

# **Fourth International Symposium on Biomolecular Archaeology**

Copenhagen, 8<sup>th</sup> – 10<sup>th</sup> September 2010

Conference Programme and Abstracts



## **Organising Institution**

Centre for GeoGenetics, Natural History Museum of Denmark,  
University of Copenhagen

## **Organising Committee**

Maria Avila

Paula Campos

Enrico Cappellini

Tom Gilbert

Ludovic Orlando

Tracey L. Pierre

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Cristina Valdiosera

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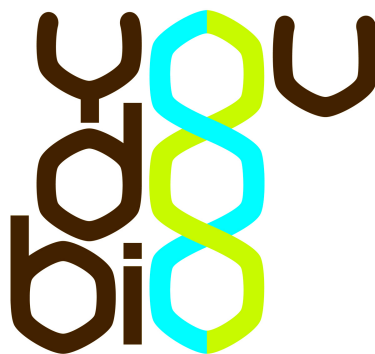


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# Programme

## Tuesday 7<sup>th</sup> September

**16.30 - 19.00** Pre-registration

## Wednesday 8<sup>th</sup> September (single session)

<b>8.00 – 9.00</b>	<b>Registration</b>
9.00 – 9.10	<b>Welcome</b> – Tom Gilbert
9.10 – 9.20	<b>Welcome</b> – Morten Meldgaard, Director of the Natural History Museum of Denmark
9.20 – 9.50	<b>Keynote Speech</b> – Eske Willerslev
<b>9.50 – 11.10</b>	<b>Session 1</b> (moderator: Eske Willerslev)
9.50 – 10.10	<b>Morten Rasmussen</b> - The nuclear genome of an ancient human
10.10 – 10.30	<b>Johannes Krause</b> - What makes us human: Insights from sequencing ancient hominin DNA
10.30 – 10.50	<b>Wolfgang Haak</b> - Ancient DNA from Early Neolithic Farmers suggests a major genetic input from the Near East
10.50 – 11.10	<b>Robert Fleischer</b> - Composition of pharmaceuticals from a 1 <sup>st</sup> century BC/AD Roman shipwreck based on chloroplast DNA sequences
<b>11.10 – 11.30</b>	Coffee Break
<b>11.30 – 13.30</b>	<b>Session 2: New World Archaeology</b> (moderator: Tracey Pierre)
11.30 – 11.50	<b>Cristina Valdiosera</b> - Tracking the genetic origins of the first Americans
11.50 – 12.10	<b>Paula F. Campos</b> - The first Americans, DNA from Pre-Clovis Human Coprolites in Oregon, USA
12.10 – 12.30	<b>Anne Stone</b> - Ancient tuberculosis before and after the Age of Exploration

12.30 – 12.50	<b>Christina Warinner</b> - Life and Death at Teposcolula Yucundaa: Mortuary, Archaeogenetic, and Isotopic Investigations of the Early Colonial Period in Mexico
12.50 – 13.10	<b>Vera Tiesler</b> - Age at death, biological ancestry and provenience of Christopher Columbus' crew at La Isabela, Santo Domingo, (1493-1498). Histological and biomolecular approaches
13.10 – 13.30	<b>Hannes Schroeder</b> - Roots revisited: Identifying source populations in the transatlantic slave trade using ancient DNA
<b>13.30 – 14.30</b>	Lunch (included, at venue)
<b>14.30 – 16.30</b>	<b>Session 3: Scandinavian Archaeology</b> (moderator: Anne Pedersen)
14.30 – 15.10	<b>Keynote Speech - Anne Pedersen</b> - The Vikings - fact and fiction
15.10 – 15.30	<b>Kerstin Lidén</b> - Tracing individual Neolithisation by stable isotope life history analysis in Stone Age Northern Europe
15.30 – 15.50	<b>Bente Philippsen</b> - Ertebølle Cuisine Reconstruction of ingredients and radiocarbon dating of Mesolithic pottery
15.50 – 16.10	<b>Sven Isaksson</b> - Pig-headed foraging – reluctant farming. A study of lipid biomarker distributions in Middle Neolithic ceramic vessel populations from eastern Sweden.
16.10 – 18.00	Coffee break and <b>Poster session</b>

#### Thursday 9<sup>th</sup> September (Parallel Sessions)

**9.00 – 9.40**      **Keynote Speech** – Doug Price - Isotopes and Biomolecular Archaeology

#### Session A

**9.40 – 11.00**      **Session 4a** (moderator: Doug Price)

9.40 – 10.00      **Guido Brandt** - Approaching the living via the dead - an interdisciplinary bioarchaeological study in a LBK population

10.00 – 10.20	<b>Angelina Siebert</b> - “Neither fish nor fowl?” - Significant increase of animal protein in human diet during the Neolithic in Central Germany
10.20 – 10.40	<b>Yaowu Hu</b> - Stable isotopic analysis of bones and teeth of human and fauna in Taojiazhai site, Xining, Qinghai
10.40 – 11.00	<b>Janet Montgomery</b> - “Gleaming, white and deadly”: the use of lead to track human exposure and geographic origins in the Roman period in Britain
<b>11.00 – 11.30</b>	Coffee Break
<b>11.30 – 13.30</b>	<b>Session 5a: Isotopic analysis in archaeology</b> (moderator: Hannes Schroeder)
11.30 – 11.50	<b>Mandy Jay</b> - The Beaker People Project isotope data: the home stretch
11.50 – 12.10	<b>Sarah Groves</b> - Illuminating “Dark Age” Northumbria: Mobility, diet and health in the Bowl Hole Early Medieval cemetery, Bamburgh
12.10 – 12.30	<b>Emilie Blaise</b> - Investigating cattle birth pattern in the late Neolithic in south- eastern France using $\delta^{18}\text{O}$ analysis of tooth enamel
12.30 – 12.50	<b>Kate Britton</b> - Palaeoclimate, palaeoenvironment and herbivore palaeoecology at the Middle Palaeolithic site of Neumark-Nord 2: analysis of tooth enamel phosphate and 120,000 year old bone collagen
12.50 – 13.10	<b>Maura Pellegrini</b> - Variations in the relationship of $\delta^{18}\text{O}_c$ and $\delta^{18}\text{O}_p$ values from enamel increments. Mechanisms of mineralization in modern teeth and/or taphonomic processes
<b>13.30 – 14.30</b>	Lunch (included, at venue)
<b>14.30 – 15.50</b>	<b>Session 6a: Residues</b> (moderator: Oliver Craig)
14.30 – 14.50	<b>Oliver Craig</b> - Food preparation and consumption among late foragers and early farmers in Northern Europe: molecular and isotopic approaches to evaluating continuity and change
14.50 – 15.10	<b>Lucy J. E. Cramp</b> - Detecting marine product processing in prehistory: recent developments using lipid biomarkers and isotopic signatures

