program, Department of Organismal Biology, Uppsala University (Sweden), 2 - Department of Terrestrial Zoology, Senckenberg Research Institute and Natural History Museum (Germany), 3 - Eurasia Department, German Archaeological Institute (Germany), 4 - Department of Natural Sciences, German Archaeological Institute (Germany), 5 - Institut für Vorderasiatische Archäologie, Ludwig-Maximilians-Universität München (Germany), 6 - Institute of Fine Arts, Academy of Sciences of the Republic of Uzbekistan (Uzbekistan)

*The domestic sheep (*Ovis aries*) is one of the oldest domesticated livestock animals in human history. It was an important step towards the sedentary lifestyle and also historical technology transfer and migration. Most of what is known about the history of sheep domestication was learned from archaeological data and most genetic information was obtained from modern populations. In the present study we investigate the history of Central Asian sheep that lived during the Bronze and Iron Age, a time frame that should also include the first introduction of wool sheep into the region. While sheep were originally domesticated in the Fertile Crescent, Central Asia is an important region for their spread into Eastern Asia and for the connection between Europe and Asia in general. We investigate the population dynamics and structure of sheep across multiple sites in Uzbekistan, with preliminary results indicating an early separation of European and Asian sheep breeds followed by substantial population stability and continuity over time. Further analyses will determine changes at genetic loci associated with certain phenotypes (polledness,*

woolly fleece, coat colouration) to directly observe the outcome of thousands of years of animal husbandry and artificial selection.

Investigation of ancient humans from Buryatia burial grounds

Dambueva Irina (1)

1 - Institute of Mongolian, Buddhist and Tibetan Studies, RAS ; Institute of Biological problems of the North, RAS ,genetics laboratory (Russia)

The region around Lake Baikal in Siberia is an important area to understand the formation of Northeast Asian human populations. In this study, we applied next-generation sequencing (NGS) to ancient bone and tooth specimens that were excavated from archaeological sites dated from Neolithic to Medieval periods in the Republic of Buryatia. To identify well-preserved specimens, we extracted DNA, prepared DNA libraries, and preliminarily sequenced them using a MiSeq sequencer. Among the 20 specimens sequenced, those with more than 10% mapping rate to the reference human genome were 5 out of 12 Neolithic specimens, 4 out of 6 presumptively Bronze or Iron Age specimens, and 2 out of 2 Medieval specimens. Then, the best-preserved specimens were sequenced using HiSeq 2500/4000 sequencers. The obtained genome data were analyzed together with the previously published genome data of ancient and present individuals. Radiocarbon dating was also performed to determine the ages of these specimens. As results, we found that individuals from Neolithic to Iron ages in Buryatia had a similar genetic background with individuals of the same periods in the Cis-Baikal region (Shamanka-II etc.). However, individuals of the Medieval period had a variety of origins: One was suggested to have originated from East Asia but another from Central Asia. The present study contributes to reveal the history of the peopling of Buryatia. Acknowledgements: The investigation was supported by project (N 14.W03.31.0016).

Genetic analysis of a c. 4,800 year old Brucella abortus genome isolated from a calcified nodule

Neumann Gunnar (1), Spyrou Maria (1, 2), Ghalichi Ayshin (1), Valtueña Aida Andrades (1), Keller Marcel (3), Koško Aleksander (4), Żurkiewicz Danuta (4), Klochko Viktor (5), Heyd Volker (6), Włodarczak Piotr (7), Haak Wolfgang (1), Krause Johannes (1, 8)

1 - Max-Planck-Institut für Menschheitsgeschichte (Germany), 2 - Eberhard Karls Universität Tübingen (Germany), 3 - University of Tartu (Estonia), 4 - Adam Mickiewicz University, Poznań, Poland (Poland), 5 - National University of Kyiv-Mohyla Academy, Kiev, (Ukraine), 6 - University of Helsinki (Finland), 7 - Instytut Archeologii i Ethnologii PAN, Krakow (Poland), 8 - Max-Planck-Institut für evolutionäre Anthropologie (Germany)

Brucellosis is a bacterial zoonotic disease caused by different Brucella species. These are moderately host specific and can infect a variety of wild as well as domesticated animals such as cattle, goat and pig, and are occasionally transmitted to humans via infected animal products, inhalation of aerosols or direct contact with infected animal. Despite intense eradication programs, brucellosis is a major health problem for animals and humans in many parts of the world. Here, we identified the cattle-specific Brucella abortus as the causative agent for the formation of a calcified thoracic nodule which was found in a kurgan burial at the site of Prydnistryanske in

modern-day Ukraine. The individual of this burial is associated with the Yamnaya culture and was radiocarbon-dated to c. 4,800 yBP. Due to the excellent preservation of bacterial DNA and minimal environmental contamination levels we were able to reconstruct a high coverage genome of >80x mean coverage without targeted enrichment. Based on its phylogenetic placement, the identified ancient strain groups with one of the earliest diverging *B. abortus* lineages currently circulating. Additionally, through Bayesian molecular dating, we calculated the divergence time of *B. abortus* from the closely related species of *B. melitensis*, which generally infects goats and sheep, in order to investigate when host specialisation most likely evolved. While this study sheds light on the evolutionary history of *B. abortus*, it also emphasizes the potential of calcified nodules as a rich archaeological source to study ancient pathogens.

Population structure of Brittany provides new insights on the introduction of steppe ancestry in western Europe

Alves Isabel (1) Giemza Joanna (1), Blum Michael G. (2), Gallien Véronique (3), Bernhardsson Carolina (4), Cabot Elodie (5), Monteil Martial (6), Margaryan Ashot (7), Racimo Fernando (8), Willerslev Eske (9), Coativy Yves (10), Paillet Yvan (11), Nicolas Clément (12), Le Bris Daniel (13), Darlu Pierre (14), Olaso Robert (15), Blanché-Koch Hélène (16), Jakobsson Mattias (4), Génin Emmanuelle (17), Deleuze Jean-François (15), Redon Richard (1), Dina Christian (1), Consortium Francegenref

1 - unité de recherche de l'institut du thorax UMR1087 UMR6291 (France), 2 - TIMC-IMAG UMR 5525 CNRS, Univ. Grenoble Alpes, Grenoble, France (France), 3 - INRAP / UMR 7264 CEPAM, CNRS-UNS Nice-Sophia Antipolis, Nice (France), 4 - Department of Organismal Biology and SciLife Lab, Uppsala University, Uppsala (Sweden), 5 - Institut National de Recherches Archéologiques Préventives, INRAP, France; Anthropologie Bio-Culturelle, Droit, Ethique et Santé, Faculté de Médecine Site Nord (UMR 7268), Marseille (France), 6 - Université de Nantes, UMR 6566 CReAAH, LARA, Nantes (France), 7 - Center for Evolutionary Hologenomics, University of Copenhagen, Denmark (Denmark), 8 - Lundbeck GeoGenetics Centre, Globe Institute, University of Copenhagen, Denmark (Denmark), 9 - Lundbeck GeoGenetics Centre, Globe Institute, University of Copenhagen, Denmark; Department of Zoology University of Cambridge, UK (Denmark), 10 - Centre de recherche bretonne et celtique, Université Bretagne Occidentale, Brest (France), 11 - Institut National de Recherches Archéologiques Préventives, INRAP, France; UMR 6554 LETG – Brest Institut Universitaire Européen de la Mer, Plouzané, France (France), 12 - UMR 8215 « Trajectoires » Maison de l'Archéologie et de l'Ethnologie, Plouzané, France (France), 13 - Faculté des Lettres, Université Bretagne Occidentale, Brest, France (France), 14 - CNRS/ MNHN/ Université Paris Diderot, UMR 7206 Eco-anthropologie et ethnobiologie, Paris, France (France), 15 - Commissariat à l'Energie Atomique, Université Paris-Saclay, Centre National de Recherche en Génomique Humaine (CNRGH), Institut de Biologie François Jacob, Evry, France and Labex GenMed (France), 16 - Fondation Jean Dausset, CEPH, Paris, France and Labex GenMed (France), 17 - UMR 1078 Génétique, Génomique fonctionnelle et Biotechnologies, Inserm, Univ Brest, EFS, CHU Brest, Brest, France (France)

Present-day France lies at the confluence of the three migration waves that mostly contributed to the genetic ancestry of modern Europeans. Therefore, understanding

the genetic makeup of modern France is key to shed light on the history of European ancestry. To do so, we generated genome-wide data for >3,000 individuals and ~850 high-coverage full genomes from the northern half of France and used hundreds of publicly available modern and ancient Europe-wide samples. Furthermore, we present here, for the first time, ancient DNA from six individuals from France dated to the medieval period (300-1100 CE) due to the complete absence of French samples from the two last millenia. Patterns of haplotype and rare allele sharing revealed extensive fine-scale population structure in northwestern France. Our analyses show relatively large differentiation of western Brittany and an overall increased population differentiation between the north and south sides of the river Loire. We observe that both sides of the river are characterised by different proportions of northwestern European- versus Mediterranean-related ancestry, with western Brittany individuals carrying unique levels (~75%) of Irish-related ancestry. Within France, western Brittany shows the largest levels of steppe ancestry and shared ancestry with Bell Beakers-associated individuals similar to other populations lying on the northwestern edge of Europe. In sum, we provide evidence that the arrival of the pastoralists from the steppe may have reached as far as present-day Brittany and massively reshaped the genetic makeup of Europeans living on the shores of the North Sea.

Palaeoproteomics identifies unexpected components in the ground layer of paintings from the Danish Golden Age

Di Gianvincenzo Fabiana (1) Mackie Meaghan (1, 2), Filtenborg Troels (3), Andersen Cecil Krarup (4), Ernst Madeleine (5), Wadum Jørgen (6), Cappellini Enrico (1)

1 - Center for EvoGenomics, The Globe Institute, University of Copenhagen (Denmark), 2 - Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Copenhagen (Denmark), 3 - National Gallery of Denmark (SMK) (Denmark), 4 - The Royal Danish Academy of Fine Arts (Denmark), 5 - Center for Newborn Screening, Department of Congenital Disorders, Statens Serum Institut (Denmark), 6 - Wadum Art Technological Studies (Denmark)

The recent application of palaeoproteomics to artistic materials has enabled the retrieval of a level of information almost unachievable with other analytical techniques. Tandem MS-based sequencing leads to the confident untargeted identification of all proteins present in a sample, including any non-traditional and undocumented materials. This study reports the protein characterisation of the ground layers of ten paintings from the Danish Golden Age. Protein residues from micro-samples were extracted using a guanidine hydrochloride solution, followed by enzymatic digestion, peptide purification on Stage-Tips, and nano-LC-MS/MS analysis. Data analysis was performed with the MaxQuant software. Collagen from bovine and/or ovicaprine species was identified in 8 of the 10 paintings, showing the presence of animal glue, the most traditional binder for canvas ground layers. However, fermentation-related proteins from baker's yeast and seed-specific proteins from multiple species of cereals (primarily barley, wheat, and buckwheat) were also identified. Also considering mechanical and preservation factors, these proteins suggest the use of a brewing product (either beer or a brewing by-product) in the ground layers. The acquired proteomic data were further processed with a metabolomics data analysis workflow, matching MS/MS spectra against GNPS reference libraries, and peptidic in silico predicted structures through

DEREPLICATOR. This approach suggested the use of a drying oil in one of the paintings, despite no protein identification was achieved in that sample. The chronology and provenance of the paintings showing this unusual recipe suggests this binder formulation was used for canvas preparation at the workshops of the Royal Danish Academy of Art.

Tracking the transformation of the domestic horse during the Bronze and the Iron Age

Liu Xuexue (1, 2), Librado Pablo (1), Lorelei Chauvey (1), Tonasso Laure (1), Schiavinato Stéphanie (1), Fages Antoine (1), Khan Naveed (1), Outram Alan (3), Orlando Ludovic (1)

1 - Centre for Anthropobiology and Genomics of Toulouse (CAGT), UMR5288 (France), 2 - Institute of Animal Science, Chinese Academy Agriculture Sciences (China), 3 - Department of Archaeology, University of Exeter (United Kingdom)

The horse represents one of the animals that most impacted human history. The characterization of extensive genome time-series for horses has started to rewrite current models of early domestication, revealing the existence of two mostly independent domestication centers. It also revealed considerable genomic changes during the last few centuries, in relation to the history of modern breed formation, and an increasing paternal influence of Oriental lineages. Except for the horses of Pazyryk Scythians, the Iron Age period has, however, received much less scholar attention. Yet, a number of key equestrian technologies have developed during the Iron Age, a time period that is also associated with the rise of cavalry. In this study, we have sequenced a total of 201 horse genomes spread across Eurasia and spanning both the Bronze and the Iron Age. Our data reveal important changes in the genetic population structure during the Iron Age, indicating increasing differentiation of two main areas in Asia and Europe. Our genomic time-series also provides new insights into the dynamics of local genomic introgression from wild progenitors and their extinction.

Runs of homozygosity suggest a reduction in inbreeding through the Holocene

Somel Mehmet (1) Gürün Kanat, Altınışık N. Ezgi, Gemici Hasan Can, Karamurat Cansu, Koptekin Dilek, Vural Kıvılcım Başak, Mapelli Igor, Sağlıcan Ekin, Sürer Elif, Erdal Yılmaz Selim, Götherström Anders, Özer Fusun, Atakuman Çiğdem, Somel Mehmet (1)

1 - Middle East Technical University (Turkey)

How the advent of agriculture and social complexity changed human inbreeding patterns is unclear. Ethnographic work suggests low levels of consanguinity in modern-day foragers compared to farmer communities. Meanwhile, the larger population sizes of farmer societies may have lowered the frequency of panmictic (i.e. drift-driven) inbreeding in these groups. Hence, the net impact of food production on autozygosity levels remains unknown. Here we show that runs of homozygosity (ROH) >1 Mb can be reliably estimated in genomes with $\geq 3x$ mean coverage per SNP. We thus calculate the ROH-based inbreeding coefficient estimate, or FROH, in 411 published ancient Eurasian genomes from the last 15,000 years. We find that FROH has significantly decreased over time, in both West and Central Eurasia. The most dramatic reduction appears associated with the Neolithic Transition. Our results hence suggest that population growth caused by food production, along with

increased rates of human mobility, reduced the frequency of panmictic inbreeding and led to a reduction in overall autozygosity. We find that cases of high consanguinity are rare in our sample and are restricted to individuals from farmer communities. Finally, we note that the high consanguinity levels observed in present-day Central Eurasia are not observed in ancient genomes, and this may therefore be a recent phenomenon.

An invasive *Haemophilus influenzae* serotype b infection in an Anglo-Saxon juvenile Plague victim.

Guellil Meriam (1), Keller Marcel (1), Dittmar Jenna (2), Inskip Sarah A. (3), Cessford Craig (4), Robb John (4), Scheib Christiana (1, 4)

1 - University of Tartu (Estonia), 2 - University of Aberdeen (United Kingdom), 3 - University of Leicester (United Kingdom), 4 - University of Cambridge (United Kingdom)

With the continuous increase of large-scale shotgun sequencing in aDNA research, our understanding of past diversity of bacterial and viral pathogenic species keeps expanding and with it our ability to detect co-infections. In this study, we report a *Haemophilus influenzae* serotype b (Hib) genome from a juvenile Anglo-Saxon plague victim (500-650 AD, Edix-Hill, England). *H. influenzae* is an opportunistic pathogen that colonizes the human nasopharynx and is capable of causing major invasive disease. Before the introduction of a conjugate vaccine in the late 1980s, Hib was the main cause of bacterial meningitis in children and one of the leading causes of childhood mortality. We recovered a full Hib genome from a tooth of a six-year-old individual showing signs of infection on its lower limbs and cranium, hinting at an invasive case of Hib. We also recovered a partial *Yersinia pestis* genome from the same individual, making it one of few known plague co-infections. We investigated the evolutionary history and virulence of the new Hib strain and characterised its genome. We found that our aDNA Hib genome clusters with the now uncommon phylogenetic group II serotype b clade, which is speculated to be the product of old recombination events. *H. influenzae* type b has not been previously recovered from aDNA datasets and opens the door for further research into respiratory pathogens and childhood mortality in osteological assemblages. Additionally, the presence of Hib in a plague victim illustrates the relevance of pre-existing health conditions for the impact of historical plague pandemics.

Assessing approaches to tracing terrestrial herbivorous mammals using ancient DNA from lake sediments

Seeber Peter A. (1), Von Hippel Barbara S. (2), Stoof-Leichsenring Kathleen R. (2), Herzs Schuh Ulrike (2, 3, 4), Epp Laura S. (1)

1 - University of Konstanz (Germany), 2 - Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Polar Terrestrial Environmental Systems, Potsdam (Germany), 3 - Institute of Environmental Science and Geography, University of Potsdam, Potsdam-Golm (Germany), 4 - Institute of Biology and Biochemistry, University of Potsdam, Potsdam-Golm (Germany)

Herbivorous mammals inhabit every major terrestrial ecosystem, apart from

Antarctica, and many large herbivores are considered keystone taxa due to their impact on vegetation and, therefore, on the entire ecosystem. These impacts include mechanisms such as facilitating nutrient cycling, promoting food web diversity, modifying vegetation structure including mycorrhizal associations, and altering hydrology. Herbivory may thus have spatial and temporal large-scale effects on ecosystems, and changes in herbivore assemblages are thus important indicators of ecosystem functioning and changes over time. The Arctic is currently experiencing dramatic ecosystem changes, with immediate effects on biodiversity, of which herbivorous mammals such as cervids are an integral part. We used ancient DNA isolated from Siberian lake sediment cores and subjected it to 1) hybridization capture enrichment of DNA of herbivorous mammals and presumed proxy organisms, 2) droplet digital PCR (ddPCR) to quantitatively amplify a 95-bp fragment of the mitochondrial control region of cervids, and 3) metabarcoding of fungi to semi-quantitatively assess coprophilous fungi as proxies of herbivore abundance. Hybridization capture yielded more information on herbivores than on proxy organisms, and ddPCR suggested variation in the abundance of cervid DNA; fungi metabarcoding produced only sporadic indications of coprophilous taxa. However, key questions that may be answered by assessing past herbivore assemblages using ancient DNA include 1) taxonomic diversity of herbivore communities and relative abundances, 2) ecology, physiology, and behavior of herbivores, and 3) evolutionary adaptations to changes in the environment, and 4) large-scale effects of herbivore population changes on ecosystem changes.