15.10 – 15.30	<b>G.C. Kirke</b> - Identifying Chemical Markers for Anomalous Radiocarbon Dates from Surface Organic Residues
15.30 – 15.50	<b>Lucija Šoberl</b> - On the Beaker trail: Investigating the function of British Beakers through organic residue analysis
<b>15.50 – 16.20</b>	Coffee Break
<b>16.20 – 18.00</b>	<b>Session 7a: Compound-specific Isotopes</b> (moderator: Colin Smith)
16.20 – 16.40	<b>Kyungcheol Choy</b> - Investigation of amino acid $\delta^{13}\text{C}$ signatures in bone collagen to reconstruct Korean palaeodiets using liquid chromatography- isotope ratio mass spectrometry
16.40 – 17.00	<b>Noah V. Honch</b> - Identifying the Consumption of Marine Resources in Prehistory: An Investigation of Bone Collagen Amino Acid $\delta^{13}\text{C}$ in Archaeological Humans and Fauna
17.00 – 17.20	<b>Yuichi I. Naito</b> - Palaeodietary reconstruction of prehistoric human populations from Japan using the $\delta^{15}\text{N}$ values of individual bone collagen amino acids
17.20 – 17.40	<b>Karyn C. Olsen</b> - Bulk and compound-specific isotope analysis of pathological bone collagen: Preliminary results
17.40 – 18.00	<b>Amy Styring</b> - Can $\delta^{15}\text{N}$ values of individual amino acids in bone collagen be used as a proxy for manuring in the past?
<b>Session B</b>	
<b>9.40 – 11.00</b>	<b>Session 4b</b> (moderator: Paula F. Campos)
9.40 – 10.00	<b>Michael Bunce</b> - Fossil eggshell: a previously unrecognised substrate for ancient DNA studies
10.00 – 10.20	<b>Charlotte L. Oskam</b> - Ancient DNA from fossil avian eggshell: insights into moa hunter sites in New Zealand
10.20 – 10.40	<b>Cecilia Anderung</b> - Vintage DNA: the use of museum specimens in assessing the impact of modern whaling on sperm whales
10.40 – 11.00	<b>Sanne Boessenkool</b> - Lost in translation or deliberate falsification? Genetic analyses reveal erroneous museum data for historic penguin specimens
<b>11.00 – 11.30</b>	Coffee Break



<b>11.30 – 13.30</b>	<b>Session 5b: DNA damage and contamination</b> (moderator: Ludovic Orlando)
11.30 – 11.50	<b>Tracey L. Pierre</b> - American Southwest Prehistory through ancient DNA
11.50 – 12.10	<b>Nienke L. van Doorn</b> - Exploring collagen and DNA damage in archaeological bone through a non-destructive method in mass spectrometry: the warm-water method
12.10 – 12.30	<b>Eveline Altena</b> - Ancient DNA research without PCR: the power of single molecule sequencing
12.30 – 12.50	<b>Odile M. Loreille</b> - Next generation sequencing of enriched libraries and its potential for ancient fixed human remains
12.50 – 13.10	<b>Helena Malmström</b> - Analysing ancient human DNA - challenges and possibilities
13.10 – 13.30	<b>Ludovic Orlando</b> - Ancient DNA: from damage characterization to repair
<b>13.30 – 14.30</b>	Lunch (included, at venue)
<b>14.30 – 15.50</b>	<b>Session 6b: Domestication</b> (moderator: Greger Larson)
14.30 – 14.50	<b>Melanie Pruvost</b> - Nuclear ancient DNA draws picture of wild and early domesticated horses
14.50 – 15.10	<b>Linus Girdland Flink</b> - The Mediterranean route: analysing early domestic pigs in Southeast Neolithic France by combining Mitochondrial and Nuclear DNA with Geometric Morphometrics
15.10 – 15.30	<b>Ben Krause-Kyora</b> - The flying pig, migration or transfer of ideas in prehistory. Molecular genetic and archaeological investigations of Mesolithic and Neolithic pigs ( <i>Sus scrofa</i> )
15.30 – 15.50	<b>Svensson Emma</b> - Signs of contrasting selection in North European cattle as revealed by single nucleotide polymorphisms
<b>15.50 – 16.20</b>	Coffee Break
<b>16.20 – 18.00</b>	<b>Session 7b: Ecology</b> (moderator: Cristina Valdiosera)
16.20 – 16.40	<b>Love Dalén</b> - Genetic change in the last remaining population of woolly mammoth

16.40 – 17.00	<b>Sophie Champlot</b> - aDNA phylogeography of the wild asses: the demise of a species?
17.00 – 17.20	<b>Tina Jørgensen</b> - Ancient plant DNA as trace fossils in Pleistocene and Holocene sediments
17.20 – 17.40	<b>Peter D. Heintzman</b> - Phylogeography of Ice Age Beringian Beetles: an Ancient DNA Approach
17.40 – 18.00	<b>Laura S. Epp</b> - Biodiversity in the dirt: reliable (mini)barcodes for DNA-based paleo-ecology

### **Friday 10<sup>th</sup> September (Single Session)**

<b>9.00 – 11.00</b>	<b>Session 8: New Directions</b> (moderator: Matthew Collins)
9.00 – 9.20	<b>Sarah L. Fordyce</b> - Ancient Transcriptomics from Archaeological Maize Kernels
9.20 – 9.40	<b>Timothy P. Cleland</b> - Molecular preservation in Mammoth and Mastodon
9.40 – 10.00	<b>Hannah Koon</b> - Scurvy bones: a collagen biomarker to detect vitamin C deficiency in archaeological bone
10.00 – 10.20	<b>Enrico Cappellini</b> - Ancient bone proteomics
10.20 – 10.40	<b>Beatrice Demarchi</b> - Smashing the egg timer: investigating the amino acid clock
10.40 – 11.00	<b>Michael Buckley</b> - ZooMS: What is it and what is it good for?
<b>11.00 – 11.30</b>	Coffee Break
<b>11.30 – 13.30</b>	<b>Session 9</b> (moderator: Terence Brown)
11.30 -11.50	<b>Robin G. Allaby</b> - Recent cotton evolution tracked through archaeogenomics
11.50 – 12.10	<b>Matti Leino</b> - Re-evaluating the history of the wheat domestication gene NAM- B1 using historical plant material
12.10 – 12.30	<b>Terry Brown</b> - Using ancient DNA to contextualise the evolution of maize within the cultural and ritual framework of pre-Columbian South America
12.30 – 12.50	<b>Silvana R. Tridico</b> - Insights into the Analyses of Human and

Animal Hairs in Archaeological, Paleontological and Forensic Contexts

12.50 – 13.10	<b>Matthew Collins</b> - SYNTHESYS II: Improving access to DNA from museum material
13.10 – 13.30	<b>Keri A. Brown</b> - Will ancient DNA analysis ever become routine in archaeology?
<b>13.30 – 14.30</b>	Lunch (included, at venue)
<b>14.30 – 16.30</b>	<b>Session 10</b> (moderator: Tom Gilbert)
14.30 – 15.10	<b>Keynote speech - Mark Thomas</b> - Modelling Biological Evolution's Misunderstood Half Sister, Cultural Evolution
15.10 – 15.30	<b>Greger Larson</b> - Pig domestication, migration, replacement, and hybridization: Preliminary results from the analysis of 60k SNPs
15.30 – 15.50	<b>Astrid M.Z. Schmidt</b> - Ice Core Genetics – Reveals Biodiversity from Ancient Ecosystems in Greenland and Antarctica
15.50 – 16.10	<b>Eline Lorenzen</b> - Combining aDNA and climate niche modeling to elucidate the relative impacts of humans and climate on the demographic trajectories of three Late Pleistocene megafauna taxa
16.10 – 16.30	<b>Ian Barnes</b> - Investigating the impact of Late Pleistocene environmental change using ancient DNA from small mammals
<b>16.30 – 17.00</b>	<b>Closing Session</b>
<b>19.00 – 23.00</b>	<b>Conference Buffet Reception, Carlsberg Akademi</b>

**Saturday 11<sup>th</sup> September**

Optional tourist-based activities (cost not included), guided by conference team. Requires sign up during conference. Examples include: walking tour of Copenhagen, visit to Carlsberg Brewery, Canal boat tour, Christiania!, guided tour of National Museum, guided tour of Ny Carlsberg Glyptotek.



# Oral Presentations

## **Session 1**

Wednesday 8<sup>th</sup> September, 2010

9.50 – 11.10

*Moderator: Eske Willerslev*

## Session 1: 9.50 – 10.10

### The nuclear genome of an ancient human

Morten Rasmussen<sup>1,2\*</sup>, Yingrui Li<sup>2,3\*</sup>, Stinus Lindgreen<sup>1,4\*</sup> *et.al.*<sup>#</sup>

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<sup>2</sup>Sino-Danish Genomics Center, BGI-Shenzhen, Shenzhen 518083, China, and University of Copenhagen, DK-2100 Copenhagen, Denmark.

<sup>3</sup>BGI-Shenzhen, Shenzhen 518083, China.

<sup>4</sup>Department of Biology, University of Copenhagen, Ole Maaloes Vej 5, DK-2200 Copenhagen, Denmark.

\*These authors contributed equally to this work.

<sup>#</sup>For complete author list see, *Nature*, **463**, 757 (2010)

We have sequenced the complete genome from an ancient human. It was obtained from 4,000-year-old permafrost-preserved hair; the genome represents a male individual from the first known culture to settle in Greenland. Sequenced to an average depth of 20, we recover 79% of the diploid genome, and identify 353,151 high-confidence single-nucleotide polymorphisms (SNPs). Comparisons with SNP data from contemporary populations allow us to explore the migrations and kinship of this extinct culture. Analyses provide evidence of a migration from Siberia into the New World, independent of that giving rise to the modern Native Americans and Inuit. The migration was dated to approximately 5,500 years BP and the closest living relatives are found in North-East Siberia showing no signs of admixture with modern Native Americans or Inuit. We use functional SNP assessment to assign possible phenotypic characteristics of the individual.

**Session 1: 10.10 – 10.30**

## What makes us human: Insights from sequencing ancient hominin DNA

Johannes Krause<sup>1,2,3</sup>

<sup>1</sup>Max-Planck Institute for Evolutionary Anthropology, Department Evolutionary Genetics, Leipzig, Germany

<sup>2</sup>Zentrum für Naturwissenschaftliche Archäologie, University of Tübingen, Germany

<sup>3</sup>Institute of Human Genetics, University of Tübingen, Germany

A genetic comparison between modern humans and their extinct relatives could both address the relationship between us and them and offer the possibility to identify genetic changes that happened specifically on the human lineage. Furthermore it may allow identifying and understanding the evolutionary history of genes and positions in the modern human genome that experienced recent positive selection after Neandertals and humans separated and that might play an important role in human evolution. Using a combination of high-throughput DNA sequencing technologies and multiple improvements in ancient DNA retrieval, library construction and targeted library enrichments, our laboratory has recently in collaboration with several groups completed a first version of the Neandertal genome. We furthermore sequenced a number of Pleistocene complete mitochondrial genomes from several different hominin forms. From the analysis of the data we were able to draw conclusion about diversity within and among hominins, determine their genetic relationship and by scanning the human genome for regions of positive selection, we identified several strong candidate genes involved in diet, cognitive traits, and skeletal morphology that were potentially selected on the modern human lineage.



**Session 1: 10.30 – 10.50**

## Ancient DNA from Early Neolithic Farmers suggests a major genetic input from the Near East

Wolfgang Haak<sup>1</sup>, Oleg Balanovsky<sup>2</sup>, Juan J Sanchez<sup>3</sup>, Christina J Adler<sup>1</sup>, Clio S I Der Sarkissian<sup>1</sup>, Guido Brandt<sup>4</sup>, Harald Meller<sup>5</sup>, Kurt W Alt<sup>4</sup>, Alan Cooper<sup>1</sup> & The Genographic Consortium

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<sup>3</sup>National Institute of Toxicology and Forensic Sciences, Canary Islands Delegation, Campus de Ciencias de la Salud, 38320 La Laguna, Tenerife, Spain

<sup>4</sup>Bioarchaeometric Group, Institute of Anthropology, Johannes Gutenberg-University of Mainz, Mainz, Germany

<sup>5</sup>Landesamt für Denkmalpflege und Archäologie und Landesmuseum für Vorgeschichte, Halle, Germany

The Neolithic transition (approx. 8000-4000 BC) is considered one of the most important demographic events in Europe's past since the initial peopling of anatomically modern humans in the Upper Paleolithic (40,000 BC). Whether this transition has been cultural or driven by large-scale population movements is subject of a long-standing scientific debate in archaeology, anthropology and human population genetics. So far, inferences about the genetic make-up of past populations have been drawn from studies of modern-day Eurasian populations, but ancient DNA studies now provide direct snapshots of specific time frames in the past.