Pydamage: automated ancient damage identification and estimation for contigs in ancient DNA de novo assembly

Borry Maxime (1), Hübner Alexander (1, 2), Rohrlach Adam Ben (1, 3), Warinner Christina (1, 4, 5)

1 - Max Planck Institute for the Science of Human History, Jena (Germany), 2 - Max Planck Institute for Evolutionary Anthropology, Leipzig (Germany), 3 - ARC Centre of Excellence for Mathematical and Statistical Frontiers, The University of Adelaide, Adelaide (Australia), 4 - Department of Anthropology, Harvard University, Cambridge, MA (United States), 5 - Faculty of Biological Sciences, Friedrich Schiller University (Germany)

DNA de novo assembly can reconstruct longer stretches of DNA (contigs), sometimes entire genes, and even genomes, from individual sequencing reads. Using this technique with metagenomics samples derived from archaeological remains, such as paleofeces and dental calculus, we can investigate the ancient functional diversity that may be lost in the modern microbiomes. While modern samples often originate from a single source, ancient samples are often formed from a mixture of ancient DNA and modern contaminants. Therefore, it is essential to be able to distinguish between truly ancient contigs and contigs assembled from contaminant DNA, possibly originating from excavation, storage environment, or any other form of modern interference. aDNA characteristic damage pattern is usually one of the key elements advocating for the authenticity of an aDNA sequence. Yet tools for inspecting and filtering aDNA damage either compute it at the read level (e.g., PMDtools), which leads to high data loss and lower overall assembly quality, or requires manual inspection by the user for each reference, which is impractical for a

typical *de novo* assembly, typically yielding tens to hundreds of thousands of contigs. To address these challenges and limitations, we designed PyDamage, an automated approach for damaged contig identification and aDNA damage estimation. PyDamage uses a statistical approach to discriminate between truly ancient contigs, and contigs originating from modern contamination. We tested PyDamage, both on simulated ancient DNA sequencing data, and real archeological samples, and demonstrated its ability to automatically retrieve and identify contigs bearing deamination based DNA damage.

The pharmaceutical content of some Late roman unguentaria from Hierapolis of Phrygia (Turkey): residue analyses and literary sources

Aquilino Maria Valentina (1) Notarstefano Florinda (1), Semeraro Grazia (1)
1 - University of Salento, Dipartimento Beni Culturali (Lecce, Italie)

The functional study of a nucleus of 30 Late Roman unguentaria (LRU) with and without stamps found in public and residential contexts in the city of Hierapolis of Phrygia (Turkey) led to the chemical characterization of their content, using the instrumental technique of GC-MS. Chemical biomarkers have been used like archaeological indicators to reconstruct the production cycle of the perishable goods, which had a large-scale circulation in the Mediterranean basin within the LRU, between the 5th and 7th centuries AD. The study of the composition of the organic residue detected, has given us the opportunity to deepen the topic of the processing of the pharmaceutical preparations, clarifying the ingredients assembly and re-enacting the recipe. The work focuses on the possibility to compare the biomolecular archaeological indicators determined by GCMS to the literary sources on pharmacological *materia medica* of plant and animal origin. This multidisciplinary approach to the study of archaeological pottery made it possible to infer that the preparation of the content, a balm, took place through the «enfleurage» technique, and that some of the ingredients probably required steam distillation. Moreover, we can hypothesize that some of the analyzed LRU hold the Moschio remedy, a poultice used for the medical treatment of traumas to the skeletal system, handed down by Galen and well known in antiquity and the spreadable preventive remedies, an ointment, handed down by Oribasius.

Genomic signals of continuity and admixture in the Caucasus

Ghalichi Ayshin (1) Reinhold Sabine (2), Posth Cosimo (1, 3), Aron Franziska (1), Wissgott Antje (1), Brandt Guido (1), Krause Johannes (1, 4), Berezina Natalia (5), Buzhilova Alexandra (5), Kalmykov Alexey (6), Belinskij Andrej (6), Hansen Svend (2), Haak Wolfgang (1)

1 - Max Planck Institute for the Science of Human History (Germany), 2 - German Archaeological Institute (Germany), 3 - Eberhard Karls Universität Tübingen (Germany), 4 - Max Planck Institute for Evolutionary Anthropology (Germany), 5 - Research Institute and Museum of Anthropology of Lomonosov Moscow State University (Russia), 6 - 'Nasledie' Cultural Heritage Unit (Russia)

Situated between the Black and Caspian Sea, the Caucasus is a key geographic region that connects the Near East and the Eurasian Steppe, with a great ecological diversity of ecotones and landscapes rich in natural resources. A recent archaeogenetic study has shown that the genetically diverse Eneolithic and Bronze Age groups of the steppe and mountains correspond to eco-geographic zones in the

Caucasus. However, the formation, interactions and population dynamics warrant further investigation. In this study we explore new genome-wide data of 68 individuals from 20 archaeological cultures across the Caucasus mountains, the piedmont and the steppe extending our temporal transect to 6000 years, doubling the number of available genomes from the region. We present the first genomic data from a Mesolithic individual (6100 calBCE) from the Northwest Caucasus that shows Eastern hunter-gatherer ancestry, Neolithic individuals from Georgia, as well as new data from genetically unexplored regions/cultures in the northeastern highlands and the dry steppe. We observe a degree of genetic continuity through time within the main mountain and steppe genetic groups, but also identify various episodes of gene flow between these and the neighboring regions. In the Late Eneolithic period, we find evidence of admixture from the south into the steppe groups, detectable through the presence of Anatolian_Neolithic-like ancestry. During the Bronze Age, we found in Steppe Maykop individuals a genetic link to West Siberian hunter-gatherers, a component that is absent from Yamnaya, North Caucasus and Catacomb groups, but reappears in Bronze Age individuals associated with the Lola culture.

The Enigma of Hyksos: An ancient DNA study

Saupe Tina (1, 2), Guellil Meriam (2), Niinemäe Helja (2), Stantis Chris (3), Scheib Christiana L. (2)

1 - Department of Evolutionary Biology, Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia (Estonia), 2 - Estonian Biocentre, Institute of Genomics, University of Tartu, Tartu, Estonia (Estonia), 3 - Faculty of Science and Technology, Department of Archaeology and Anthropology, Bournemouth University, Dorset, UK (United Kingdom)

The Second Intermediate Period (~1640-1530 BCE) signalled a time of change in Egyptian history. It was marked by the occupation of Egypt by foreign rulers, known as the Hyksos, who left their marks on Egyptian culture well beyond the New Kingdom. While previous studies have pointed to a Western Asian origin for the Hyksos, potentially in the Levant (Schuenemann et al. 2017), the actual geographical and genetic origin of the Egyptian conquerors remains unknown. Here, we present the first full human mitochondrial genomes from the archaeological site of Tell el Dab'a in the Eastern Nile Delta (Egypt), where the capital of Hyksos once stood, and whole nuclear and mitochondrial genomes from new sites in the Levant. We extracted DNA from teeth and petrous bones of 49 individuals in the dedicated ancient DNA laboratory at the Estonian Biocentre, Institute of Genomics, University of Tartu, Estonia. After raw data processing, samples ($n=13$) with more than 4% endogenous human content were additionally sequenced to increase coverage, and samples ($n=16$) with a minimum of 0.1% of endogenous human content were selected for mtDNA target enrichment to generate full mtDNA genomes. We will analyse our new aDNA data using previously published datasets from the same geographical region and period to estimate the genetic components and gain new insights into the Hyksos genetic make-up (Agranat-Tamir et al. 2020; Skourtanioti et al. 2020).

Reduced virulence of late medieval *Yersinia pestis* strains could have contributed to the disappearance of plague from Europe.

Bonczarowska Joanna (1), Susat Julian (1), Pētersone-gordina Elina (2), Immel Alexander (1), Nebel Almut (1), Gerhards Guntis (2), Krause-Kyora Ben (1)
1 - Institute of Clinical Molecular Biology, Kiel University (Germany), 2 - Institute of Latvian History, University of Latvia, (Latvia)

Yersinia pestis (*Y. pestis*) is responsible for three major plague pandemics, including the medieval pandemic (14th-18th centuries) which began with the infamous Black Death (1347-1353). Although several *Y. pestis* genomes from that time period have been analysed, the majority of the findings come from western Europe. Therefore, the diversity and microevolution of this pathogen in the eastern part of the continent remain elusive. In the present study, skeletal remains from two graveyards located in Riga (Latvia) were examined. Historical sources indicate that at least two plague outbreaks took place in the area while the cemeteries were in use (15th-18th centuries). It is thus possible that the sites constituted a burial ground for the victims of plague. DNA was extracted from teeth of 16 individuals and subjected to shotgun sequencing. Four samples exhibited molecular evidence of *Y. pestis* confirming the plague hypothesis. The analysis of two reconstructed bacterial genomes revealed a depletion in the *pla* region of the pPCP1 plasmid, suggesting the presence of two plasmid variants (*pla+* and *pla-*). The same phenomenon was discovered for other strains responsible for the post Black Death outbreaks of the disease. As *pla* is an important virulence factor for *Y. pestis* - essential for infection and transmission in humans - a decreased number of *pla+* plasmids could have possibly contributed to the disappearance of plague from Europe in the 18th century.

Insights into ancient Egyptian genomes in the first Millennium BC

Salem Nada (1), Gneccchi Ruscone Guido Alberto (1, 2), Furtwängler Anja (1), Freund Caecilia (1), Bianco Raffaella (1), Martins Nuno Filipe Gomes (3), Burri Marta (4), Tiliakou Anthi (1), Spyrou Maria (5), Mötsch Angela (1), Teßmann Barbara (6), Frenzel Hannah (7), Francken Michael (8), Harvati Katerina (5, 9), Krause Johannes (1, 2), Stockhammer Philipp W. (1, 10)

1 - Department of Archaeogenetics, Max Planck Institute for the Science of Human History, Jena (Germany), 2 - Max Planck Institute for Evolutionary Anthropology, Leipzig, (Germany), 3 - Evolutionary Genomics Section, GLOBE Institute, University of Copenhagen, Copenhagen (Denmark), 4 - Swiss Ornithological Institute, Sempach, (Switzerland), 5 - Institute for Archaeological Sciences (INA), University of Tübingen, Tübingen (Germany), 6 - Berlin society of Anthropology, Ethnology and Prehistory, Berlin, (Germany), 7 - Institute of Anatomy, Leipzig University, Leipzig (Germany), 8 - Cultural Heritage Management, Government office of Stuttgart, Baden Württemberg (Germany), 9 - Senckenberg Centre for Human Evolution and Palaeoenvironment, University of Tübingen, Tübingen (Germany), 10 - Institute for Pre- and Protohistoric Archaeology and Archaeology of the Roman Provinces, LMU Munich, Munich (Germany)

Egypt provides a privileged location to study historical population dynamics as it is at the crossroads between the ancient civilizations in Africa, Asia, and Europe. In the first millennium BC, ancient Egypt witnessed foreign domination by the neighboring populations including Libyans, Nubians, Assyrians, Greeks, Romans, and others, whose roles vary from trade exchange and interaction to invasion and rule. Despite being potential to addressing questions on the population's demographic, retrieval of

ancient DNA from the Egyptian mummies has greatly been challenged by the presence of contamination. Here we report a preliminary, rigorously tested genome-wide dataset from mummies using high-throughput DNA sequencing and targeted capture techniques. The individuals in our study are recovered from Upper and Lower Egypt sites and spanning around 900 years of ancient Egyptian history, from the Third Intermediate to the Roman period. Our study aims to characterize the major ancestry components for ancient Egyptians and to explore the genetic continuation and admixture through times and regions.

A new look at the evolutionary and cultural history of the pig in Corsica, from its Neolithic introduction to modern populations.

Manin Aurélie (1), Cucchi Thomas (2), Stanton David (3), Fulgione Domenico (4), Petrelli Simona (4), Istria Daniel (5), Vigne Jean-Denis (6), Frantz Laurent (3), Larson Greger (7)

1 - Department of Archaeology, University of Oxford (United Kingdom), 2 - Archéozoologie, archéobotanique : sociétés, pratiques et environnements (France), 3 - Queen Mary University in London (United Kingdom), 4 - University of Naples Federico II, Department of Biology (Italy), 5 - CNRS (France), 6 - MNHN: Archéozoologie, Archéobotanique : sociétés, pratiques et environnements, Paris (France), 7 - PalaeoBarn, University of Oxford (United Kingdom)

Palaeontological and archaeological evidence indicates that pigs (*Sus scrofa*) were present in Corsica from about 8,000 years ago. Corsica has been separated from continental Europe since the beginning of the Pliocene (5 million years ago), and it is very unlikely that wild boars would have been able to naturally dispersed to the island. Pigs were thus likely introduced by people, at least once, from a wild or domestic stock. Previous studies, based on ancient and modern mitochondrial data, suggested that Corsican pigs possessed some degree of ancestry related to the earliest domestic pigs that were first introduced into Europe, from the Near East, during the Neolithic expansion. It remains possible, however, that pigs were introduced more than once since. In fact, modern pigs in Corsica now possess a myriad of phenotypes along the wild and domestic continuum, including wild boars, domestic pigs, as well as feral and hybrid populations. We do not know whether these different forms represent genetically homogenous populations, or exchange gene flow, nor do we know whether they evolved from different introductions or if they diversified in-situ. To address these questions, we generated genomic data from 15 modern Corsican domestic pigs, wild boars and hybrids, along with that of several Neolithic and medieval individuals. We compared them to an extensive set of published modern and ancient genomes to explore the genetic diversity and evolutionary history of suids in Corsica.

Exploring the population history of Patagonia & Tierra del Fuego, Chile, using ancient DNA

Balentine Christina M. (1, 2), Alfonso-Durruty Marta (3), Vilar Miguel (4), Morello Flavia (5), San Román Manuel (5), Springs Lauren C. (6), Archer Samantha M. (2), Wing Natalie (7), Bolnick Deborah A. (2, 8)

1 - Department of Integrative Biology, University of Texas at Austin (United States), 2 - Department of Anthropology, University of Connecticut (United States), 3 - Department of Sociology, Anthropology, and Social Work, Kansas State University

(United States), 4 - Department of Anthropology, University of Maryland (United States), 5 - Instituto de la Patagonia, Universidad de Magallanes (Chile), 6 - Department of Anthropology, University of Texas at Austin (United States), 7 - Harvard University (United States), 8 - Institute for Systems Genomics, University of Connecticut (United States)

Humans have occupied the extreme southerly reaches of South America, today known as Patagonia and Tierra del Fuego (Fuego-Patagonia), for more than 10,000 years BP. These first terrestrial hunter-gatherers subsisted mainly on guanaco (*Lama guanicoe*) for thousands of years. Around 6,000 years BP, a sudden influx of marine-specialized cultural materials, including barbed harpoons and navigation technology, appears in the archaeological record. Researchers have long debated the origins of these seemingly novel cultural materials. Did terrestrial hunter-gatherers shift their subsistence strategy at this time to rely more on large marine mammals, or did a new group of marine hunter-gatherers migrate into the region? Recent ancient DNA (aDNA) research has attempted to answer these questions and has begun to shed more light on the population history of Fuego-Patagonia. In this study, we add to the growing body of aDNA research in Fuego-Patagonia by analyzing mitochondrial DNA (mtDNA) from 49 ancient individuals who lived 6,800-304 years cal. BP in what is now Chile. To characterize the population history of Chilean Fuegian-Patagonians, we calculated indices of molecular diversity, built haplotype networks, and conducted demographic simulations. *F_{ST}* results, exact tests, and haplotype networks show that marine hunter-gatherers are genetically differentiated from both terrestrial and mixed-diet hunter-gatherers. These results support the hypothesis that marine hunter-gatherers in the region after 6,000 BP originate from a different source population than terrestrial or mixed-diet hunter-gatherers. These findings are consistent with previous aDNA research in the region, and helps paint a fuller picture of the population history of Fuego-Patagonia.

Genomic ancestry during Antiquity in France

Mendisisco Fanny (1), De Belvalet Harmony (2), Gleize Yves (3), Bel Valerie (3), Thiol Sandrine (3), Oudry-Braillon Sophie (3), Grimaud Julie (3), Rochette Marie (3), Pinard Estelle (3), Chenal Fanny (3), Barrand-Emam H el ene (4), Hernandez J erome (3), Pruvost M elanie (5)

1 - De la Pr ehistoire   l'Actuel : Culture, Environnement et Anthropologie (France), 2 - De la Pr ehistoire   l'Actuel : Culture, Environnement et Anthropologie (PACEA), Pessac, (France), 3 - INRAP (France), 4 - Antea Arch eologie (France), 5 - De la Pr ehistoire   l'Actuel : Culture, Environnement et Anthropologie (France)

The aim of the ANCESTRA project (ANR Ancestra, dir. M.Pruvost) is to reconstruct the peopling of the territory of present-day France by characterizing the different waves of human populations since the Neolithic and until the beginning of the Middle-Ages. Following the publication of data concerning the Neolithic and Bronze Age, this part of the project focuses more particularly on the unexplored period of Antiquity. Thanks to a close collaboration with the archaeologists involved in the project, we report here genome wide data from about a hundred samples from South, North and East of France. Antiquity, like most periods of transition, could be marked by new social rules, new networks of exchange as well as the arrival of new groups of migrants. Crossing archaeological, anthropological and genomic data, we investigate demographic processes and social structures during the Ancient Era, marked, among other things, by the expansion of the Roman Empire across Europe. Despite an

overall diversity for this Antiquity period which echoes that currently observed in the France territory, we were able to identify a subtle structure of this genetic diversity according geographic regions. We also could identify that the local population were affected by gene flow from eastern Mediterranean region but the admixture with Roman individuals remains sporadic. Finally, these genomic data, confronted with more local archaeological issues, raise new questions about cultural continuities across France, and allow us to discuss the different components of the society of Antiquity.

Paleogenomic insights into Nubian ancestry from ancient Middle Nile populations

Breidenstein Abigail (1), Neukamm Judith (1), Barbieri Chiara (2), Ferrari Giada (3), Gondek-Wyrozemska Agata T. (4), Abdallah Mohammed Saad (5, 6), Bushara Murtada (6), Stark Robert J. (7), Ciesielska Joanna (8), Obluski Artur (8), Star Bastiaan (3), Hassan Hisham Yousef (9), Rühli Frank (1), Schuenemann Verena (1), Bigham Abigail W. (10)

1 - Institute of Evolutionary Medicine (Switzerland), 2 - Department of Evolutionary Biology and Environmental Studies (Switzerland), 3 - Centre for Ecological and Evolutionary Synthesis (Norway), 4 - Norwegian College of Fishery Science (Norway), 5 - Bolheim Bioarchaeology Laboratory (Sudan), 6 - National Corporation for Antiquities and Museums (Sudan), 7 - McMaster University, Hamilton, Ontario (Canada), 8 - Polish Centre of Mediterranean Archaeology (Poland), 9 - Banoon ART & Cytogenetics Centre, Bahrain Defense Force Hospital (Bahrain), 10 - Department of Anthropology, UCLA (United States)

The kingdoms of Nubia, located in the Nile River Valley of modern-day northern Sudan and southern Egypt, served as an important corridor of migration for millennia. Little is known of the ancient genetic landscape, but this biological perspective can further our understanding of population movements before this event. Here, we created a time-transect of genetic diversity in the Middle Nile region, using whole mitochondrial (MT) genome analysis of ancient DNA samples obtained from several archaeological sites spanning nearly two thousand years, from the Meroitic period (ca. 350 BCE) to before the Arab expansion (ca. 1450 CE). We trialed 43 individuals, extracting DNA using newly developed 8, including petrosal bone extraction, non-heat sample processing, enzymatic pretreatments, and DNA capture techniques, optimized for samples with very poor DNA preservation. Following strict contamination and authentication assessments, we retrieved whole mitogenomes for six individuals: two with African ancestry and four with Eurasian ancestry. The ancient Nubians showed most genetic affinity with modern East Africans, Middle Easterners, and Egyptians. These results indicate that Nubians had a strong African component with evidence of gene flow from Eurasia dating back to at least Meroitic through Christian times. Although these individuals encompass varying archaeological contexts and span two millennia, these initial results hint at the complexity of the region's genetic makeup and begin to reconstruct the impact of migrations from outside Africa. Lastly, our work represents the first successful retrieval of full MT sequence data from Middle Nile inhabitants, further demonstrating the viability of paleogenomic work in Sudan.

Ancient DNA links genetic admixture to language shift in the medieval Upper Volga

Peltola Sanni (1, 2), Majander Kerttu (3), Makarov Nikolaj (4), Dobrovolskaya Maria (4), Nordqvist Kerkko (5), Krause Johannes (6), Salmela Elina (1, 2, 7), Onkamo Päivi (7)

1 - Faculty of Biological and Environmental Sciences, University of Helsinki (Finland), 2 - Max Planck Institute for the Science of Human History (Germany), 3 - Institute of Evolutionary Medicine, University of Zürich, Zürich (Switzerland), 4 - Institute of Archaeology, Russian Academy of Sciences (Russia), 5 - Faculty of Arts, University of Helsinki (Finland), 6 - Max-Planck-Institut für evolutionäre Anthropologie (Germany), 7 - Department of Biology, University of Turku (Finland)

Uralic languages span a wide geographic area across northern Eurasia, and most of their speakers share a distinct genetic component. The extant members of the language family, however, represent only a subset of the former linguistic diversity. At least three other Uralic languages—Merya, Murom, and Meschera—endured in the Upper Volga region of central Russia until the Middle Ages. However, during the second half of the first millennium, the rising importance of Slavic language led to their gradual demise. Today, traces of these extinct Uralic languages survive only in historical literature and place names. Thus, the linguistic history of the Upper Volga region is relatively well known, but it remains unclear how Slavicization affected the local gene pool over time. In this study, we produced genome-wide data from 32 ancient individuals from the Upper Volga Suzdal region to investigate the subtle population dynamics associated with the language shift. We find that the Iron Age population, predating the arrival of the Slavs, was genetically similar to present-day Uralic-speaking populations of northeastern Europe. This local Iron Age population contributed a substantial proportion of ancestry to their medieval successors at the initial stage of Slavicization. In the following centuries, Slavic-like ancestry became the major component, while Uralic-like ancestry gradually decreased. Both ancestries, however, remain present in modern-day Central Russians. Finally, we observed several outlier individuals representing non-local ancestries in our medieval dataset. These individuals highlight the importance of the medieval Upper Volga region as a center of far-reaching trade and cultural contacts.

Tracking the spatio-temporal spread of the cultivated olive with archaeogenomics

Raimondeau Pauline (1), Wales Nathan (2), Wagner Stefanie (3, 4), Jean-Frédéric Terral, Bouby Laurent, Kaniewski David (5, 6), Galili Ehud, Barazani Oz, Dag Arnon, Figueiral Isabel, Newton Claire (7), Pagnoux Clémence, Rovira Núria (8), Tillier Margaux (8), Orlando Ludovic (2, 4), Besnard Guillaume (9)

1 - Laboratoire Évolution and Diversité Biologique (EDB UMR 5174) (France), 2 - Center for GeoGenetics, National History Museum of Denmark, University of Copenhagen (Denmark), 3 - BIOGECO, INRA, Univ. Bordeaux (Cestas, France), 4 - Centre for anthropobiology and Genomics of Toulouse, Toulouse (France), 5 - Laboratoire Ecologie Fonctionnelle et Environnement, Toulouse (France), 6 - Institut Universitaire de France, Maison des Universités, Paris (France), 7 - Laboratoire d'Archéologie et de Patrimoine, Université du Québec à Rimouski (Canada), 8 - Archéologie des Sociétés Méditerranéennes, Lattes (France), 9 - Laboratoire Evolution et Diversité Biologique, TOULOUSE (France)

Olive is an iconic crop of the Mediterranean cultural heritage. Yet, disentangling its

domestication history is challenging due to multiple origins and recurrent gene flow between cultivated and wild compartments. Olive domestication started approximately 6000 years ago in the eastern Mediterranean Basin. The crop was then spread to westernmost regions, where it was introgressed by local wild populations allowing a secondary diversification. Two highly differentiated wild gene pools have thus contributed to current olive cultivars. The confrontation of archaeological and genetic evidence may bring new insights on the crop origins, diffusion, and secondary diversification. Using ancient DNA (aDNA) extracted from olive stones originating from diverse archaeological sites around the Mediterranean Basin (from the Chalcolithic to the Roman period), we aimed to chart the dispersal of maternal DNA lineages through space and time. Our methodology focused on diagnostic SNPs and indels in the plastome and mitogenome, filtering out regions with low-coverage and reads showing signatures of aDNA degradation. Most samples were successfully assigned to one of the three main maternal haplogroups, and in some cases represented a unique haplotype. In particular, our data suggest that haplotype E1.1, which accounts for 80% of present cultivars, was already introduced in the Greek city of Massalia, in southern France, during the first Iron Age. Nuclear information was also investigated to study admixture profiles of the different samples and gain insights on olive introgression process during secondary diversification. This study demonstrates the huge potential of archaeogenomics to uncover the olive history.

Genomic and anthropological analysis on the human skeletal remains recovered in the House with Garden in Pompeii, Italy

Zaro Valentina (1), Cannariato Costanza (1), Zaro Valentina (1), Pilli Elena (1), Vai Stefania (1), Diroma Maria Angela (1), Amoretti Valeria (2), Osanna Massimo (2), Lari Martina (1), Caramelli David (1)

1 - Department of Biology, University of Florence, Florence, Italy (Italy), 2 - Archaeological park of Pompeii, Pompei, Italy (Italy)

Life in the city of Pompeii has stopped in 79 C.E. when the eruption of Vesuvio covered the town leaving it unchanged over the years. For this reason, the archeological site of Pompeii has an importance unmatched in the world. Starting from XVIII c. around 50 hectares have been excavated and more than one thousand corps unearthed. Most of the human remains consist in skeletons of people died during the first phase of eruption: when a dense fall of lapilli and stones hit the city. Thanks to recent excavations, additional skeletal remains from specific excavation contexts have been discovered. This is the case of the so-called «Stanza degli scheletri», where several human remains belonging to different individuals have been unearthed. First recognition evidenced disturbing activities of the deposition site during historical times, and the skeletal elements were displaced from the original position of the corpses. We performed anthropological and molecular analysis in order to determine the number, sex and age of individuals found inside the room and their biological relationships. Even with differential degree of preservation, we were able to obtain endogenous DNA from alle the analyzed remains. Using both forensic and genomic approaches we demonstrated that the number of different individuals was 10. Interestingly, only women and six children were present in the house. Moreover, we identified two maternal relationships, one of them confirmed as first-grade relationship. Our analysis contributed to reconstruct the facts that people faced during the tragic event in Pompeii.

Improving ancient DNA extraction from waterlogged grape pips

Chauvey Lorelei (1), Wagner Stefanie (1, 2), Bouby Laurent (3), Mata Xavier (1), Wales Nathan (4), Orlando Ludovic (1)

1 - CAGT, Centre for Anthropobiology and Genomics of Toulouse (France), 2 - INRAE - Institut National de la Recherche pour l'Agriculture, l'Alimentation et l'Environnement - 33140 Villenave-d'Ornon (France), 3 - Institut des Sciences de l'Évolution de Montpellier (France), 4 - University of York [York, UK] (United Kingdom)

Pips are common to many archaeological assemblages and can provide invaluable information on the diversity of plants that were part of past ecosystems, agrosystems and diet. They also represent important source of ancient plant DNA material. Within the framework of the ANR-funded Viniculture programme, we aim to use pips as the main source of ancient DNA to map the genomic makeup of ancient grape cultivars in France during the last 4,000 years. Optimizing DNA extraction procedures that maximize access to endogenous DNA content is, thus, instrumental. Here, we present an improved methodology for the extraction of DNA from ancient plant specimens, especially waterlogged grape pips. The method is based on the protocol previously published by Wales et al. (2014). Amongst the five purification procedures compared, we found that a procedure in which the DNA is concentrated and purified in a single step using a large volume column Roche® provides best performance. Our approach can increase the endogenous DNA content obtained following library construction and shotgun sequencing. Importantly, while our method thus facilitates whole genome sequencing of ancient plant remains and minimizes hands-on time, it generally under-performs when applied to bone powder.

Detecting Modern Contamination in Ancient DNA Samples with Deep Learning

Oskolkov Nikolay (1)

1 - Lund University, Lund (Sweden)

Presence of modern contamination can introduce a severe bias into the ancient DNA data analysis. Popular computational techniques using deamination pattern to correct for the bias: 1) require a reference genome that is not always available, 2) perform statistical analysis on a number of DNA sequences, i.e. do not provide a per-sequence estimate, which becomes a limiting factor for the analysis of rare ancient microbial species. Here I present a computational model based on Convolutional Neural Network (CNN) to discriminate between ancient and modern DNA sequences. The model provides a per-sequence ancient vs. modern classification, and does not require the sequences to be aligned to a reference genome. Interpreting the model reveals that it is able to learn *k*-mer patterns, predominantly on the ends of the sequences, that are indicative for modern contamination, and use those patterns for the ancient vs. modern classification.

Population genomic analyses of ancient Hungarians and related peoples in the Volga-Kama and Ural regions

Gyuris Balázs (1), Szeifert Bea (1), Ringbauer Harald (2), Türk Attila (3), Mende Balázs (1), Reich David (4), Szécsényi-Nagy Anna (1)

1 - Institute of Archaeogenomics, Research Centre for the Humanities, Eötvös Loránd Research Network (Hungary), 2 - Department of Archeogenetics, MPI for Evolutionary Anthropology, Leipzig (Germany), 3 - Faculty of Humanities and Social Sciences, Institute of Archeology, Pázmány Péter Catholic University (Hungary), 4 - Department of Genetics, Harvard Medical School (United States)

Ancient Hungarians are considered to originate from the Ural and Volga-Kama regions of present-day Russia. The location of the ancient homeland and settlement territories of the Hungarians are debated, although archaeological and fresh evidence of uniparentally inherited genetic markers show parallelism between Hungarian conquerors in the Carpathian basin (10th century) and the population of the southern Trans-Ural and Volga-Kama regions (6-10th century AD). Here we focus on the main migration events that shaped the Western Siberian-Ural region from the Late-Iron Age and the Western-Eurasian steppe from the Migration Period to the early Medieval Era, which are largely unexplored yet. In our whole genome analysis of 52 samples we use enrichment and target capture analyses of 1.24 million single nucleotide polymorphisms (SNPs). Understanding the distribution of the ancestry proportions in this wide area helps us detect possible source populations of the early Medieval populations of the Volga-Ural region and the population genetically heterogeneous Hungarian conquerors in the Carpathian Basin. Moreover, modeling population transformations in the Ural region has the potential to answer fundamental but highly debated questions about the origins not only of proto-Hungarians and later conqueror Hungarians but also closely related populations. In this talk, we will discuss admixture events leading to the formation of these populations, discuss the extent of genetic continuity and genealogy, and provide insight into the dynamics of complex migration patterns

Archaeogenomics of prehistoric Iberian sheep: insights on early European sheep populations and their relationship with modern sheep.

Morell Miranda Pedro (1), Günther Torsten (2), Iriarte Eneko (3), Galindo-Pellicena Marian (4), Valdosiera Cristina (5), Carretero Jose-Miguel (3)

1 - Uppsala Universitet [Uppsala] (Sweden), 2 - Human Evolution, Dep. Organismal Biology, Uppsala University (Sweden), 3 - Laboratorio de Evolución Humana. Dept. Historia, Geografía y Comunicación, Universidad de Burgos (Spain), 4 - Centro Mixto UCM-ISCIH de Evolución y Comportamiento Humanos (Spain), 5 - Archaeology and History department, La Trobe University (Australia)

The advent and expansion of farming is one of the most important events in human prehistory and sheep has been one of the most influential species during this revolution. Not only was it among the first livestock species to be domesticated but was also the most abundant species in Anatolian Early Neolithic settlements such as Aşıklı Höyük and Çatalhöyük. The role of sheep during the expansion and later development of farming throughout Europe, however, is less well known. In this study we present seven new high quality sheep genomes (0.5-23.3x) from the El Portalón cave (Atapuerca, Northern Spain) that span from the Neolithic to the Bronze Age. We use an extensive modern genomic dataset comprised of landraces and commercial breeds, along with several genomes of sheep's wild relative, the oriental mouflon to put the prehistoric sheep into the context of modern genetic variation. The time series of genomes from the same location allows us to analyze how this population changed between their arrival to the Iberian Peninsula and the Bronze Age, and how much they contributed to modern Iberian breeds. Preliminary analyses suggest a large degree of population continuity in prehistoric Iberia, and that there are clear genetic similarities between our ancient sheep and European modern breeds.

Deciphering the evolutionary history of a bacterial crop pathogen: insights from historical herbarium specimens

Campos Paola (1), Groot Crego Clara (1), Boyer Karine (1), Gaudeul Myriam (2), Baider Claudia (3), Richard Damien (4), Pruvost Olivier (1), Roumagnac Philippe (5), Szurek Boris, Becker Nathalie (2), Gagnevin Lionel (6), Rieux Adrien (1)

1 - Peuplements végétaux et bioagresseurs en milieu tropical, Faculté des Sciences et techniques - Université de La Réunion (France), 2 - Institut de Systématique, Evolution, Biodiversité UMR 7205 (France), 3 - The Mauritius Herbarium (Mauritius), 4 - Peuplements végétaux et bioagresseurs en milieu tropical, La Réunion (France), 5 - Biologie et génétique des interactions plantes-parasites pour la protection intégrée, Montpellier (France), 6 - Plant Health Institute of Montpellier (France)

Over the past decade, the field of ancient genomics has triggered considerable progress in the study of various pathogens, including those affecting crops. In this context, herbarium collections have been shown to be an enormous source of dated, identified and preserved DNA material that can be used in comparative genomic and phylogeographic studies to shed light into the emergence and evolutionary history of plant pathogens. In this study, we reconstructed the first complete historical genomes of a bacterial crop pathogen, *Xanthomonas citri* pv. *citri* (Xci), from infected citrus herbarium specimens using a shotgun-based deep sequencing strategy. This allowed us to describe the whole taxonomic diversity contained within our historical herbarium specimens. Authenticity of our historical samples was verified by assessing DNA damage patterns. We compared the historical strains sequences to a large set of modern genomes to reconstruct their phylogenetic relationships and estimate several evolutionary parameters, using Bayesian tip-calibration inferences. Our results reveal that Xci originated in Asia ~8,000 years ago and diversified during the beginning of the 14th century, subsequently spreading to the rest of the world, including the South West of the Indian Ocean islands. In this area, we dated the arrival of Xci to the 19th century in Mauritius Island and hypothesized its emergence was linked to human migrations following the abolishment of slavery. Our study shows the great potential hidden in herbarium collections to bring light on the evolutionary dynamics that drive pathogens invasion, ultimately helping us to better control current and future crop epidemics.