We present new mitochondrial and Y-chromosomal data from Neolithic individuals from a Central European early farming site, Derenburg (Germany), which significantly extends the genetic dataset of the *Linearbandkeramik* (LBK; n=42), and provides the first detailed genetic picture of the earliest Neolithic culture in Central Europe (5500-5000 cal BC). Comprehensive population-genetic analyses utilizing a large database of modern-day Western Eurasian populations (n=23,394) reveal unique genetic features of the LBK population and a clearly distinct mitochondrial haplogroup frequency distribution. Importantly however, the LBK population shows an affinity to populations in the modern-day Near East, suggesting a major genetic input from this region at the time of the advent of farming in Europe.

## Composition of pharmaceuticals from a 1<sup>st</sup> century BC/AD Roman shipwreck based on chloroplast DNA sequences

Robert Fleischer<sup>1</sup>, Alain Touwaide<sup>2</sup>, Emanuela Appetiti<sup>2</sup>, Danica Harbaugh<sup>1,2</sup> & John Kress<sup>2</sup>

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<sup>2</sup>Department of Botany, MRC-166, National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, Washington, DC 20013-7012

We report here on chloroplast DNA sequences obtained from multiple extractions and PCR amplifications from two large “pills” obtained from a box of medicines found in a 1<sup>st</sup> Century BC/AD Roman shipwreck with the collaboration of the Soprintendenza per i Beni Archeologici di Firenze, Italy. We conducted the extractions and PCR setups in an isolated, ancient DNA laboratory usually used for animal DNA analysis, and obtained multiple short sequences from each pill. Many of these sequences could be aligned by “blast” comparisons to sequences on Genbank. Some sequences turned out to be artifacts or of bacterial, human or other contaminant origin, but most appropriately matched Angiosperm sequences. In total we found sequences reflecting more than ten different types of plants. We obtained mostly replicated evidence (multiple extractions, PCRs and different gene regions) of *Hibiscus*, *Apium* (celery), a bean, an aster, pepper, *Persea*, alfalfa and other taxa, although in some cases resolution of exact taxa could not be achieved because of short length of sequence reads. Nearly all of the sequences we obtained could not be ruled out as items that would potentially be found in pharmaceuticals from this period and region based on historical documentation.

**Session 2**

New World Archaeology

Wednesday 8<sup>th</sup> September, 2010

11.30 – 13.30

*Moderator: Tracey Pierre*

**Session 2: 11.30 – 11.50**

## **The Genetic Traces of the First Americans: Insights From Ancient DNA**

Cristina Valdiosera<sup>1</sup>, Morten Rasmussen<sup>1</sup>, Gustavo Politis<sup>2</sup>, Josefina Mansilla<sup>3</sup>, Tracey Pierre<sup>1</sup>, Tom Gilbert<sup>1</sup>, Eske Willerslev<sup>1</sup>

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<sup>2</sup>CONICET-INCUBA, Facultad de Ciencias Sociales UNCPBA y Museo de La Plata UNLP; Argentina

<sup>3</sup>Dirección de Antropología Física, Instituto Nacional de Antropología e Historia, México

Evidence based on cranial morphology shows that the earliest known human remains from the Americas (the so called Clovis culture) are most similar to north eastern Asians and thus it has been suggested that the first American populations were derived from northeast Asia after several waves of migration (c. 13ka) and were the direct ancestors of modern Amerindians. However, in the last few years increasing archaeological and genetic evidence have suggested that the processes involved in the colonization of the New World were much more complex. Here, we conduct the first large-scale palaeogenetic investigation of the first Americans and their descendants in order to shed light on the details of the colonization of the New World. This will be carried out by the genetic analyses of ancient human remains from the Americas including the oldest known samples in the Americas (in C14 years BP): The Tuqan Man (~9000), -The Arlington Springs Man (~11 000) in the United States, the oldest Chinchorro mummy (~ 8000) in Arica, Chile, Luzia specimen from Lagoa Santa, Brazil, Arroyo de Frías, from the Pampean region, Argentina (~10 500), plus younger prehispanic material from all these areas, and importantly, the Mexican Pericú – those believed to be the direct descendants of first palaeoamericans. In addition, and for comparison purposes, the complete genome of the Native American Sitting Bull will be sequenced. This dataset will be able to determine 1) the genetic source of these populations and 2) the genetic relationships (if any) between the Clovis, Pericú and contemporaneous palaeo-indian populations.

**Session 2: 11.50 – 12.10**

**The first Americans, DNA from Pre-Clovis Human Coprolites in Oregon, USA**

Paula F Campos<sup>1</sup>, Dennis L Jenkins<sup>2</sup>, Thomas W Stafford<sup>3</sup>, Jr., Morten Rasmussen<sup>1</sup>, M Thomas P Gilbert<sup>1</sup> & Eske Willerslev<sup>1</sup>

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<sup>2</sup>Museum of Natural and Cultural History, 1224 University of Oregon, Eugene, OR 97403-1224, USA

<sup>3</sup>Stafford Research Laboratories, 200 Acadia Avenue, Lafayette, CO 80026, USA

The timing, route and origin of the first human migration into the Americas are still heavily debated. The most widely accepted dates of occupation relate to the Clovis complex, ~11,000 to 10,800 14C years before the present (yr B.P.) (13.2–13.1 to 12.9–12.8 ka), a distinct technology that appears to have originated and spread throughout North America in as little as 200 to 300 years.

However, human mitochondrial DNA recovered from coprolites found at the Paisley 5 Mile Point Caves, in south-central Oregon, suggest human presence as early as 12,300 14C years B.P (coprolites were directly dated by accelerator mass spectrometry). These coprolites are > 1000 14C years older than the accepted dates for the Clovis complex.

Here we present new genetic, archaeological and stratigraphical data that further confirm these results. Nothing is known about the genetic relationship between these Pre-Clovis human remains and the Clovis culture or other modern humans. Our current work is looking at using PEC (primer extension capture) coupled with state-of-the-art high throughput sequencing to target the complete mitochondrial genome of Clovis and Pre-Clovis people in order to help resolve the phylogenetic placement of these first Americans and possibly the timing and route of these migrations.

## Session 2: 12.10 – 12.30

### Ancient tuberculosis before and after the Age of Exploration

Alicia K Wilbur<sup>1,2</sup>, Kelly Harkins<sup>1</sup>, Tessa Campbell<sup>3</sup>, Jane E Buikstra<sup>1</sup> & Anne C Stone<sup>1</sup>

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<sup>3</sup>Department of Archaeology, University of Capetown, South Africa

The Age of Exploration resulted in contact between human populations that were previously isolated from each other, initiating exchange of ideas, cultigens, and diseases. The modern biogeography of tuberculosis (TB) strains appears to reflect this with, for example, the presence of European type strains in the Americas and elsewhere. Until recently, it was thought that TB originated in the Old World in the last 10,000 years and the presence of TB in the Americas prior to contact was debated. Current estimates of TB's origins, however, range from 3-6 million years ago. In our research, we attempt to characterize ancient mycobacterial strains from cases of disseminated bone TB in order to understand the phylogenetic relationships between strains of tuberculosis prior to and after the Age of Exploration. DNA was extracted from over 115 samples exhibiting classic tuberculosis lesions obtained from both the New and Old Worlds and ranging in age from 5800 BCE to A.D. 1800. Then, four quantitative PCR assays were used to gauge the preservation of host and pathogen DNA. Human nuclear and mitochondrial, and mycobacterial repetitive (IS6110) and single copy (rpoB) loci were analyzed. These results show that while approximately one third of the samples contain human nuclear and/or mitochondrial DNA, only 10% were positive for mycobacterial DNA. Mycobacterial DNA was usually recovered in the presence of human DNA (75%). In addition, our results suggest that TB strains in the Americas dating prior to European contact did not contain the IS6110 repeat element. From the samples that tested positive for host and mycobacterial DNA, we first selected two from Peru and one from Canada, for subsequent analyses using high-throughput pyrosequencing. Our analyses indicate that both slow-growing (pathogenic) and fast-growing (environmental) species of mycobacteria are present in the samples. However, our analyses also indicate that new methods for targeting specific sequences of interest are necessary to obtain sufficient genome coverage for evolutionary analyses. We will discuss ways of doing this and our current progress in this effort.

**Session 2: 12.30 – 12.50**

## **Life and Death at Teposcolula Yucundaa: Mortuary, Archaeogenetic, and Isotopic Investigations of the Early Colonial Period in Mexico**

Christina Warinner<sup>1</sup>, Nelly Robles Garcia<sup>2</sup>, Ronald Spores<sup>3</sup> & Noreen Tuross<sup>4</sup>

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Two mid-16<sup>th</sup> century cemeteries are investigated at the Mixtec site of Teposcolula Yucundaa and shown to be related to the unidentified pandemic of 1545-1550. Through archaeogenetic and oxygen stable isotope analysis it is shown that the interred individuals are local Mixtecs, and mortuary analysis sheds light on both Christian and traditional religious practices at the site. Mitochondrial haplogroup frequencies do not support population bottlenecking during the 16<sup>th</sup> century epidemic period, and carbon and nitrogen stable isotope analysis does not support a shift away from maize consumption, despite evidence for increased wheat production at the site.

**Session 2: 12.50 – 13.10**

Age at death, biological ancestry and provenience of Christopher Columbus' crew at La Isabela, Santo Domingo, (1493-1498).

Histological and biomolecular approaches

Vera Tiesler<sup>1</sup>, Andrea Cucina<sup>1</sup>, T Douglas Price<sup>2</sup>, James Burton<sup>2</sup> & Hannes Schroeder<sup>3</sup>

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The site of La Isabela, in the Dominican Republic, was the first colonial town in the Americas. It was settled by Christopher Columbus and his crew at the beginning of AD 1494, and initially housed some 1,500 individuals from a wide array of social, economic and probably ethnic backgrounds. Its graveyard quickly accumulated the mortal remains of those who succumbed to the harsh conditions of the Atlantic crossing and life in the colony. In this study we present the preliminary results of a series of histological and molecular (isotopic and DNA) studies that expand on the macroscopic skeletal information in combination with detailed historical records on the lives of the deceased. Considered jointly, the data sets provide deepened insights into age at death, disease, nutrition, biological ancestry and geographic origins of 49 individuals unearthed between 1983 and 1991 and currently stored at the Museo del Hombre Dominicano in Santo Domingo, Dominican Republic. The analyses were largely funded by the Universidad Autónoma de Yucatan, Merida, Mexico, and National Geographic Society, Washington D.C., US, and received logistical support from the Museo del Hombre Dominicano, Dominican Republic.



## Session 2: 13.10 – 13.30

### Roots revisited: Identifying source populations in the transatlantic slave trade using ancient DNA

Hannes Schroeder<sup>1</sup>, Pia Bennike<sup>2</sup>, Jay Haviser<sup>3</sup>, Fatimah Jackson<sup>4</sup>, Kate Robson-Brown<sup>5</sup>, Kristrina Shuler<sup>6</sup>, Eske Willerslev<sup>1</sup> & Tom Gilbert<sup>1</sup>

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Between the sixteenth and nineteenth centuries, approximately twelve million Africans were kidnapped from Africa and transported to the Americas. This process caused immeasurable human suffering and led to the loss of significant aspects of African history, identity, and culture for succeeding generations of Africans in the diaspora. European shipping records and other documents provide useful information regarding the volume and structure of the slave trade but, unfortunately, they tend to reveal relatively little about the slaves' actual geographic or ethnic origins in Africa. DNA analyses provide an alternative way to address this issue. Lineage based analyses have been widely used to infer the genetic ancestry of present-day African Americans but these analyses are complicated by non-African admixture and gene-flow between various African groups in the New World. This is why the genetic analysis of archaeological remains like those found at the African Burial Ground in New York is of immense interest. Here we report the initial findings of an EU-funded project that employs ancient DNA analyses to trace the biogeographical origins of enslaved Africans who were brought to the Americas with the slave trade.

**Session 3**

Scandinavian Archaeology

Wednesday 8<sup>th</sup> September, 2010

14.30 – 16.30

*Moderator: Anne Pedersen*

**Session 3: 15.10 – 15.30**

## Tracing individual Neolithisation by stable isotope life history analysis in Stone Age Northern Europe

Kerstin Lidén<sup>1</sup> & Gunilla Eriksson<sup>1</sup>

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Recent years have seen developing theories and methods moving beyond the view of groups and populations as the smallest unit of analysis of prehistoric people, putting the individual at the centre of attention. Especially within the realm of burial archaeology, there has been an expansion of data concerning individual life biographies. The possibility to trace individual life history is, however, not limited to carefully excavated, neatly preserved, single burials with articulate skeletal remains. Even collective burials, disturbed graves, disarticulated human remains in cultural layers, or other depositions that deviate from what we as archaeologists often consider as “proper burials”, offer the possibility to look at individual life history. We will present here how stable isotope analysis of bone and teeth can do just that – what kind of requirements need to be fulfilled in order for it to work, and what the constraints are. We are concerned here only with carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope analysis of bone and dentine collagen from humans. Summarizing intra-individual data for some 125 Mesolithic and Neolithic individuals, with a geographical focus on present-day Sweden and Latvia, we will demonstrate how both the sources and various kinds of variation one is likely to find, and how the data can be explained and transformed into an archaeologically meaningful interpretation in our specific case, the Neolithisation process.