Surface sediment DNA metabarcoding reveals DNA distribution patterns in lake

Wang Yi (1), Gutbrod Lisa (1), Schmidt Myriam (1), Epp Laura (1)

1 - University of Konstanz (Germany)

Lake sedimentary ancient DNA has become a recognized source of information on past biodiversity change, but our understanding of its distribution and taphonomy is still limited. Here we collected 40 surface sediment samples from Lake Constance in southern Germany and characterized sedimentary DNA (sedDNA) heterogeneity through metabarcoding PCRs on general eukaryotes, vascular plants, cyanobacteria and copepods. Redundancy analysis on eukaryotic DNA shows 10.7% of sample variability is attributed to geographical locations. When examining the read abundance of each taxon across all samples, the majority (69%) of taxa show strong right-skewed distributions, with absence in most and occurrence in only a few samples. This result indicates that sedDNA is not distributed uniformly across the lake, and this pattern varies across taxa. When tracking through time, sedDNA abundance and presence/absence data should be interpreted accordingly. To

validate our findings, this result will be further integrated into the analysis of two short cores from the same lake spanning the last 110 years.

Study of collagen crosslinking and associated modifications in bones using proteomics

Gilbert Catherine (1), Claverol Stéphane (1), Tokarski Caroline (1), Bathany Katell (1), Scanvion Quentin (2), Bertrand Benoit (2), Hédouin Valéry (2)

1 - Institute of Chemistry and Biology of Membranes and Nanoobjects, UMR CNRS 5248, Proteome Platform, University of Bordeaux, Bordeaux (France), 2 - Univ. Lille, CHU Lille, ULR 7367 - UTML&A - Forensic Taphonomy & Anatomy, Lille (France)

Palaeoproteomics allows us to study archaeological materials in order to reveal new insights; human habits, commercial exchanges from the past, ancient pathologies and pathogens, evolutionary links and phylogeny are few examples. Considering the preserved proteins in fossilized bones, a deeper understanding of the patterns of degradation and chemical modifications are of major interest. Their use as markers for identification of modern contaminants, through the study of deamidation, is one of the recent application. Possible diagenesis-related modifications such as loss of hydroxylation/glutamic semialdehyde, carboxymethyllysine were described in an extinct taxon. Hydroxylysine glucosyl galactosylation potentially involved in collagen fiber formation and bone mineralization was recently identified in a 120,000-year-old bone remains. Using a combination of 8 adapted from biological proteomics, we are studying structural and chemical properties of bone collagens with a focus on protein crosslinking and related modifications in human bones from various ages, from forensic to ancient. Collagen cross-linking in bones is important for good biomechanical strength, but is challenging to study mainly due to the chemistry involved and the complexity of the associated data processing. This presentation will describe and discuss several crosslinking chemical markers (e.g. hydroxylysines) and products showing the importance of revealing an unknown part of the mass spectrometry data. On another aspect, the presented work will show how the sample preparation (e.g. demineralization reagents and duration, alternative extraction, ...) is impacting the chemical protein patterns in terms of chemical modifications and protein breakdown. The original experimental designs and optimized 8 will be detailed.

Variation in Genetic Relatedness Patterns among Co-burials in Anatolian Neolithic Societies

Yaka Reyhan (1, 2), Mapelli Igor, Kaptan Damla, Doğu Ayça, Chyleński Maciej, Erdal Ömür Dilek, Vural Kıvılcım Başak, Bayliss Alex, Koptekin Dilek, Mazzucato Camilla, Fer Evrim, Lagerholm Vendela Kempe, Krzewińska Maja, Yurtman Erinç, Çokoğlu Sevim Seda, Gemici Hasan Can, Kılınç Gülşah Merve, Adams Donovan, Munters Arielle R., Sağlıcan Ekin, Milella Marco, Schotsmans Eline M.j., Yorulmaz Sevgi, Sevkar Arda, Karamurat Cansu, Altınışik Ezgi, Juras Anna, Bilgin C. Can, Günther Torsten, Storå Jan, Jakobsson Mattias, Kleijn Maurice De, Mustafaoğlu Gökhan, Fairbairn Andrew, Pearson Jessica, Togan Inci, Kayacan Nurcan, Marciniak Arkadiusz, Larsen Clark Spencer, Hodder Ian, Atakuman Çiğdem, Pilloud Marin, Sürer Elif, Gerritsen Fokke, Özbal Rana, Baird Douglas, Erdal Yılmaz Selim, Duru Güneş, Özbaşaran Mihriban, Haddow Scott D., Knüsel Christopher J., Götherström Anders (1), Özer Füsün (3), Somel

Mehmet (2)

1 - Centre for Palaeogenetics, Stockholm (Sweden), 2 - Department of Biology, Middle East Technical University (METU), Ankara (Turkey), 3 - Human G Lab, Department of Anthropology, Hacettepe University, Ankara (Turkey)

The Neolithic Transition to village life and food production first emerged in the Fertile Crescent (c.10th and early 9th millennium BCE) and fundamentally reshaped human history. Although this transition involved major changes in human lifestyle, the social organization and traditions of the earliest sedentary communities is poorly understood. Here, we investigate genetic relatedness patterns among co-buried individuals within domestic structures in Neolithic Anatolia by studying 22 newly generated ancient genomes from Aşıklı Höyük and Çatalhöyük and combining these with published genomes from other Anatolian Neolithic sites. We focus on the sites that span the early (Aşıklı Höyük and Boncuklu) and late Neolithic (Çatalhöyük and Barcın) to understand temporal variation in genetic relatedness patterns in association with burial location. During the early Neolithic period (late 9th and early 8th millennium BCE), represented by Aşıklı Höyük and Boncuklu, siblings and parent-offspring pairs are at relatively high frequency among co-burials. This suggests the existence of close genetic kinship components within the social organization of these settlements. In other settlements, such as the late Neolithic period (7th millennium BCE) Çatalhöyük and Barcın, the frequency of genetically close relatives among co-burials is much lower. Despite the shortcomings of the small sample size, our results provide the first insights into the genetic kinship patterns between co-buried individuals, and how burial traditions of Neolithic societies in Anatolia varied among settlements, and may possibly have changed over time in conjunction with changing architecture, growing settlement size and cultural traditions.

The Ancient American Dog Project: Understanding the History of Dogs in the Americas

Charlton Sophy (1), Larson Greger (1), Manin Aurélie (2), Ostrander Elaine (3), Parker Heidi (3), Hogan Andrew (3)

1 - PalaeoBARN, School of Archaeology, University of Oxford (United Kingdom), 2 - Department of Archaeology, University of Oxford (United Kingdom), 3 - National Institutes of Health (United States)

After their domestication in Eurasia, dogs were introduced into America prior to 10,000 years ago. This monophyletic clade of pre-contact dogs (PCD) then dispersed throughout the continent and evolved in isolation for at least 9,000 years. However, it has been suggested, based on limited amounts of data, that this lineage left virtually no legacy in the modern dog population of the Americas, including amongst breeds of so-called pre-contact origin (e.g. xoloitzcuintle, Peruvian hairless dog, chihuahua, Carolina dog), despite some being phenotypically similar to representations of dogs in pre-contact American cultures. Here we present a new project which aims to develop a greater understanding of the earliest dogs within the Americas. In particular, through aDNA analysis of archaeological canid samples from across North, Central and South America, we are assessing the genomic variation of PCD, characterising the replacement of PCD following European contact, determining levels of admixture between PCD and indigenous canids, and exploring the phenotypic characteristics of PCD. By combining aDNA analyses with archaeological data, the project will deepen our knowledge of both the earliest PCD and also post-contact dogs across the American continent.

Human demographic history of the western Trans-Himalayan region

De La Fuente Constanza (1), Bandyopadhyay Esha (1), Witonsky David (1), Nautiyal Vinod (2), Raghavan Maanasa (1), Rai Niraj (3)

1 - Department of Human Genetics, University of Chicago (United States), 2 - Garhwal University, Uttarakhand (India), 3 - Birbal Sahni Institute of Palaeosciences, Lucknow, Uttar Pradesh (India)

The Trans-Himalayan region has a complex and understudied genetic history, owing to its rough terrain. Recent genomics research has revealed genetic affinities between ancient populations inhabiting the Upper Mustang region of north-central Nepal and East Asian populations, likely those adapted to high-altitude environments, with genetic continuity spanning at least the last three millennia. Further ancient genomics studies exploring population origins and high-altitude adaptations more broadly along the Himalayan arc can add to our current understanding of population relationships and movements into this region. In this study, we focused on two sites, Lippa and Kanam, from the high-Himalayan region of Kinnaur in northern India. Archaeological evidence found at these sites suggests a cist-burial culture that developed around the 6th century BCE, with a common ceramic tradition that connects the trans-Himalayan and Upper Tibetan Plateau regions. We generated genomic data for four individuals from Kinnaur (three from Lippa, one from Kanam) and investigated their genomic diversity and affinities to worldwide populations. We observed high genetic affinity of the studied ancient individuals to populations from the broader trans-Himalayan region and the Tibetan Plateau, suggesting long-term population continuity in the region. Although previous analysis of ancient individuals from neighboring Nepal has suggested a genetic continuity in the region, this study enables us to spatially extend current human demographic inferences to include the understudied western half of the Trans-Himalayan region.

Rapid inference of demographic history with ADMIXTOOLS 2

Maier Robert (1, 2, 3), Flegontov Pavel (1, 3), Patterson Nick (1, 2, 3), Reich David (1) (2, 3, 4)

1 - Department of Human Evolutionary Biology, Harvard University (United States), 2 - Broad Institute of MIT and Harvard (United States), 3 - Department of Genetics, Harvard Medical School (United States), 4 - Howard Hughes Medical Institute, Harvard Medical School (United States)

ADMIXTOOLS is one of the most widely used tools for inferring demographic history from genetic data. It computes statistics which describe the relationship between two to four populations (f -statistics), and uses those statistics to model the relationships between more than four populations. The exponential growth in the number of available samples poses computational as well as methodological challenges to the traditional approach of using ADMIXTOOLS. To address these challenges, we developed ADMIXTOOLS 2, which facilitates fast and robust analyses by re-implementing, and extending the existing toolset in a number of ways: It provides fast and accurate algorithms for automatically inferring admixture graphs. It facilitates the rapid testing of large numbers of qpWave, qpAdm, or qpGraph models. It offers new δ for distinguishing between competing models and for obtaining bootstrap confidence intervals. It makes working with admixture graphs easy through a graphical user interface. ADMIXTOOLS 2 is currently being used in a wide range of projects, some of which challenge our understanding of human

prehistory. ADMIXTOOLS 2 is freely available as an R package under <https://uqrmaie1.github.io/admixtools>.

Modelling protein decay

Nair Bharath (1)

1 - *Globe Institute, University of Copenhagen (Denmark)*

Mass spectrometry is an excellent tool for identifying proteins, but in more degraded ancient samples, only fewer of the queries result in identified peptides. Modelling of protein decay aims at exploring this phenomenon of unidentified fragment scans by degrading a simple model protein, here beta-lactoglobulin (BLG), in acidic (pH 3), basic (pH 11), and neutral (pH 7) conditions and analyzing the decay products by amino acid racemization and mass spectrometric techniques. We sampled the decay products at exponentially increasing time points starting from 0, 0.5, 1, 2, 4, 8, 16, 32, 64, and 128 days. Herein, we use the data generated on decay products at an unprecedented level of detail to understand the underlying mechanisms behind protein degradation across time. High D/L ratios for Ser, Tyr, Phe, Asx, and Glx after 16 days particularly in basic condition, reveals high extent of degradation at high pH as compared to acidic and neutral conditions. Furthermore, LC-MS2 reveals heavy protein damage in acidic and basic conditions after 64 and 128 days. The low rate of peptide spectrum matches (PSMs) in a single protein system directs towards the integration of multiple experimental and computational proteomics workflows to explore dark proteome in detail.

Assessing the predictive taxonomic power of the bony labyrinth 3D shape in horses, donkeys and their F1-hybrids

Clavel Pierre (1), Dumoncel Jean (1), Der Sarkissian Clio (1), Seguin-Orlando Andaine (1), Calvière-Tonasso Laure (1), Schiavinato Stéphanie (1), Chauvey Lorelei (1), Onar Vedat (2), Clavel Benoît (3), Lepetz Sébastien (3), Braga José (4) (1), Orlando Ludovic (1), Erc Pegasus Consortium (5)

1 - *Centre for Anthropobiology and Genomics of Toulouse, University of Toulouse 3 Paul Sabatier, Toulouse, France (France)*, 2 - *Osteoarchaeology Practice and Research Centre & Department of Anatomy, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, Istanbul (Turkey)*, 3 - *Archéozoologie, Archéobotanique, Sociétés, Pratiques et Environnements, Muséum National d'Histoire Naturelle (MNHN), Centre National de Recherche Scientifique (CNRS), Alliance Sorbonne Université, Paris, France (France)*, 4 - *Evolutionary Studies Institute, University of the Witwatersrand, Johannesburg, (South Africa)*, 5 - *Centre for Anthropobiology and Genomics of Toulouse, Toulouse, France (France)*

Horses and donkeys have had a far-reaching impact on human history but also their F1-hybrids, especially mules, which were massively employed for their exceptional strength, endurance and resistance. The reconstruction of the respective role that equids played in past societies and economies, however, requires their prior identification in archaeological assemblages. This task remains extraordinary difficult based on morphological data alone, as available material is most often fragmentary. While DNA sequencing provides almost certain identification success, this approach requires dedicated infrastructure and sufficient ancient DNA preservation. In this study, we assessed the performance of a cost-effective alternative approach based on geometric morphometric (GMM) analysis of the bony labyrinth, a structure carried within the petrosal bone. To assess the GMM performance, we first genetically

identified 41 horses, 24 donkeys, 36 mules and one hinny from 11 archaeological sites from France and Turkey spanning different time periods. This provided a panel of 102 ancient equine remains for micro-computed tomography and GMM assessment of the variation of the bony labyrinth shape (cochlea and semicircular canals). The new method developed shows good-to-excellent prediction rates (85.7%-95.2%) for the identification of species and hybrids when the cochlea and semicircular canals are considered together. It, thus, provides a cheap, non-destructive alternative to aDNA for the taxonomic identification of past equine assemblages.

Using extremely low-coverage sequence data to accurately assign structural variation haplotypes in ancient specimens

Atmore Lane M. (1), Ferrari Giada (1), Martínez-García Lourdes (1), Cuevas Angélica (1), Mackowiecki Daniel (2), Barrett James H. (3, 4), Star Bastiaan (1)

1 - Centre for Ecological and Evolutionary Synthesis, Department of Biosciences, University of Oslo (Norway), 2 - Department of Environmental Archaeology and Human Paleoeology, Institute of Archaeology, Nicolaus Copernicus University (Poland), 3 - McDonald Institute for Archaeological Research, Department of Archaeology, University of Cambridge (United Kingdom), 4 - Department of Archaeology and Cultural History, NTNU University Museum, Trondheim (Norway)

Structural variants, such as large-scale chromosomal inversions, can play a fundamental role in adaptation to local environments. The ability to identify the haplotypes of such structural variants in ancient specimens can therefore provide diagnostic information on population continuity, evolution, climate change, species migration, and trade. Yet, identification of structural variants often relies on high-coverage sequence data, which remains challenging to generate for most ancient DNA specimens due to poor sample preservation and low rates of endogenous DNA. We have developed a novel approach that efficiently identifies haplotypes of structural variants in extremely low-coverage sequence data. First, we generated custom-made databases from modern reference data, populating these databases with a subset of the most highly-diverged SNPs within a structural variant. BAM files generated for ancient specimens are subsequently compared to these databases to ascertain allelic state and scored for similarity to each haplotype. To assess the efficacy of this approach, we assigned haplotypes in species with segregating structural variants - Atlantic cod, Atlantic herring, and *Heliconius* butterflies - using both modern and ancient samples. The method accurately assigns structural variant haplotypes from extremely low-coverage (e.g. 0.004X) data and can also be generalized to determine population membership based on genome-wide SNPs. This approach will therefore increase the number of ancient samples in ecological and bioarchaeological research for which relevant biological information can be obtained.