### Session 3: 15.30 – 15.50

## Ertebølle Cuisine - Reconstruction of ingredients and radiocarbon dating of Mesolithic pottery

Bente Philippsen<sup>1</sup>

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Pottery was a remarkable innovation for the Terminal Mesolithic Ertebølle society of southern Scandinavia: Boiling in vessels over direct heat made food resources available that otherwise are indigestive, while preserving all nutrients in the liquid. Did this innovation occur contemporaneously throughout northern Germany, or were inland groups some hundreds of years ahead of those at the coast? Reliable dating is an important precondition for identifying the origin of pottery, as it has to be known which other cultural groups were contemporaneous with the Ertebølle culture.

<sup>14</sup>C-dating of pottery uses charred food remains (“food crusts”) that are frequently found on Ertebølle pottery. However, crusts made of freshwater food can result in radiocarbon ages that are too high. Freshwater systems with hard water, a high content of dissolved minerals, contain considerable amounts of “<sup>14</sup>C-dead” carbon. Those minerals originate from carbonate rocks, having infinite ages compared to the <sup>14</sup>C time scale. This effect is called the “hardwater effect”.

Thus the question of what Ertebølle people cooked in their pots is not only interesting in itself, but also helps in finding out whether the radiocarbon dating is affected by the hardwater effect. I used the stable isotopes of carbon and nitrogen, <sup>13</sup>C and <sup>15</sup>N, to identify the foodstuffs that formed the crust. Experiments with copies of stone age vessels were conducted to test the reliability of the method: Was it possible to retrieve the recipe of a food crust by stable isotope measurements? Is a fish’s reservoir age the same as that of the food crust made from this fish?

In addition to recent materials, I examined archaeological remains from the two inland sites Kayhude and Schlamersdorf, where the presumably oldest Ertebølle pottery had been found. The fact that this pottery was much older than pottery from coastal Ertebølle sites, combined with the sites being located close to freshwater rivers, aroused suspicion: Is the oldest Ertebølle pottery really that old? Measurements of food crusts, terrestrial and fluvial samples from the same context gave the answer.

### Session 3: 15.50 – 16.10

Pig-headed foraging – reluctant farming. A study of lipid biomarker distributions in Middle Neolithic ceramic vessel populations from eastern Sweden.

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Agriculture was introduced quite rapidly in eastern Sweden around 4000 BC, but it took it some 2000 years to be generally recognized as the appropriate way of life. This ambiguity of lifestyle resulted in a cultural complexity evident through the both qualitative and spatial disparity of the material remains from the Middle Neolithic. The basic outlines usually given are that people living in the interior parts of Scandinavia practiced agriculture, while foraging was still the standard of living along the coasts. What this diversity actually represents is still debated, however; separate ethnic or cultural groups or not, ritual or economic specializations of the same group, etc. The interpretations provided are still to a great extent caught in reproducing conventional concepts of archaeological culture, untouched by recent advances made in fields like Material Culture Studies, Innovation Research and Cultural Evolution Studies. Analyses of lipid residues from prehistoric pottery have the potential of providing evidence necessary to address a number of questions along these lines of enquiry, and also to pull out new data from old materials. Embedded as it is in the Culinary Arts, Pottery Use can tell of more than only diet. In this study the frequencies of biomarkers, for terrestrial and aquatic animals, vegetables, and of heating events, in lipid residues from well-defined vessel populations were investigated. The results show a great difference in Pottery Use between early farmers and coastal foragers even though the foragers pottery is stylistically and technologically derived from the former. The discovery of this inconsistency allows a discussion in terms of innovation resistance. The cultural evolution of the foragers Pottery Use indicates a strong vertical social transmission of knowledge, a connected tradition. The directionality is however towards agriculture as the tradition is connected to Late Neolithic and Bronze Age Pottery Use without any clear break. On a highly general level this study also find chronological co-variation between marine/terrestrial signals in pots and diet during the Neolithic and the Bronze Age, possibly providing some support for the utility of the lipid biomarker approach applied in this study.

**Session 4A**

Thursday 9<sup>th</sup> September, 2010

9.40 – 11.00

*Moderator: Douglas Price*

## Approaching the living via the dead - an interdisciplinary bioarchaeological study in a LBK population

Guido Brandt<sup>1</sup>, Corina Knipper<sup>1</sup>, Nicole Nicklisch<sup>1</sup>, Angelina Siebert<sup>1</sup>, Vicky Oelze<sup>2</sup>, Robert Ganslmeier<sup>3</sup>, Wolfgang Haak<sup>4</sup>, Harald Meller<sup>3</sup> & Kurt W Alt<sup>1</sup>

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On the site of the Karsdorf LBK settlement (Saxony-Anhalt, Germany) dating to 5240-5000 BC, 30 burials were associated with 24 longhouses. The results of the archaeological, anthropological, biogeochemical, and molecular genetic analyses point to high fluctuations of individuals spanning time and generations. The large variability of mtDNA maternal lines and the scant evidence for kinship relations among adjacent individuals let one conclude that the buried individuals do not represent families. Analyses concerning human mobility point to patrilocal residential rules. The non-local individuals, as identified by Sr isotope analyses, are not restricted to the oldest features within the settlement, but give evidence for long-lasting contacts to neighbouring communities. With one exception, non-local individuals were found near all the houses and show that the place of origin of a person was irrelevant for its burial in association to a house. Furthermore, there is no apparent correlation between the sparse grave goods and the position and orientation of the burials. Moreover, C and N isotope analyses demonstrate a homogenous diet of the Karsdorf community with low proportions of animal derived protein. These multiple lines of evidence depict a dynamic and mobile settlement community in Neolithic Central Germany.

## **“Neither fish nor fowl?” - Significant increase of animal protein in human diet during the Neolithic in Central Germany**

Angelina Siebert<sup>1</sup>, Viktoria M Oelze<sup>2</sup>, Nina Ulrich<sup>1</sup>, Nicole Nicklisch<sup>1</sup>, Robert Ganslmeier<sup>3</sup> & Kurt W Alt<sup>1</sup>

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The most significant historic change in human diet was probably the transition from hunting and gathering to sedentary farming, which started in Central Germany at about 5.500 BC. Many studies have focused on this specific Meso-Neolithic change in diet in various geographical areas, but only few took a closer look on the developments within the Neolithic. Acting on the assumption that the adoption of this completely novel lifestyle was a long and laborious path of experimentation phases, rebounds and learning processes, one must expect that husbandry and farming strategies were subjected to a specific development dependent on experience and ecological resources of the Neolithic populations. Even if corn is regarded to be the major basis of Neolithic foodstuff supply, the influence of animal products in human diet should not be underestimated. Our study focuses on the impact of animal protein, whether from meat or dairy products, in human diet during 3.500 years of agricultural history. Stable isotope analysis of carbon and nitrogen from bone collagen has been carried out on 258 human bone samples from all over Saxony-Anhalt, covering archaeological sites of the early, middle and late Neolithic periods. The results of the  $\delta^{15}\text{N}$  investigation show a significant increase of animal protein in human food consumption, which correlates with a chronological sequence of archaeological cultures. While animal products did not seem to play a major role in human diet in the Early Neolithic, the importance of meat and dairy products increases during the Middle Neolithic and reaches its highest level in populations of the Late Neolithic and Early Bronze Age. These developments may be a result of various factors, e.g. a growing stabilization of the supply of animal products due to sophisticated husbandry practices, a shift of farming focuses as well as climatic factors or sociocultural developments. Our aim is to provide further insight into human food consumption and husbandry strategies and to determine when and for what reasons people started to intensify livestock breeding and dairy farming.



**Session 4A: 10.20 – 10.40**

**Stable isotopic analysis of bones and teeth of human and fauna  
in Taojiazhai site, Xining, Qinghai**

Yaowu Hu<sup>1,2</sup>, Jinglei Zhang<sup>3</sup>, Ying Ma<sup>4</sup>, Yi Guo<sup>1,2,4</sup>, Hong Zhu<sup>5</sup> & Changsui Wang<sup>1,2</sup>

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Qiang Minority is one of important ethnics who played a great role in the formation of Chinese in Chinese history. Archaeological, anthropological and aDNA evidences show that Taojiazhai site, Xining, Qinghai, was occupied by Qiang Minority as well as some people from central China from East Han (25-220AD) to Weijin Period (220AD-420AD). To investigate the dietary changes and interactions between the two ethnics, stable isotopic analysis of bones and teeth of humans and fauna were undertaken. The isotopic values of animals, including mouse, sheep, horse, cattle, pig and dog, lay a great foundation to reconstruct the isotopic baseline in that site. The carbon isotope values of most of individuals indicated the diets mixed with C3 and C4 foods, which is quite similar to the animals, such as sheep, cattle, pig and dog. Two individuals have much higher carbon isotopic values, strongly indicative of that they were immigrants and might come from central China. Quite high nitrogen isotopic values are observed in all individuals compared to those of the animals, suggesting that humans had consumed quite large proportion of animal protein. In addition, the isotopic differences among individuals.

## “Gleaming, white and deadly”: the use of lead to track human exposure and geographic origins in the Roman period in Britain

Janet Montgomery<sup>1</sup>, Jane Evans<sup>2</sup>, Simon Chenery<sup>3</sup>, Vanessa Pashley<sup>2</sup>, Kristina Killgrove<sup>4</sup> & Julia Beaumont<sup>1</sup>

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When one reads of lead it is rarely long before the Roman world is mentioned. Their stories are closely intertwined in a tale of necessity, progress, and the comforts and “enticing vices” of civilization (Tacitus, *Agricola* 21) that has left the world a deadly legacy. The attractions of lead are manifold: it is cheap, malleable, waterproof, simple to smelt and its compounds improve the qualities of paints, pigments, make-up and sweeten and whiten foodstuffs. But lead is toxic and deleterious to calcium metabolism and the nervous, digestive and reproductive systems. The dysfunctional society created by a people poisoned by smelters, piped water, lead cooking vessels, cosmetics and wine sweetened and preserved with sapa, defrutum and sugar of lead (lead acetate) has been proposed, and just as strongly refuted, as a contributory factor in the fall of the Roman Empire.

Building on the initial study of Molleson et al. (1986), this paper presents lead isotope and concentration data obtained from British burials dating from the Neolithic through to the 19<sup>th</sup> century AD to investigate the change in human lead exposure using enamel lead concentrations and fluctuating lead isotope ratios in response to large-scale lead production in the Roman period. The data show that throughout prehistory, individuals had uniformly low concentrations coupled with highly variable lead isotope ratios derived from country rocks. In the Roman period, human lead isotope ratios converge within the English ore lead field as a result of exposure to anthropogenic lead and this is accompanied by a rise in their lead levels. Although this uniformity makes it difficult to use lead to discriminate between British individuals in this period, people exposed to non-British sources are much easier to identify. In the Roman period, therefore, lead isotope analysis provides a highly effective discriminant where strontium and oxygen isotope ratios do not. To illustrate this, we use comparative data for burials from Imperial Rome, to propose that an exceptional burial of a young woman from Spitalfields in London was not of British origin but consistent with inhabitants of Rome.

**Session 4B**

Thursday 9<sup>th</sup> September, 2010

9.40 – 11.00

*Moderator: Paula Campos*

## Fossil eggshell: a previously unrecognised substrate for ancient DNA studies

Michael Bunce<sup>1</sup>, Charlotte Oskam<sup>1</sup>, James Haile<sup>1,2</sup>, Morten Allentoft<sup>1,3</sup>, Richard N Holdaway<sup>3</sup>, Tom Gilbert<sup>2</sup>, Eske Willerslev<sup>2</sup> & Chris Jacomb<sup>4</sup>

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Due to exceptional biomolecule preservation fossil eggshells have been used extensively to reconstruct palaeoecology, palaeodiet, and they also serve as an exceptional medium for a variety of dating methods, including radiocarbon, amino acid racemization and uranium-series disequilibrium. Despite the widespread use of eggshell in both paleontological and archeological applications it has been a widespread belief for a number of years that there is no ancient DNA preserved within the calcite matrix of fossil eggshells. Our data show when careful attention is paid to the DNA isolation method, fossil eggshell is actually a very rich source of ancient DNA (Oskam *et al.*, Proc. B., 2010). We speculate that previous attempts to recover DNA from fossil eggshell have failed primarily due to the fact that inappropriate DNA isolation chemistry was used.