Ancient DNA investigation at Photangkhun Longkhap, Nagaland, India

Bandyopadhyay Esha (1), De La Fuente Constanza (1), Witonsky David (1), Jamir Tiatoshi (2), Rai Niraj (3), Raghavan Maanasa (1)

1 - Department of Human Genetics, University of Chicago, USA (United States), 2 - Nagaland University, Kohima Campus, India (India), 3 - Birbal Sahni Institute of Palaeosciences, Lucknow, India (India)

Genetic studies of present-day populations suggest past gene flow into Northeast India from Eastern Eurasia. From existing archaeological records, it is evident that prominent features of Northeast Indian farming culture, or the Neolithic, such as ground stone tools, cord-mark pottery, and rice/millet cultivation, display similarities with East Asia and Southeast Asia. Concurrently, the archaeological datasets from cave and rockshelter sites of the Naga Hills report evidence of hunter-gatherer societies that survived in the forested uplands of Northeast India spanning the mid-Holocene to as late as 1600 BCE prior to the beginnings of agriculture. This raises the question: what is the genetic relationship between resident hunter-gatherers/foragers, early farmers who are proposed to have advanced into the region at a later period equipped with Neolithic technologies, and present-day inhabitants of the region? In this study, we aim to integrate archaeological and genetic data to better understand past human movements and interactions in Northeast India. By analyzing genome-wide data from three mid-Holocene (archaeologically dated to ~3,700 - 4,300 BP) human samples excavated from the rockshelter site of Photangkun Longkhap, Nagaland, India, this study will contribute to our understanding of the regional demography over the last ~4000 years. Keywords: Northeast India, ancient DNA, migrations/gene flow, mid-Holocene, population genetics

Comparison of proteomic, genomic, and osteological methods of archaeological sex estimation at two ancestral Ohlone sites in Central California (2440 – 100 cal BP)

Buonasera Tammy (1), Eerkens Jelmer (1), Parker Glendon (1)
1 - University of California (United States)

Biological sex is fundamental to archaeological studies of human health, identity, kinship, evolution, and gender. Although both genomic and proteomic techniques can extend accurate sex estimation to skeletal remains that are fragmentary, or from individuals too young to have distinctive osteological markers, little is known about the relative reliability of these 8 in applied settings. We present matching osteological, shotgun-genomic, and proteomics data to estimate the sex of 55 individuals, each with an independent radiocarbon date between 2440 and 100 cal BP, from two ancestral Ohlone sites in Central California. Sex estimation was possible in 100% of this burial sample using proteomics, in 91% using genomics, and in 51% using osteology. Genomic sex estimates were 100% consistent with proteomic and osteological estimates when DNA reads were above 100,000 total sequences. However, more than half the samples had DNA read numbers below this threshold, producing high rates of conflict with osteological and proteomic data where nine out of twenty conditional DNA sex estimates conflicted with proteomics. Proteomic sex estimation was independent of DNA quality and extended accurate sex estimation to human skeletal remains that were too degraded for the successful application of genomic or osteological techniques. Together, this information provided highly confident and comprehensive sex estimates for reconstructing male and female life expectancies and detecting sex-biased infant mortality. Broader application of proteomic sex estimation could provide more comprehensive comparisons of male and female life histories across a wide range of archaeologically known societies.

First Genomic Insights into Pre-pottery Neolithic of Upper Mesopotamia

Altınışik N. Ezgi (1), Aydoğan Ayça (1), Kazancı Duygu Deniz (1), Vural Kıvılcım Başak (2), Koptekin Dilek (2), Özkan Mustafa (2), Gemici Hasan Can (3), Karamurat Cansu (3), Erdal Ömür Dilek (4), Götherström Anders (5, 6), Sürer Elif (7), Atakuman Çiğdem (8), Erim Aslı (9), Özer Füsün (1), Somel Mehmet (2), Erdal Yılmaz Selim (1)

1 - Human-G Laboratory, Department of Anthropology, Hacettepe University, Ankara, Turkey (Turkey), 2 - Department of Biology, Middle East Technical University (METU), Ankara, Turkey (Turkey), 3 - Graduate School of Social Sciences, Middle East Technical University (METU), Ankara, Turkey (Turkey), 4 - Husbiol Laboratory, Department of Anthropology, Hacettepe University, Ankara, Turkey (Turkey), 5 - Department of Archaeology and Classical Studies, Stockholm University, Stockholm, Sweden (Sweden), 6 - Centre for Palaeogenetics, Stockholm, Sweden (Sweden), 7 - Department of Modeling and Simulation, Graduate School of Informatics, Middle East Technical University (METU), Ankara, Turkey (Turkey), 8 - Institute of Social Sciences, Middle East Technical University (METU), Ankara, Turkey (Turkey), 9 - Department of Archaeology, Çanakkale Onsekiz Mart University, Çanakkale 17100, Turkey (Turkey)

Recent studies showed that Neolithic populations in southwest Asia included distinct gene pools in the Levant, in Central Anatolia, and in the Zagros. Further, genomic comparisons suggested that all three populations adopted sedentism and farming without major admixture or replacement from other regions. Meanwhile, the population genetic characteristics of the geographic midpoint of these regions, namely upper Mesopotamia, has not been investigated so far. Here in this study, we present the first genomic data of individuals excavated from the PPNB phase of Çayönü. Çayönü, near the upper stretches of river Tigris, is one of the early settlements discovered in southeast Anatolia at the upper-most edge of Fertile Crescent. Material culture data indicate that the Çayönü population interacted intensely with nearby regions - Anatolia, Levant, and Zagros. Despite poor DNA preservation due to harsh environmental conditions, after screening the remains of 33 individuals we managed to obtain genomic data enough for population genetics analyses from 14 individuals. We revealed that Çayönü individuals were genetically similar to early Holocene groups of C Anatolia, Levant, and Zagros, with higher affinity to the C Anatolia-Levant cline. We also modelled Çayönü as a three-way admixture utilizing qpAdm and found that pre-pottery Neolithic population of Çayönü harbored ancestry from all surrounding populations. Overall, in line with archaeological evidence, Çayönü appears to have been a melting pot of neighbouring Neolithic populations during the 9th and 8th millennia BC.

Oceanic island museomics: human impact and the natural laboratory paradigm

De Vries Annabelle (1, 2), Carine Mark (2), Allaby Robin (1)

1 - University of Warwick, Coventry (United Kingdom), 2 - The Natural History Museum, London (United Kingdom)

Oceanic islands have long been recognised as ideally suited for in-situ studies of evolution. They provide some of the most striking, textbook examples of adaptive radiations and have played - and continue to play - a major role in the development of key concepts in biology. At the same time, the island biota that are so rich in unique diversity, are also among the most globally endangered. This project aims to better understand how human impact has affected island biodiversity patterns and

question how robust islands are as model system for studying evolution. For this study, we focus on the plant genus *Trochetiopsis*, an endemic of St Helena, to test the assumptions of the natural laboratory paradigm. This island is interesting as it highlights classic examples of evolutionary processes such as adaptive radiation, has historical records of human impact, and there is a history of botanical collecting spanning the last three centuries. The genus *Trochetiopsis* consists of three species of which two extinct (in the wild) and one re-discovered. Its collections spanning 320 years, allows us to look closer at these extinction events. During this project, a *de novo* whole genome of *Trochetiopsis ebenus* will be assembled using PacBio long reads obtained from sequencing modern material. This assembled genome will function as a reference genome for the ancient DNA from herbarium collections. The ancient and modern DNA will be compared to investigate the change of species over time.

Modelling and profiling the biomolecular mechanisms of mummification

Sharpen Jack (1), Boggi Byron (1), Palmer Karren (1), Drosou Konstantina (1)
1 - University of Manchester, Manchester (United Kingdom)

Mummification has long been hypothesised to preserve ancient biomolecules such as DNA and proteins due to the rapid desiccation of tissues inhibiting wholesale hydrolysis. As such, mummified remains are a very important source of bioarchaeological knowledge, but the underlying mechanisms behind mummification are still not fully understood. A novel porcine micro-modelling approach that combines modern omics technologies has been designed to determine the mechanisms of mummification and profile the rate and extent that biomolecule degradation occurs. Pig tissues from skin, the underlying dermal muscle and brain were subjected to conditions replicating both spontaneous (in sand) and anthropogenic mummification of Ancient Egypt, with an emphasis on altering natron's chemical composition and role as a desiccant due to its historical significance. DNA and proteins were subsequently extracted, quantified and analysed for changes induced by desiccation; specifically, the decrease in total biomolecule concentrations and post-mortem modifications found within ancient biomolecules such as hydroxylation, deamidation, deamination and hydrolytic fragmentation. Preliminary data was successful in its ability to fully mummify all tissue models compared to previous attempts on whole organisms. Both a significant decrease in tissue water percentage and DNA mass (ng/uL) was found within natron desiccation after only a 24-hour period, whilst similar measurements were seen in spontaneous mummification after a longer period. Initial omic analyses has supplemented macroscopic changes by showing degradation of biomolecule content is highly dependent on the types of mummification. Ongoing work will then explore the potential applications and importance within the fields of both biomolecular archaeology and forensic taphonomy.

15th Century Genomic Evidence of Indian Ocean Slave Trade beneath Kota Melaka

McCull Hugh (1) Demeter Fabrice (1, 2), Mokhtar Saidin Mohd (3), Shahidan Shaiful (3), Ken Khong Eng (3), Hung Hsiao-Chun (4), Sikora Martin (1), Willerslev Eske (1)

1 - Centre for GeoGenetics, GLOBE Institute, University of Copenhagen (Denmark),

2 - Sorbonne Universités, Université Pierre et Marie Curie, Univ. Paris 06, Institut du Calcul et de la Simulation Paris (France), 3 - Centre for Global Archaeological Research, Universiti Sains Malaysia, Penang (Malaysia), 4 - Department of Archaeology and Natural History, Australian National University, Canberra, ACT (Australia)

The 15th century city of Melaka was a key trading port linked to China, India, the Middle East and East Africa. Historical written reports describe the city as consisting of ethnically diverse free traders and slaves, under the rule of the Melaka Sultanate. We generated low coverage whole genomes from three burials from within the Palace grounds, revealing unadmixed individuals from East Africa and South Asia, and Malaysia. We also present C14 dates for the two previously undated individuals, consistent with previous dating and archaeological estimates. The burial location and arrangement, anatomical positioning and ethnicities are consistent with royal slaves or companions of the Sultanate.

Bridging the Divide: Mutually Beneficial Collaboration Between Indigenous and European Scientists in aDNA Research

Collin Yvette (1), Orlando Ludovic (1)

1 - Centre for Anthropobiology and Genomics of Toulouse (France)

The technologies underlying ancient DNA research have emerged within a Western paradigm and their access remains difficult to most Native researchers. As a result, current policies, practices, techniques and scientific studies have been developed largely without Indigenous input. However, the benefit of co-developing a truly shared scientific experience that goes beyond agreements for ethical access to samples and training appears increasingly clear. In fact, several initiatives have committed to bridging this divide in which Native and European researchers supported by the Marie Skłodowska-Curie IF actions have united in the field of equine ancient DNA research to create meaningful and mutually respectful collaboration. Such collaboration would allow for the inclusion of a unique scientific methodology specific to the geographic and cultural areas of focus, as well as additional data of import that may not otherwise be available to the greater scientific community. This poster provides an overview of the underlying aDNA research process, with highlights from a Native scientific perspective. The highlights indicate the ways in which stakeholder consultation can be transformed into mutually beneficial and active stakeholder participation that can be utilized to significantly enhance the field of ancient DNA research and its outcomes for future generations.

Imputation of ancient genomes

De Sousa Mota Bárbara (1, 2), Sikora Martin (3), Rubinacci Simone (1, 2), Willerslev Eske (3), Malaspinas Anna-Sapfo (1, 2), Delaneau Olivier (1, 2)

1 - Department of Computational Biology, University of Lausanne (Switzerland), 2 - Swiss Institute of Bioinformatics (Switzerland), 3 - Centre for GeoGenetics, Natural History Museum of Denmark and Department of Biology, University of Copenhagen (Denmark)

Ancient DNA (aDNA) is characterized by damage accumulated through time, such as fragmentation and deamination. Consequently, missing data and noise undermine downstream analyses for ancient genomes compared to modern ones. Imputation of ancient genomes can alleviate these challenges by producing more complete and

reliable genotype and haplotype calls. Here we provide a computational roadmap for aDNA researchers to perform imputation by demonstrating the potential of GLIMPSE, a phasing and imputation tool, and by assessing the imputation effects on standard demographic analyses. While GLIMPSE was shown to accurately impute low-coverage present-day genomes with reduced computational costs, its performance for ancient data remains unclear. We assessed its accuracy by imputing downsampled high-coverage (>10x) ancient genomes from around the world. At the sample level, we accurately imputed most of the ancient genomes, with error rates decreasing as sequencing coverage increases. In particular, for coverages as low as 0.8x, most samples are imputed with error rates below 5% at genotypes with one copy of the minor allele or more. We also find higher genotyping error rates for populations underrepresented in the reference panel, which is the case of some African populations. Transversion sites are imputed more accurately than transitions at rare variants (MAF).

Resolving the timescale of South-Central African palaeoenvironments and their impact on human behaviour and evolution

Bartlet Chloë (1), Penkman Kirsty (1), Barham Larry (2), White Tom (3)

1 - University of York (United Kingdom), 2 - University of Liverpool (United Kingdom), 3 - Natural History Museum (United Kingdom)

Dating is integral to the study of archaeology and palaeoenvironments, but is problematic beyond the limit of radiocarbon dating, ca. 60 ka. Several dating techniques are widely applied, with none able to span the entire Quaternary (2.58 Ma) for all sample types. Analysis of intra-crystalline protein degradation (IcPD) exploits the time-dependent breakdown of proteins (such as racemisation of amino acids) within biominerals (mollusc shell, eggshell, coral, tooth enamel). IcPD targets the intra-crystalline fraction of protein, which having effectively formed a closed system, minimises the effects of contamination, leaching and other impacts of the depositional environment. IcPD has provided robust and reliable relative geochronologies on Pleistocene timescales at archaeological and palaeoenvironmental sites across the world. The South-Central African region played an important role within mammalian (especially hominin) evolution during the Quaternary, yielding the Kabwe cranium (*Homo heidelbergensis* (*rhodesiensis*)), changing tool technology and early pigment use (~265 ka). Ecologically, the region was once an extensive palaeo-wetland, encompassing the Okavango delta, the Makgadikgadi pan and the Zambezi and Kafue rivers, providing both a migratory corridor between Southern and Eastern Africa, and an ideal environment for hominin habitation. By targeting several biominerals (notably *Achatina* mollusc shell and tooth enamel from a number of mammalian species) from regional archaeological and palaeoenvironmental sites, we aim to provide a series of well-constrained geochronologies with potential for wider application across Africa, helping to elucidate critical archaeological and palaeoenvironmental questions. This poster presents the first IcPD studies using biominerals from the Twin Rivers site in Zambia.

Insights from unprecedentedly large family trees from the Neolithic site of Gurgy in France

Rivollat Maïté (1, 2), Ringbauer Harald (3), Childebayeva Ainash (2), Rohrlach Adam Benjamin (2, 4), Barquera Rodrigo (2), Szolek András (5), Le Roy Mélie

(6), Rey Léonie (1), Goude Gwenaëlle (7), Balter Vincent (8), Rottier Stéphane (1), Deguilloux Marie-France (1), Haak Wolfgang (2)

1 - *De la Préhistoire à l'Actuel : Culture, Environnement et Anthropologie (PACEA)* (France), 2 - *Max Planck Institute for the Science of Human History, Department of Archaeogenetics, Jena (Germany)*, 3 - *Max Planck Institute for Evolutionary Anthropology, Department of Archaeogenetics, Leipzig (Germany)*, 4 - *ARC Centre of Excellence for Mathematical and Statistical Frontiers, School of Mathematical Sciences, The University of Adelaide, Adelaide (Australia)*, 5 - *Applied Bioinformatics, Department of Computer Science, University of Tübingen, Tübingen (Germany)*, 6 - *Queen's University Belfast, School of Natural and Built Environment, ArcPal, Belfast (United Kingdom)*, 7 - *Laboratoire Méditerranéen de Préhistoire Europe Afrique (LAMPEA) (France)*, 8 - *Laboratoire de Géologie de Lyon (France)*

In parallel to population-level studies, archaeogenetic research has drawn attention to intra-site studies. With the optimization of ancient DNA 8, it is now feasible to obtain genome-wide data for multiple individuals from a single site, allowing us to reconstruct biological relationships in archaeological contexts and shed light onto the demographic structure and social organization of past societies/communities. Here, we present new data from the Middle Neolithic French site of Gurgy. Due to an extensive sampling and the use of the 1240K capture array, we obtained genomic data from 94 of 128 individuals. Using a multi-proxy approach and following established 8 to determine biological relatedness, we reconstructed two large pedigrees, one connecting 62 individuals over seven generations, and the other 10 individuals over at least three generations. Genealogies were reinforced by HLA classes I and II haplotypes. From our data, we inferred a patrilocal and patrilineal system, and the practice of female exogamy. The absence of genetic affinities between non-local females and the overall length of runs of homozygosity in the group suggest a wide regional network. Strontium analyses confirm the non-local origin of adult females, but also reveal non-local signatures in the first-generation founders of the site. Using age-at-death from the first and last generations, we narrowed the chronological range of the site use. These unprecedentedly large genealogies provide insights that go beyond the immediate genetic relatedness and allow us to study the group structure, its size, funerary and settlement practices in a much broader social and economic context.

Palaeoproteomics and Diet at the Romano-British Site of Northstowe

Evans Miranda (1), Mazzilli Francesca (3), Hagan Richard (2), Aldred Oscar (3), Evans Chris (3), Neil Benjamin (3), Collins Matthew (1), Hendy Jessica (2)

1 - *Department of Archaeology, University of Cambridge (United Kingdom)*, 2 - *BioArCh, Department of Archaeology, University of York, York (United Kingdom)*, 3 - *Cambridge Archaeological Unit (United Kingdom)*

In this preliminary investigation, a palaeoproteomic approach was used to investigate diet at the Romano-British site of Northstowe in Cambridgeshire. We sought to explore the variability in dietary protein preservation in different mediums. Food is the most tangible part of our daily lives yet arguably the most intangible in the archaeological record. Diet tells us about more than just what people ate; it reveals insights into trade, technology, and cultural identity and how these facets vary temporally and geographically. While other bioarchaeological 8 have been used to investigate food in Roman Britain, the field of paleoproteomics offers tissue and taxonomic specificity facilitating more detailed dietary identifications. This study

draws on samples of both human dental calculus, and lime-scale from a range of vessel forms and fabrics to provide a more nuanced interpretation of food prepared and consumed at Northstowe. The initial aim of the study was to confirm the viability of bottom up palaeoproteomic approaches to obtaining dietary information from lime-scale residues at the site. Also investigated was the variability of dietary protein preservation related to ceramic fabric and vessel form, both of which were observed. In addition, the differences between lime-scale and dental calculus as a medium for protein preservation were explored, with observations supporting this variability in residues related to food preparation and those related to consumption. Finally, a dietary profile of the inhabitants of Northstowe was revealed which included a range of species and tissue specific findings.

Grapevine palaeogenomics across the Mediterranean Basin

Wales Nathan (1)

1 - University of York, York (United Kingdom)

The Mediterranean Basin is home to some of the world's most celebrated winemaking regions, with some traditions stretching back millennia. Despite its economic and cultural significance, there are significant gaps in our knowledge of how domesticated grapevines were transported across the region and which varieties were used at different points in time. The DREGS project, funded by a Marie Skłodowska-Curie Fellowship, seeks to explore the history of winemaking using palaeogenomic testing of archaeological grape seeds. Genome-wide data indicate that grapevine varieties can be identified in historic contexts and that past changes in viticulture reflect broader cultural changes.

Uncanny genetic proportions from Hungary suggest a long lasting Hunter-Gatherer ancestry in Central Europe at the Bronze Age

Gerber Dániel (1, 2), Ari Eszter (2, 3), Szeifert Bea (1, 2), Kustár Ágnes, Fábrián Szilvia (4), Bondár Mária (5), Kiss Viktória (5), Köhler Kitti (5), Mende Balázs Gusztáv (1), Szécsényi-Nagy Anna (1)

1 - Research Centre for the Humanities, Institute of Archaeogenomics, Eötvös Loránd Research Network (ELKH), Budapest, Hungary (Hungary), 2 - Department of Genetics, ELTE Eötvös Loránd University, Budapest, Hungary (Hungary), 3 - Synthetic and Systems Biology Unit, Biological Research Centre, Eötvös Loránd Research Network (ELKH BRC) (Hungary), 4 - Natural History Museum (Hungary), 5 - Research Centre for the Humanities, Institute of Archaeology, Eötvös Loránd Research Network (ELKH), Budapest, Hungary (Hungary)

Bronze Age was a critical turning point in the formation of today's European populations when especially Central Europe became genetically really colourful. Recent large-scale studies tend to focus on the non-European connections and impacts of incoming populations, however, many factors that shaped the European gene pool remains mostly uninvestigated. A number of neglected genetically outlier individuals left an anomalous proportion of Hunter-Gatherer (HG) heritage unrecognized in the post-Neolithic period. Rivollat et al. 2020 have already described elevated HG ancestry from Middle-Late Neolithic sites in present-day Germany, but without further uncovering its exact source and subsequent history. In our study communities of a single site, called Balatonkeresztúr in Western Hungary, from the Early-Middle Bronze age Kisapostag/Encrusted Pottery culture was genetically

analysed for the first time, owing to their extensive cremation practices. The site provides further evidence of an increased, yet mosaically appearing HG component in Central Europe even at the Bronze Age, which likely originated in the region, as suggested by additional novel and published outlier samples. The hunt for the HG components' origin is presented in this talk, where yet barely used high coverage genomes were co-analysed and set to new directions by our dataset. We applied low coverage shotgun and genome-wide capture data of 20 individuals from the site, who belong to at least three distinct archaeological horizons. We were able to pinpoint rare genetic diseases, pigmentation, kinship, social organisation and population affinities of these people, thus providing the complex and multidisciplinary interpretation of these Bronze Age communities.

Unraveling the genetic history of Italians: a genome-wide study of Iron Age Italic populations

Zaro Valentina (1), Vergata Chiara (1), Cannariato Costanza (1), Modi Alessandra (1), Vai Stefania (1), Pilli Elena (1), Diroma Maria Angela (1), Caramelli David (1), Lari Martina (1)

1 - Department of Biology, University of Florence, Florence, Italy (Italy)

The high genetic variability of present-day Italians reflects a complex scenario of past population dynamics dating back not only to Late Paleolithic and Neolithic but also Metal Ages. Although many archaeogenetic studies have been recently carried out to investigate the peopling of Europe, only few genomic data have been reported from Italic populations so far, especially the ones belonging to the last phase of Metal Ages: the Iron Age. To outline a picture of Iron Age genetic variability within the Italian context and infer potential gene flow patterns, we collected 78 human remains from 8 Iron Age necropolises covering 5 different regions of Italy (Emilia-Romagna, Umbria, Marche, Latium and Sicily). Double stranded half-UDG libraries were produced and then shotgun sequenced on an Illumina NovaSeq6000 platform to allow for an initial screening of the samples. Raw reads were processed using the EAGER pipeline and then assessment of DNA authenticity and sex determination were performed. Preliminary population genetics tests were run on genotyped data by building a west Eurasian PCA including all the samples with at least 10.000 SNPs covered on the Affymetrix Human Origins panel. The first results highlight an affinity of the majority of the samples with previously reported Iron Age individuals from Italy, while all samples from Sicily overlap with the genetic variability observed in this area during the Bronze Age. Our aim is to deeper investigate these samples which can significantly contribute to better understand past peopling dynamics of the Italian peninsula and reconstruct modern Italians' genetic history.

Can ancient biomolecules resolve controversy surrounding the identity of the extinct Australian thunderbirds (Genyornis)?

Demarchi Beatrice (1), Stiller Josefin (2), Grealy Alicia (3), Deng Yuan (2), Gilbert Tom (4), Clarke Julia (5), Magee John (6), Zhang Guojie (2), Bunce Michael (3), Collins Matthew (7, 8), Miller Gifford (9)

1 - Department of Life Sciences and Systems Biology, University of Turin (Italy), 2 - Centre for Biodiversity Genomics, University of Copenhagen (Denmark), 3 - Trace and Environmental DNA (TrEnD) laboratory, Curtin University, Perth (Australia), 4 - Center for Evolutionary Hologenomics, The GLOBE Institute, University of

Copenhagen (Denmark), 5 - Department of Geological Sciences, The University of Texas at Austin (United States), 6 - Research School Earth Sciences, Australian National University, Canberra, 2601, Australian Capital Territory (Australia), 7 - Section for Evolutionary Genomics, The GLOBE Institute, Faculty of Health and Medical Sciences, University of Copenhagen (Denmark), 8 - McDonald Institute for Archaeological Research, University of Cambridge (United Kingdom), 9 - INSTAAR and Department of Geological Sciences, University of Colorado Boulder (United States)

«Thunderbirds» (Aves: Dromornithidae), the giant flightless birds that inhabited Australia during the Oligocene and Pleistocene, are among the most iconic extinct megafauna from the «dreamtime». It is believed that the latest surviving member of this fossil clade, *Genyornis newtoni*, became extinct ~50k years ago, having interacted for several millennia with the first humans to colonise Australia (Miller et al., 2016, Nat. Commun. 7, 10496). Since the 1980s the identity of Pleistocene fossil eggshell found in South Australia has been surrounded in controversy. Although the large, thick eggshell was originally identified as belonging to *Genyornis* (Williams, 1981. *Alcheringa: An Australasian Journal of Palaeontology* 5, 133-140), more recent morphological analysis (Grellet-Tinner et al., 2016. *Quat. Sci. Rev.* 133, 147-164) suggests the eggshell belonged to an extinct megapode, *Progura*-like (Aves: Galliformes: Megapodiidae). Here, we explore the potential of ancient biomolecules to resolve this dispute. After ancient DNA analysis failed to yield any sequence data, amino acid racemisation analysis was used to guide selection of samples for palaeoproteomics. We present the first ancient protein sequences (up to 80% coverage) from eggshells of this «mystery bird» and discuss its phylogenetic placement using a comparative analysis comprising more than 150 extant species from the Bird 10,000 Genomes (B10K) Project. We find that the protein sequences recovered from the «mystery» eggshell are placed within the Galloanseres clade as expected, but that the eggs are unlikely to have been laid by a megapode parent.

Origin and spread of the Iron Age eastern Scythians revealed through a one-thousand-year transect of ancient genomes across the Central Asian steppe. Gnecci-Ruscione Guido Alberto (1, 2), Khussainova Elmira (3), Kahbatkyz Nurzhibek (3, 4), Musralina Lyazzat (2, 3, 4), Spyrou Maria (2), Djansugurova Leyla (3), Jeong Choongwon (5), Krause Johannes (1, 2)

1 - Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany (Germany), 2 - Department of Archaeogenetics, Max Planck Institute for the Science of Human History, Jena, Germany (Germany), 3 - Laboratory of Population Genetics, Institute of General Genetics and Cytology, Almaty, Kazakhstan (Kazakhstan), 4 - Kazakh National University by al-Farabi, Almaty, Kazakhstan (Kazakhstan), 5 - School of Biological Sciences, Seoul National University, Seoul, Republic of Korea (South Korea)

The Iron Age nomads of the Eurasian steppe, commonly referred altogether as Scythians, are renowned in historiography through indirect sources from the neighboring sedentary civilizations, among them, the ancient Greeks followed by the Romans, the Persians and the ancient Chinese. Nevertheless, due to lack of direct written records, little is known about their origins and the actual relation between the many different cultures that lived in such a vast territory and long time period. In order to understand the genetic structure of the different eastern Scythian cultures as well as the demographic events associated with their origins and decline, we generated

genome-wide data from 111 human remains retrieved from 39 different sites across the Kazakh Steppe (Kazakhstan, Kyrgyzstan and Russia), covering a time period from the 9th century BCE to the 4th century CE. We identified signals of extensive genetic admixture between genetically distinct Late Bronze Age sources from which we identified two main gene-pools emerging and giving rise to the different groups. The decline of the Scythian cultures was mirrored by new genetic turnovers, that started as early as the 4th century BCE and continued with the spread of the eastern nomad empires in the first centuries CE along with the expansion of Persian-related civilization from the south.

Women and children in Roman Thessaloniki: an investigation of breastfeeding and weaning through delta-13C and delta-15N incremental analysis.

Ganiatsou Elissavet (1), Vika Efrossini (2) (1), Georgiadou Aggeliki (1), Protopsalti Tania (3), Papageorgopoulou Christina (1)

1 - Laboratory of Physical Anthropology, Department of History and Ethnology, Democritus University of Thrace, Komotini, Greece (Greece), 2 - Department of Archaeology, University of York (United Kingdom), 3 - Ephorate of Antiquities of Thessaloniki City, Ministry of Culture and Sports, Thessaloniki, Greece (Greece)

The ancient city of Thessaloniki was conquered by the Romans in 168 BC and quickly became the capital of the Provincia Macedonia. Having the great advantage of being located on the sea front and along the Via Egnatia, the monumental Roman road that connected the Adriatic to the Black Sea, Thessaloniki developed into one of the most prominent trade centres of the Roman Empire. At the same time, the flourishing local artisanality and cultural countenance created the unique regional character of the city: although it was the affluent capital of the province, Thessaloniki was declared a *Liberam Civitatem* with many privileges, attested through the city's exceptional architecture and influential artistic expression. The recent excavations of the two main necropoleis provide an unprecedented opportunity to investigate the bioarchaeological expressions of the city's distinctive cultural character as part of the Empire. In this study, we explore the role of women in the domestic sphere as mothers and care-givers in Roman Thessaloniki. For this purpose, we employed $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis from dental increments to study breastfeeding and weaning patterns, under the premise that breastfeeding is a complex interaction between biological necessity and cultural evolution and a concept critical to the understanding of parental investment and the liminality of childhood. Our holistic approach aims to enhance the fruitful integration of historical and bioarchaeological research to better explain the cultural complexities during a time of social transformation in the south of the Roman Empire.

Genetic Structure and Pathogen Diversity of a Moche Tomb in Northern Peru

Guerra Amorim C. Eduardo (1, 2), Neuenschwander Samuel (1, 2), Arizmendi-Cárdenas Yami Ommar (1, 2), Mota Bárbara (1, 2), Cruz Dávalos Diana I. (1, 2), Barbieri Chiara (3, 4), Verano John (5), Fehren-Schmitz Lars (6), Malaspina Anna-Sapfo (1, 2)

1 - University of Lausanne (Switzerland), 2 - Swiss Institute of Bioinformatics (Switzerland), 3 - University of Zurich (Switzerland), 4 - Max Planck Institute (Germany), 5 - Tulane University (United States), 6 - University of California, Santa Cruz (United States)

The Moche culture flourished in the Northern Peruvian coast between the years 100 BCE to 800 CE, extending over a 500km region at its apogee. The Moche engineered many elaborate metal artifacts and figurative pottery, had a remarkably complex social structure with leaders, warriors, ritual specialists, artisans farmers, and fishermen, but did not leave any written record. The Huaca Cao Viejo, which is part of the El Brujo archaeological complex in the Chicama Valley, is one of the most prominent Moche sites. It is mostly known for the undisturbed burial of a female leader known as the Señora de Cao, discovered in 2006. Here we study the low depth whole genomes of $n=7$ individuals found in the Tomb 2 of Huaca Cao Viejo (~350 - 600 CE) and other $n=7$ individuals from different El Brujo sites. We show that the genetic ancestry of the ancient individuals is similar to that of the modern Indigenous populations from Northern Peru. These analyses also indicate that one individual from Huaca Cao Viejo, dated at ~580 CE, presented East Asian ancestry (~13%), not shared with the other ancient individuals from El Brujo. Furthermore, we identify potential viral ancient DNA molecules in these samples. In particular, we find that all seven individuals from Tomb 2 present evidence of human parvovirus B19 ancient DNA, absent in the other samples outside this tomb. We discuss these findings in light of the growing catalog of ancient pathogens in pre- and post-contact Native American samples.

Palaeoreconstruction of East Siberian Pleistocene glacial and interglacial biodiversity based on ancient DNA analyses of sediments from the Batagay Megaslump exposure - Providing a picture of past ecosystems

Courtin Jeremy (1), Perfumo Amedea (1), Stoof-Leichsenring Kathleen R. (1), Herzschuh Ulrike (1, 2, 3)

1 - Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Polar Terrestrial Environmental Systems, Potsdam (Germany), 2 - Institute of Environmental Science and Geography, University of Potsdam, Potsdam-Golm (Germany), 3 - Institute of Biology and Biochemistry, University of Potsdam, Potsdam-Golm (Germany)

Amplified arctic warming causes Northern boreal ecosystems to experience even stronger climate change than in the rest of Northern Hemisphere. Intricate interactions between and within the abiotic and biotic components of the system make challenging to predict the evolutionary dynamics of arctic ecosystems. During the Pleistocene, glacial and interglacial cycles involved drastic changes in past ecosystems. Consequently, the investigation of Quaternary records can strengthen the current 8 to forecast the effects of global warming on ecosystems. The Batagay megaslump, in northern Yakutia, is the world's largest known retrospective thaw slump discontinuously exposing Middle Pleistocene to Holocene permafrost formations. In 2019, 70 sediment samples were collected with the aim to characterise biodiversity changes between Quaternary glacials and interglacials in East Siberia. Using the ancient DNA extracted from these environmental samples, we performed a metabarcoding analysis (chloroplast *trnL*) to investigate past vegetation composition as well as shotgun metagenomic analysis, which enabled us to access the entire biodiversity, from viruses to Mammoths. This approach makes possible not only to investigate and provide an comprehensive picture of past biodiversity but also to infer on potential interactions across taxa and kingdoms. This work on past and modern biodiversity of permafrost regions holds great potential to reveal new insights into the evolution of this fragile ecosystem.

nf-core/eager: reproducible, portable, and efficient ancient genome reconstruction

Fellows Yates James A. (1), Lamnidis Thiseas C. (1), Borry Maxime (1), Andrades Valtueña Aida (1), Fagnäs Zandra (1), Clayton Stephen (1), Neukamm Judith (2), Peltzer Alexander (3)

1 - Department of Archaeogenetics, Max Planck Institute for the Science of Human History (Germany), 2 - Institute of Evolutionary Medicine (IEM), University of Zürich (Switzerland), 3 - Quantitative Biology Center (QBiC), Eberhard Karls Universität Tübingen (Germany)

Rapid expansion of palaeo- and archaeogenomics is seeing an increasing diversity in the types of ancient DNA (aDNA) being studied. The scale of output and heterogeneity in the types of computational contexts and analyses being performed is correspondingly growing. We present a new version of the Efficient Ancient Genome Reconstruction pipeline (EAGER doi:10.1186/s13059-016-0918-z), which adapts to the challenges facing the rising utilisation of ancient DNA by the natural and social sciences. nf-core/eager expands the repertoire of tools to include increasingly routine analyses such as metagenomic screening for ancient microbes of interest and human biological sex-determination, while maintaining ease of use for those without computational backgrounds. A rewrite of the pipeline in the domain-specific-language Nextflow (doi:10.1038/nbt.3820) has been performed to allow portability and scalability across a wide range of computing environments, from personal computers to integration within High-Performance-Clusters (HPCs) and cloud platforms. nf-core/eager has also been developed within the nf-core community (doi:10.1101/610741) to enforce best-practice bioinformatics and software development guidelines. This helps to ensure accessibility for new contributors, continuous integration testing and feedback. Finally, nf-core/eager comes with extensive user-friendly, illustrated documentation, which assists in the general understanding of NGS aDNA analysis for newcomers to the field. In this presentation, I will introduce the pipeline, encourage the greater community involvement to ensure long-term development and maintenance, and demonstrate how it will help democratise access and improve the quality of reproducible ancient DNA analysis for research groups of all sizes.

Human mitochondrial haplogroups and ancient DNA preservation across Egyptian history

Urban Christian (1), Neukamm Judith (1), Eppenberger Patrick (1), Brändle Martin (2), Rühli Frank (1), Schuenemann Verena (1)

1 - Institute of Evolutionary Medicine (University of Zurich Switzerland), 2 - Faculty of Biology, Philipps-Universität Marburg (Germany)

Egypt represents an ideal location for genetic studies on population migration and admixture due to its geographic location and rich history. However, there are only a few reliable genetic studies on ancient Egyptian samples. In a previous study, we assessed the genetic history of a single site: Abusir el-Meleq from 1388 BCE to 426 CE. We now focus on widening the geographic scope to give a general overview of the population genetic background, focusing on mitochondrial haplogroups present among the whole Egyptian Nile River Valley. We collected 81 tooth, hair, bone, and soft tissue samples from 14 mummies and 17 skeletal remains. The samples span approximately 4000 years of Egyptian history and originate from six different excavation sites covering the whole length of the Egyptian Nile River Valley. NGS

based ancient DNA 8 were applied to reconstruct 18 high-quality mitochondrial genomes from 10 different individuals. The determined mitochondrial haplogroups match the results from our Abusir el-Meleq study. Our results indicate very low rates of modern DNA contamination independent of the tissue type. Although authentic ancient DNA was recovered from different tissues, a reliable recovery was best achieved using teeth or petrous bone material. Moreover, the rate for successful ancient DNA retrieval between Egyptian mummies and skeletal remains did not differ significantly. Our study provides preliminary insights into population history across different regions and compares tissue-specific DNA preservation for mummies and skeletal remains from the Egyptian Nile River Valley.

Extraction and Analysis of ancient DNA from Mineralized Tissue Formations
Hiss Alina N. (1), Teschler-Nicola Maria (2, 3), Winter Eduard (2), Herbig Alexander (1), Krause Johannes (1, 4)

1 - Max Planck Institute for Evolutionary Anthropology (Germany), 2 - Department of Anthropology, Natural History Museum Vienna (Austria), 3 - Department of Evolutionary Anthropology, University of Vienna (Austria), 4 - Faculty of Biological Sciences, Friedrich Schiller University Jena (Germany)

Mineralized tissue formations, such as bladder-, kidney- or gallstones are a rare finding in archaeological excavations, even though they were likely not uncommon in past human populations. However, they can be a valuable source for both host and pathogen DNA. The formation of such calculi can be linked to diet and hydration, metabolic diseases, tumors and metastases, as well as chronic and infectious diseases. Especially the latter can be of great interest when studying pathogens, as they become enclosed in the course of formation. Even though calculi most often form inside the gallbladder, kidney, urinary bladder, and associated ducts, they can be found in various other locations of the body, such as the salivary glands, pancreas, the veins, and as nodules inside the lungs, where they can be associated with infectious diseases such as tuberculosis or brucellosis. However, as the archaeological record is sparse, not many objects have been studied yet regarding ancient host and pathogen DNA. During the 19th century in Vienna, surgeon and urologist Leopold von Dittel had collected numerous different calculi, that are - together with a variety of other ancient stone formations - stored in the «Pathologisch-anatomischen Sammlung im 'Narrenturm', Naturhistorisches Museum Wien (PASiN-NHM)». Using calculi from this extensive collection, we explore different sampling strategies aiming to preserve stone formations as intact as possible, while still obtaining enough DNA to extract and analyze, in order to both quantitatively and qualitatively assess host and pathogen DNA as well as species composition across different sample types.

Estimating age at death from ancient DNA methylomes

Mendez Katterinne (1), Diekmann Yoan (1), Blöcher Jens (1), Burger Joachim (1)

1 - Palaeogenetics Group, Institute of Organismic and Molecular Evolution (iomE), Johannes Gutenberg University (Germany)

Aging affects DNA methylation. Hundreds of genomic sites whose DNA methylation levels are strongly correlated with age have been identified, defined as epigenetic clock, and shown to be accurate predictors of age across various fresh human

tissues, including bone. In archaeology, age-at-death is most often unknown but highly relevant for palaeodemography, the demographic study of ancient human populations. The availability of high-quality ancient genomes has allowed for the inference of DNA methylation levels through the observation of post-mortem damage (PMD) patterns. However, the accuracy of epigenetic clocks in ancient DNA (aDNA) is currently unknown. Here, we evaluate how accurately epigenetic clocks work on aDNA. First, we assess site-specific accuracy of DNA methylation inference via simulations of aDNA sequencing data with DNA methylation-dependent cytosine deamination patterns. Simulations are carried out with different PMD profiles and at different sequencing depths to quantify their effect on the inference. Our simulations show that DNA methylation is inferred with a mean error of ~20% at sequencing depth $\geq 10X$. Next, we quantify the effect of this error on age-at-death estimates at different PMD levels and depths and capture the statistical uncertainty in form of confidence intervals. To illustrate the usefulness of our age interval estimates from archaeological human bones, we compare mean age-at-death of a group of early Neolithic farmers against a group of Mesolithic hunter-gatherers, fully accounting for the statistical uncertainty. Our work demonstrates that age-at-death estimation from aDNA is accurate enough to answer relevant palaeodemographic questions.

Shedding light on the evolutionary past of the world's largest tropical lake using sedaDNA

De Cahsan Binia (1), Zhao Meng (1), Monchamp Marie-Eve (1, 2), Muschick Moritz (3) (4), Marques David Alexander (3, 4), Jemmi Eliane Inessa (3, 4), Ngoepe Dora Nare (3, 4), Cuenca Cambroner Maria (3, 4), King Leighton Rebecca (3, 4), Matthews Blake (4, 5), Gilbert Marcus Thomas Pius (1), Pedersen Mikkel Winther (1), Seehausen Ole (3, 4)

1 - GLOBE Institute, University of Copenhagen, Copenhagen, Denmark (Denmark), 2 - Department of Biology, McGill University, Montreal, Quebec, Canada (Canada), 3 - Aquatic Ecology and Evolution, Institute of Ecology and Evolution, University of Bern, Bern, Switzerland (Switzerland), 4 - Department of Fish Ecology & Evolution, EAWAG, Swiss Federal Institute for Aquatic Science and Technology, Kastanienbaum, Switzerland (Switzerland), 5 - Institute of Integrative Biology, ETH-Zurich, Zurich, Switzerland (Switzerland)

Lake Victoria is the largest tropical lake in the world by surface. It is a global biodiversity hotspot and is widely known for its endemic adaptive radiation of more than 500 species of cichlid fish among others. Despite its large endemic species diversity, this lake system and its associated food web are only ~15,000 years old. This young but complex system represents a natural opportunity to study how evolution and ecosystem function have been influenced by environmental change over thousands of years. Throughout the lake's history, minerogenic and organic debris from organisms living within and around the lake, have been deposited on the bottom stacking a layered sedimentary archive. To track environmental change and the evolutionary diversification of both fish and other biological assemblages (phyto-, zooplankton and zoobenthos communities) through time, we are extracting and analysing sediment ancient DNA (sedaDNA). We combine our results with geological, micro- and macrofossil evidence and hope to obtain an unprecedented paleoenvironmental reconstruction, providing important pieces in the development of the unique biome and endemic cichlid species radiation of today's Lake Victoria.

Tropical cichlid fish subfossils as source of ancient DNA and tool to study adaptive radiation

Muschick Moritz (1, 2), Jemmi Eliane (1, 2), Seehausen Ole (1, 2)

1 - EAWAG Center for Ecology, Evolution & Biogeochemistry (Switzerland), 2 - Institute of Ecology & Evolution, University of Bern (Switzerland)

Many evolutionary processes unfold over time periods longer than we can observe directly, with environmental conditions and ecological settings constantly changing. Hence, inferring the causes and consequences of past diversification, extinction and migration from data of extant populations alone is often difficult. Cichlid fishes of the East African Great Lakes are renowned for their exuberant species-richness and ecological specialisations and are a famous study system in evolutionary biology. Their extant diversity and phylogenetic relationships within and between lake radiations are largely known, but the conditions at the beginning and during these radiations are much less studied. Here we present findings from paleogenetic analyses of fish remains from the sediment record of Lake Victoria, the largest tropical lake in the world, where haplochromine cichlid fishes radiated into hundreds of species since the basin refilled just 15'000 years ago. A very sensitive cichlid-specific qPCR assay allows us to screen hundreds of individual fish remains for endogenous DNA and generate mitochondrial and low-coverage nuclear genome sequences from the most promising samples through single-stranded library preparation and sequence capture. We gauge mitochondrial haplotype diversity and allelic diversity stemming from an ancestral admixture event through time, leveraging a reference database with hundreds of re-sequenced modern species for comparison. We discuss our findings in the context of environmental and anthropogenic change, and highlight the scientific opportunities - but also methodological challenges - of this approach for the study of cichlid fish biodiversity and evolution.

Koch's bacillus genome, literally?

Sejdiu Donikë (1), Akgül Gülfirde (1), Nelson Elizabeth A., Merkel Kevin, Leendertz Fabian H., Molak Martyna, Schnalke Thomas, Schuenemann Verena J. (1), Calvignac-Spencer Sébastien

1 - Institute of Evolutionary Medicine (Switzerland)

During the 19th century, tuberculosis (TB) devastated Europe and is estimated to have killed 20% of the adult population. Today tuberculosis remains a global health burden with sustained high mortality and the emergence of antibiotic resistant strains, largely due an incomplete understanding of evolutionary factors driving this resistance. The first isolation of its causative agent, the tubercle bacillus, was performed in 1882 by Robert Koch, confirming the infectious nature of TB. However, an understanding of Koch's isolates on a genomic level has yet to be realized. In this study, we performed high-throughput sequencing on two 19th century samples from Robert Koch's own experiments, including a tubercle bacilli isolate culture (Koch1891) and tuberculin, a suspension of tuberculous bacteria developed by Koch to serve as curing drug (Koch1896). Here, we present the reconstruction and characterization of two TB genomes from Koch's samples, with genome coverage of 79-fold (Koch 1891) and 1.2-fold (Koch 1896). Phylogenetic analysis revealed these genomes cluster with the Euro-American lineage (L4) of *M. tuberculosis* and are most closely related to the H37Rv strain which was isolated from a clinical sample in 1905 and is now used as the primary TB reference genome. Our results add to the

relatively small set of pre-antibiotic genomes and thereby contributes to providing a useful reference to study the recent evolutionary trajectory of drug resistance and virulence. This contribution provides a better understanding of early variants of *M. tuberculosis* in the continuation of Robert Koch's legacy to the study of tuberculosis.

Improving the taxonomic identification of lipids preserved in pottery: towards a detailed characterisation of ancient culinary practices through MALDI MS?

Drieu Léa (1), Bergström Ed (2), Smith Rachel K. (2), Lundy Jasmine (1), Thomas-Oates Jane (2), Craig Oliver E (1)

1 - Department of Archaeology, BioArCh, University of York (United Kingdom), 2 - Department of Chemistry [York, UK] (United Kingdom)

The analysis of lipids preserved in archaeological pottery is now a common approach to studying the economy of ancient populations. Commonly used $\delta^{13}C$ in lipid analysis provide global trends in the consumption of natural substances: ruminant and non-ruminant carcass fats, dairy products, marine and freshwater products, plant oils and waxes, etc. However, the taxonomic resolution of these analytical $\delta^{13}C$ is very restricted, and only allows a limited approach to the dietary and culinary diversity of ancient populations. Here we present the analysis by MALDI MS and MALDI MS/MS of triacylglycerols preserved in Sicilian pottery dating from the 9th to the 14th century A.D., with the aim of assessing what level of taxonomic precision we can achieve to identify the commodities consumed by the early medieval populations in Sicily. The analysis of triacylglycerols by MALDI-MS proves to be limited to samples where the overall triacylglycerol profile is particularly well preserved. This bias, also observed with conventional gas chromatography analyses, is due to the degradation of the most diagnostic triacylglycerols. In order to overcome this problem, we used MALDI MS/MS to study the fatty acid composition of triacylglycerols that are most resistant to degradation. This approach revealed the specific profiles of certain commodities, which were used in particular to distinguish between different dairy products and plant oils. Despite the prevailing poor preservation of lipids in Mediterranean contexts, it was possible to propose precise identifications of the contents of some vessels and to broaden our understanding of early medieval consumption patterns in Sicily.

Ancient DNA traces the history of some of the southernmost polar bear fossil remains

Cabrera Andrea A. (1), Westbury Michael V. (1), Lorenzen Eline D (1)

1 - GLOBE Institute, University of Copenhagen, Øster Voldgade 5-7, Copenhagen, Denmark (Denmark)

Polar bears (*Ursus maritimus*) are uniquely adapted to the extreme Arctic environment. They spend most of their lives on the sea ice and have become a flagship species of the threats global warming has on biodiversity. Although their current native range lies largely within the Arctic Circle, fossil records show the presence of polar bears more than 2,000 km south of their current range limits. Fossil evidence from southern Scandinavia represent some of the southernmost remains, and date to the Younger Dryas, 12,500-8,000 years BP, where glacial conditions temporarily returned, and sea ice expanded into large parts of Northern Europe. Little is known about this polar bear population. Here, we analyzed genomic information from nine Scandinavian polar bear fossil remains, to elucidate their evolutionary and

demographic history. We compared our data against previously published whole-genome sequence data from modern polar bears across their geographic range. Endogenous content of the specimens varied among samples from <0.1% to ~65%. Preliminary phylogenetic results based on the mitochondrial genome show that Scandinavian polar bears were more closely related to lineages currently found in Siberia and Alaska. To investigate the sex of the polar bear remains, we compared the read depth of the chromosome X and autosomal chromosomes. Preliminary results show an excess of males among the nine identified samples (66% males). This information can provide insights into the population structure, life history and demography of past polar bear populations.

Exploring the Influence of Caloric Restriction (CR) on Nitrogen and Carbon Stable Isotope Ratios on Mouse Bones

Eléa Gutierrez (1, 2) Sharon Mitchell (2), Catherine Hambly (2), John R. Speakman (2), Kate Britton (1)

1 - School of Geosciences, Department of Archaeology, University of Aberdeen (United Kingdom), 2 - School of Biological Sciences, Energetics Research Group, University of Aberdeen (United Kingdom)

Stable isotope analysis is widely used in archaeology for the reconstruction of past diets. While nitrogen isotope analysis of preserved proteins, such as bone collagen, is commonly employed to reconstruct the source/trophic level of dietary protein, there are uncertainties surrounding the influence of dietary composition on the excretion of nitrogen and therefore diet-tissue offsets. In particular, the impact of i) caloric restriction/famine and ii) dietary macronutrient composition warrants further investigation. Here, we take advantage of a series of carefully past controlled feeding experiments to explore the relationship between these aspects of dietary manipulation and diet-tissue enrichment in ^{15}N . In this pilot study, we utilised preserved materials from a previous unrelated experiment to explore the effect of caloric restriction on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of mouse fur and bone collagen. Mice had been exposed at 21 weeks of age (close to their skeletal maturity) to five different levels of caloric restriction: 0, 10, 20, 30 and 40% lower calories than their ad libitum intakes for nineteen months (around 70% of their lifespan). Tissues were obtained at the end of the experiment, allowing exploration of the impact of long-term nutritional stress on archaeologically relevant tissues.

Advancing Ancient Mollusk Shells as Multi-Proxy Archives of the Past

Der Sarkissian Clio (1), Thyrring Jakob (2, 3), Mata Xavier (1), Dumoncel Jean (1), Ivorra Sarah (4), Evin Allowen (4), Orlando Ludovic (1), Samir Chafik (5), Braga José (1)

1 - Centre for Anthropobiology and Genomics of Toulouse (France), 2 - Aarhus University, Department of Bioscience (Denmark), 3 - University of British Columbia, Department of Zoology (Canada), 4 - Institute of Evolutionary Science of Montpellier (France), 5 - Laboratory of Informatics, Modelling and Optimization of the Systems (France)

Mollusk shells were recently revealed as metagenomic archives of the past, potentially covering the last 100,000 years. This has not only opened new avenues for ancient DNA research but also added DNA to the list of morphological, structural and biogeochemical shell proxies that provide a wealth of information about the

evolution, life history, stress responses and past environments of mollusks. Multi-proxy studies thus offer the unique opportunity to characterize, at high resolution, the dynamics of their different biological responses to environmental changes. We present here a multi-disciplinary approach combining 3D computed tomography imaging, statistical shape analyses, diffeomorphometry, paleo-environmental reconstruction and ancient genomics to investigate the relationships between the phenotype and genomic composition of wild *Mytilus* mussels across environmental gradients at both the spatial and temporal scales. Our novel methodological framework is highly relevant to address the on-going and future environmental challenges faced by our societies, such as global warming and pollution intensification, and their damaging consequences on biodiversity. Other goals include advancing responsible ancient mollusk DNA research by maximizing information output while minimizing destruction of shells.

Assessing the resolution of rare variation for inferring human history from ancient genomes

Lamnidis Thiseas (1), Schiffels Stephan (2)

1 - Max Planck Institute for the Science of Human History (Germany), 2 - Max Planck Institute for the Science of Human History, Department of Archaeogenetics, Jena (Germany)

Understanding how population structure changes through time and space is key for reconstructing human population history. Most analytical methods for assessing population structure rely on allele frequency differences between individuals and groups. Previous research has suggested that rare genetic variation shared between groups can be utilised to detect and model population structure, providing a higher resolution than is available from common genetic variation, while requiring relatively low sequencing coverage compared to other more high resolution methods. Here, we simulate a structured population with known per-generation admixture rates between neighbouring sub-populations to assess and compare the resolution provided by analysis of common and rare variants respectively. By varying the degree of genetic structure within the simulated population, we were able to assess and compare the resolution of methods focusing on the sharing of common or rare variation between populations. We find that for detecting population structure, rare variation methods outperform those focused on common variants, retaining resolution even in weakly structured populations. Our results indicate that analysis of rare variation is a good candidate method for inferring the population history between closely-related populations in a cost-effective manner.

No particular genomic features underpin the dramatic economic consequences of 17th century plague epidemics in Italy

Seguin-Orlando Andaine (1), Costedoat Caroline (2), Der Sarkissian Clio (1), Tzortzis Stefan (3), Kamel Celia (2), Telmon Norbert (1), Dalén Love (4), Thèves Catherine (1), Signoli Michel (2), Orlando Ludovic (1)

1 - Centre for Anthropobiology and Genomics of Toulouse UMR5288 CAGT (France), 2 - Anthropologie bio-culturelle, Droit, Ethique et Santé (France), 3 - Direction Régionale des Affaires Culturelles Provence-Alpes-Côte d'Azur (France), 4 - Centre for Palaeogenetics (Sweden)

The second plague pandemic started with the Black Death, an infection caused by a

Yersinia pestis strain that killed approximately 30-60% of the European population in the mid-14th century. Since then, several epidemic waves came back to haunt human populations until the 18th century, when this pathogenic form vanished. During the 17th century, plague only added to an already long list of various scourges, including climate cooling, famines and wars. At the time, plague had a stronger demographic toll on populations from southern Europe, especially Italy, where it caused long-lasting economical damage. It remains, however, unknown whether sanitation control of the epidemic and its animal vectors was less effective there, or whether more harmful pathogenic strains circulated. Screening the DNA content of 26 human bodies excavated from the 1629-1630 plague cemetery of Lariey (French Alps), we identified two teeth particularly rich in plague genetic material. Further sequencing revealed two *Y. pestis* genomes phylogenetically closest to those previously characterized from the 1636 plague outbreak of San Procolo a Saturno, Italy. They both belonged to a cluster extending from the Alps to northern Germany, that probably propagated during the Thirty Years war (1618-1648). Patterns of sequence variation did not support faster evolutionary rates in the Italian genomes and instead revealed only rare private non-synonymous mutations, which did not affect virulence genes. This, and the limited spread of such genomes outside of Italy, suggest environmental, social and/or institutional rather than biological causes for the severe Italian epidemic trajectory observed during the 17th century.

Ancient mitochondrial genomes from the Argentinian Pampas inform the peopling of the Southern Cone of South America

Roca-Rada Xavier (1), Politis Gustavo (2, 3), Messineo Pablo (3), Scheifler Nahuel (3), Scabuzzo Clara (4), González Mariela (3), M. Harkins Kelly (5), Reich David (6, 7, 8) (9), Souilmi Yassine (1), C. Teixeira João (1), Llamas Bastien (1), Fehren-Schmitz Lars (5)

1 - Australian Centre for Ancient DNA - University of Adelaide (Australia), 2 - División Arqueología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Buenos Aires (Argentina), 3 - INCUAPA-CONICET, Facultad de Ciencias Sociales, Universidad Nacional del Centro de la Provincia de Buenos Aires, Buenos Aires (Argentina), 4 - CICYTTP-CONICET, Provincia de Entre Ríos-UADER-División Arqueología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata. (Argentina), 5 - University of California, Santa Cruz (United States), 6 - Department of Genetics, Harvard Medical School, Boston (United States), 7 - Department of Human Evolutionary Biology, Harvard University, Cambridge (United States), 8 - Broad Institute of MIT and Harvard, Cambridge, (United States), 9 - Howard Hughes Medical Institute, Harvard Medical School, Boston, (United States)

The Southern Cone of South America (SCSA)-formed by Argentina, Chile, Uruguay and Southern Brazil- is a key region for investigations about the peopling of the Americas. However, only a few recent studies involved populations from the eastern sector of the SCSA, the Argentinian Pampas. We analysed whole mitochondrial genomes from 18 human skeletal remains from three Early to Late Holocene archaeological sites in the Argentinian Pampas: Arroyo Seco 2, Laguna de los Pampas and Laguna Chica. Results revealed a distinctive genetic makeup in the Pampas when compared with other Middle to Late Holocene pre-Columbian SCSA populations. We also report the earliest individuals carrying SCSA-specific mitochondrial haplogroups D1j and D1g from Early and Middle Holocene,

respectively. Using these deep calibration timepoints in Bayesian phylogenetic reconstructions, we suggest the first settlers of the Pampas were part of a single and rapid dispersal ~15,600 years ago, synchronous with the initial peopling of the Americas. Finally, we propose that present-day genetic differences between the Pampas and the rest of the SCSA result from a combination of founder effects, genetic drift and a partial population replacement in the Early Holocene.

Evaluating the effect of genotype imputation of ancient whole genome *Sus scrofa* DNA

Erven Jolijn (1), Çakırlar Canan (1), Raemaekers Daan (1), Bradley D. (2), Madsen Ole (3)

1 - Groningen Institute of Archaeology, University of Groningen (Netherlands), 2 - Smurfit Institute of Genetics, Trinity College Dublin (Ireland), 3 - Animal Breeding and Genomics Centre, Wageningen University (Netherlands)

Advances in sequencing techniques of the last decades have increased the amount of sequenced ancient DNA. However, sequencing ancient DNA to high coverage is often limited by sample quality or cost. Imputing missing genotypes has the potential to increase information content, cost-effectiveness, and quality of downstream analyses in ancient data. Ancient DNA has certain methodological and computational challenges compared to modern DNA, and requires a different imputation approach than imputation of modern samples. Recent studies on imputation of ancient human data have shown promising results but it is still unclear to what extent the human approach can be applied to other species with different genetic architecture, demography history and less available genetic resources (e.g. whole genome sequencing data). *Sus scrofa* is an important farm animal that played a key role in understanding the emergence of animal husbandry in Europe and is therefore an interesting case study. In this study we present a systematic evaluation of imputation on whole genome ancient *Sus scrofa* DNA from Early to Late Neolithic (~7,100-4,500 BP). We investigate how issues like genetic architecture, sample quality, low coverage, and reference divergence and size affect imputation accuracy. We will present results from different imputation strategies and provide guidelines on best imputation practices from ancient pig samples.

Epigenetic variation in Neolithic hunter-gatherer and farmer populations from Scandinavia

Thalmann Olaf (1), Hanghøj Kristian (2), Edlund Hanna (3), Bernhardsson Carolina (3), Orlando Ludovic A. (4), Herzig Karl-Heinz (1), Walkowiak Jarosław (1), Jakobsson Mattias (3)

1 - Poznan University of Medical Sciences, Dept. of Pediatric Gastroenterology and Metabolic Diseases (Poland), 2 - The Bioinformatics Centre, Department of Biology, University of Copenhagen (Denmark), 3 - Uppsala University, Department of Organismal Biology (Sweden), 4 - University Paul Sabatier, Centre for Anthropobiology and Genomics of Toulouse (France)

Centuries of research have unraveled the many facets of human's singularity, and with the recent advances in molecular biology we are now to directly link molecular footprints with the adaptation to lifestyle changes in the past. One epoch with important alterations in human lifestyle is the Neolithic, where humans transitioned from hunting-gathering to farming, and consequently populations started to increase

and modern lifestyle diseases became manifested. Applying paleoepigenomic approaches and more specifically, by describing methylation landscapes within the genomes of Neolithic Scandinavians we shed new light at the adaptive potential of our Northern ancestors. We analyzed 10 newly generated high-coverage genomes including five individuals originating from the Ajvide site at the island of Gotland in Sweden, a site known for its assemblage of hunter-gatherers from the Pitted Ware Culture that occupied the island from the Mesolithic to the Bronze age. By reconstructing the methylation profiles from the high-coverage genome data obtained from all five individuals we assessed the width of epigenetic variation within a single hunter-gatherer Neolithic population at a specific point in time and compared those patterns to the methylation landscapes generated from Scandinavian farmers living contemporaneously. While such a set-up allows us to highlight epigenomic variation within ancient human populations and monitor it through a transition in life-style, by incorporating our newly generated data into a growing assemblage of temporarily spaced methylomes we are setting another milestone in the expanding field of paleoepigenomics.

Population dynamics and demographic history of Eurasian collared lemmings
Edana Lord (1), Marangoni Aurelio (1), Baca Matuesz (2), Popović Danijela (2), Goropashnaya Anya (3), Stewart John R. (4), Germonpré Meitje (5), Vartanyan Sergey (6), Prost Stefan (7), Olsen Remi-André (8), Federov Vadim (3), Dalén Love (1)

1 - Centre for Palaeogenetics (Sweden), 2 - Centre of New technologies, University of Warsaw, Warsaw (Poland), 3 - Institute of Arctic Biology, University of Alaska Fairbanks (United States), 4 - Faculty of Science and Technology, Bournemouth University (United Kingdom), 5 - Department of Palaeontology, Royal Belgian Institute of Natural Sciences (Belgium), 6 - N.A. Shilo North-East Interdisciplinary Scientific Research Institute, Russian Academy of Sciences (Russia), 7 - LOEWE-Centre for Translational Biodiversity Genomic (Germany), 8 - Science for Life Laboratory (SciLifeLab), Stockholm University (Sweden)

Ancient DNA studies have shown that climatic cycles during the Late Pleistocene likely had a significant effect on population structure and demographic processes in Arctic species. The Eurasian collared lemming (*Dicrostonyx torquatus*) is a keystone species in the Arctic ecosystem and is currently restricted to the tundra of northern Siberia. Earlier studies have indicated that Late Pleistocene climatic fluctuations were important drivers of genetic diversification in the collared lemming. To further investigate this, we analysed 44 ancient and 54 modern mitogenomes from across Eurasia, as well as one modern nuclear genome. Our results suggest that collared lemmings underwent a genetic bottleneck during the Eemian interglacial, implying that warming during this period had severe consequences for this cold-adapted taxon in Eurasia. Furthermore, we find that the population structure identified using complete mitogenomes is largely consistent with previous Cytochrome B studies. Lastly, our results show that the population in northeastern Siberia maintained genetic diversity and a constant population size after the Last Glacial Maximum, suggesting there were more suitable conditions for collared lemmings in this region during the Pleistocene/Holocene transition. This study provides important insights into the impacts of climate on the evolutionary history of collared lemmings and shows that future ancient genomic studies are feasible in non-permafrost small animal remains.

Variations in the stable isotope ratios (d18O, d13C, d15N, d34S) of human bone tissue at the individual scale: is one bone enough?

Clauzel Thibault (1), Richardin Pascale (2, 3), Ricard Jannick (4, 5), Le Béchennec Yves (4, 5), Amiot Romain (1), Fourel François (6), Phouybanhdyt Brian (2, 7), Vinçon-Laugier Arnaud (1), Flandrois Jean-Pierre (8), Lecuyer Christophe (1)

1 - Laboratoire de Géologie de Lyon - Terre, Planètes, Environnement, Lyon (France), 2 - Centre de recherche et de restauration des musées de France (France), 3 - Préhistoire et Technologie (France), 4 - Textes, Représentations, Archéologie, Autorité et Mémoires de l'Antiquité à la Renaissance (France), 5 - Archéologie et Philologie d'Orient et d'Occident (France), 6 - Laboratoire d'Écologie des Hydrosystèmes Naturels et Anthropisés (France), 7 - Laboratoire des Sciences du Climat et de l'Environnement, Gif-sur-Yvette (France), 8 - Laboratoire de Biométrie et Biologie Evolutive - UMR 5558 (France)

The bone tissue is a mix of hydroxy-apatite crystals and organic matrix. While dental tissue grows by accretion and does not renew afterwards, there is a set-up of re-absorption of old bone (both organic and inorganic matrix) and deposition of new bone tissue. At maturity, this mechanism works continuously and the bone is said to «turnover». For a young adult, the total bone tissue is expected to turnover every five to six years on average, therefore changing its stable isotope composition during this interval. Most isotope studies devoted to bone material are realized on one bone because of poor conservation of the material in the field, the lack of articulations between bones or the preciousness of samples. This sampling strategy implies to assume that single point-in-time analyses are representative of the whole individual characteristics. However, if bone turnover is variable at the intra- and inter- individual scale, we can wonder to what extent the isotope study of one bone is representative of the whole skeleton? To investigate this question, various bones from the cephalic, axial and appendicular skeleton of archaeological sedentary human individuals were sampled. The variations in oxygen isotope composition of bone apatite, and carbon, nitrogen and sulfur isotope compositions of bone collagen have been investigated. Our results show that all isotope compositions were homogenous (within the range of analytical uncertainties) for each individual, hence validating the use of one bone as representative of the whole skeleton in the case of stable isotope studies of sedentary archaeological populations.

Characterisation of Bronze and Iron Age Italian Wool Textiles by Palaeoproteomics

Mackie Meaghan (1, 2), Solazzo Caroline (3), Gleba Margarita (4), Olsen Jesper V. (1), Cappellini Enrico (2)

1 - Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Copenhagen (Denmark), 2 - Center for EvoGenomics, The Globe Institute, University of Copenhagen, Copenhagen K (Denmark), 3 - Smithsonian's Museum Conservation Institute, Suitland (United States), 4 - McDonald Institute for Archaeological Research, University of Cambridge, Cambridge (United Kingdom)

Mass spectrometry (MS)-based sequencing of proteins recovered from archaeological contexts (palaeoproteomics) is a rapidly advancing field, allowing archaeologists to better understand people and their activities in the past. For example, with wool textiles (~97% protein), preserved at some archaeological sites, palaeoproteomics can help identify the species that produced the wool, as well as

examine the post translational modifications (PTMs) that have occurred through damage and other processes. We used palaeoproteomics to analyse 12 pre-Roman Italian wool specimens, dated between 16th and 4th century BCE, from seven different archaeological sites. We observe that all the wool was produced from sheep hair, an attribution sometimes challenging based on morphological analysis. Different specimens were made of different quality wool and experienced different preservation conditions. Accordingly, we observe differences in PTMs between samples. Those specimens recovered from frozen deposits, or made of generally thicker wool fibres, generally carry the least biomolecular damage. Reversely, the oldest specimens, those made of generally thinner fibres, and those from the southernmost warmer sites are the most damaged. In addition, evidence of other PTMs associated with UV damage and other degradation products supports previous work that shows there are more protein damage products to be explored in ancient samples than the classical deamidation patterns, which can be highly affected by other factors. These products may help authenticate recovered proteins or give indications of their history. These results are highly valuable for a better understanding of wool production in the past, and for conserving this delicate class of archaeological objects.

The preservation of ancient DNA in archaeological fish bone

Giada Ferrari (1), Angélica Cuevas, Agata T. Gondek-Wyrozemska, Rachel Ballantyne (2), Oliver Kersten, Albina Hulda Pálsdóttir (3, 4), Inge Van Der Jagt (5), Anne Karin Hufthammer (6), Ingrid Ystgaard (7, 8), Stephen Wickler, Gerald F. Bigelow, Jennifer Harland (9), Rebecca Nicholson (10), David Orton (11), Benoît Clavel (12), Sanne Boessenkool (3), James H. Barrett, Bastiaan Star (1)
1. Centre for Ecological and Evolutionary Synthesis, Department of Biosciences, University of Oslo, 2 - McDonald Institute for Archaeological Research, Department of Archaeology, University of Cambridge, 3- University of Oslo, 4 - Agricultural University of Iceland, 5 - Cultural Heritage Agency of the Netherlands, Department of Archaeology, Amersfoort, 6 - The University Museum, Department of Natural History, University of Bergen, Bergen, 7 - Department of Historical and Classical Studies, NTNU, 8 -Department of Archaeology and Cultural History, NTNU University Museum, Trondheim, 9 - Archaeology Institute, University of the Highlands and Islands, Kirkwall, Orkney, 10 - Oxford Archaeology, Oxford, 11- BioArCh, Department of Archaeology, University of York, York, 12 - Muséum national d'histoire naturel de Paris

The field of ancient DNA is dominated by studies focusing on terrestrial vertebrates. This taxonomic bias limits our understanding of endogenous DNA preservation for species with different bone physiology, such as teleost fish. Teleost bone is typically brittle, porous, lightweight, and is characterized by a lack of bone remodeling during growth. All of these factors potentially affect DNA preservation. Using high-throughput shotgun sequencing, we here investigate the preservation of DNA in a range of different bone elements from over 200 archaeological Atlantic cod (*Gadus morhua*) specimens from 38 sites in northern Europe, dating up to 8000 years before present. We observe that the majority of archaeological sites (79%) yield endogenous DNA, with 40% of sites providing samples containing high levels (> 20%). Library preparation success and levels of endogenous DNA depend mainly on excavation site and pre-extraction laboratory treatment. The use of pre-extraction treatments lowers the rate of libraries that can be sequenced, although — if

successful — the fraction of endogenous DNA can be improved by several orders of magnitude. This trade-off between library preparation success and levels of endogenous DNA allows for alternative extraction strategies depending on the requirements of down-stream analyses and research questions. Finally, we do not find particular bone elements to yield higher levels of endogenous DNA, as is the case for denser bones in mammals. Our results highlight the potential of archaeological fish bone as a source for ancient DNA and suggest a possible role of bone remodeling in the preservation of endogenous DNA.