Using confocal imaging we are able to image DNA in fossil eggshell and significantly, were able to isolate both mitochondrial and nuclear DNA from a variety of extinct avian taxa, such as New Zealand's moa and the Madagascan elephant bird. DNA from eggshell up to 19,000 years old was recovered from environments not usually conducive to long term DNA preservation (e.g. Australia and Madagascar). Quantitative PCR experiments also demonstrate that moa eggshell has approximately 125 times lower bacterial load when compared with bone, making it a highly suitable substrate for high-throughput sequencing approaches. Taken together, these data indicate that fossil eggshell preserves a cache of relatively "pure" DNA that can survive in a number of hostile environments. The ability to isolate and amplify DNA from fossil eggshell will, when combined with stable isotope data and dating methods, provide a powerful tool in a number of paleontological and archaeological applications.

**Session 4B: 10.00 – 10.20**

## Ancient DNA from fossil avian eggshell: insights into moa hunter sites in New Zealand

Charlotte L. Oskam<sup>1</sup>, Chris Jacomb<sup>2</sup>, Richard Walter<sup>2</sup>, Morten E Allentoft<sup>1,3</sup>, Richard N Holdaway<sup>3</sup> & Michael Bunce<sup>1</sup>

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Avian eggshells are frequently described in palaeontological and archaeological deposits from across the globe. Their resilient nature slows destructive taphonomic processes, which is why eggshell has been extensively utilised as an accurate substrate for studying palaeoecology and palaeodiets, as well as for geochronology. When humans arrived in New Zealand ~700 years ago, large flightless herbivorous birds, including the moa (Ave: Dinornithiformes), soon became a primary food source. Many of New Zealand's flightless birds and their eggs were consumed by the early Polynesian inhabitants, as evidenced by the vast quantities of moa bone and eggshell remains found within archaeological middens. Unfortunately, the moa became extinct within only a century or so.

Although species identification of intact bones is reasonably robust, identification of eggshell is more problematic. Eggs are almost universally found as fragments, and there is little morphological variation between moa species. Lack of uniformity on eggshell thickness also precludes species Identity. Our recent work, which describes the isolation of both mitochondrial and nuclear DNA from fossil eggshell, provides a means to investigate both the species composition and timing of moa eggshell deposition in moa hunter sites across the country.

Wairau Bar located at the northern end of the South Island is New Zealand's most renowned archaeological site. Dating evidence suggests Wairau Bar was occupied during the late 13<sup>th</sup> - 14<sup>th</sup> centuries. Moa and their eggs were consumed here in considerable numbers. This presentation will showcase how mtDNA sequences and STR profiling of fossil avian eggshell collected from Wairau Bar middens can provide valuable insights into both hunting practices and extinction processes.

## Vintage DNA: the use of museum specimens in assessing the impact of modern whaling on sperm whales

Cecilia Anderung<sup>1</sup>, Adrian Glover<sup>2</sup>, Richard Sabin<sup>2</sup> Victoria Nardell<sup>2</sup> & Thomas G Dahlgren<sup>3,4</sup>

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Whaling in the 18th - 20th centuries reduced the numbers of whales and it remains unclear to what extent whale populations have recovered since the worldwide pause of all commercial whaling activities in the 80s. Industrial whaling can be roughly divided into two periods, an early phase starting around 1750 in the North Atlantic, and the more modern industrialised whaling from the beginning of the 1900s that applied new technological developments. The modern style of whaling spread quickly from the North Atlantic to the Antarctic and into the South Pacific. Large scale harvesting continued in this manner until 1986 when growing concerns regarding the long term sustainability of the hunt led to a moratorium.

The sperm whale (*Physeter macrocephalus*) is the largest species of toothed whale, and is highly mobile and globally distributed throughout the oceans. They were extensively hunted in two phases of industrial whaling, initially in the 18th and 19th centuries, after which whaling between 1880 and 1946 focused on the larger and more valuable blue whale thanks to technical developments. The subsequent decline of blue whales led to the beginning of the second phase of sperm whale hunting in the 1950s. Over the last decade the population structure in sperm whales has been investigated using DNA from the current post-whaling population. The general trend that has been found is a low mtDNA genetic diversity on a global scale and the results so far suggest a low differentiation between ocean basins and little subdivision within ocean basins. We will present preliminary results from a project we have initiated in which we are investigating how the sperm whale population has been affected by past industrial whaling, by extracting and analysing DNA from ancient and historical specimens of sperm whales kept in museum collections. This type of vintage DNA will give us a unique opportunity to directly assess what genetic diversity was removed. In so doing, we will provide a historical baseline pivotal to understanding the present status of sperm whales.

**Session 4B: 10.40 – 11.00**

**Lost in translation or deliberate falsification? Genetic analyses reveal erroneous museum data for historic penguin specimens**

Sanne Boessenkool<sup>1,2</sup>, Bastiaan Star<sup>3</sup>, R Paul Scofield<sup>4</sup>, Phillip Seddon<sup>1</sup> & Jonathan Waters<sup>1</sup>

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Historic museum specimens are increasingly used to answer a wide variety of questions in scientific research. The potential problems of working with low quality DNA are well known, but possible errors in specimen data are potentially much more complex and have received little attention in the literature. Here we use individual-based genetic analyses to demonstrate erroneous locality information for archive specimens from the late nineteenth century. Using 10 microsatellite markers, we analysed 350 contemporary and 43 historic yellow-eyed penguin (*Megadyptes antipodes*) specimens from New Zealand's South Island and sub-Antarctic regions. Factorial correspondence analysis and an assignment test strongly suggest that eight of the historic specimens purportedly of sub-Antarctic origin were in fact collected from the South Island. Interestingly, all eight specimens were obtained by the same collector, and all are currently held in the same museum collection. This study highlights a promising extension to the well-known applications of assignment tests in molecular ecology, which can complement methods that are currently being applied for error detection in specimen data. Our results also serve as a warning to all who use archive specimens to invest time in the verification of collection information.

**Session 5A**

Isotopic Analysis in Archaeology

Thursday 9<sup>th</sup> September, 2010

11.30 – 13.30

*Moderator: Hannes Schroeder*



## **The Beaker People Project isotope data: the home stretch**

Mandy Jay<sup>1,2</sup>, Janet Montgomery<sup>3</sup>, Maura Pellegrini<sup>3</sup>, Olaf Nehlich<sup>2</sup>, Mike Richards<sup>1,2,4</sup>, Mike Parker Pearson<sup>5</sup> & Jane Evans<sup>6</sup>

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The Beaker People Project has been a five year multi-disciplinary and -institutional project including the isotopic analysis of skeletal material from 250 Late Neolithic and Early Bronze Age burials from across the UK. The organic collagen analyses have been done on both bone and dentine ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$ ) and tooth enamel has been analysed for  $^{87}\text{Sr}/^{86}\text{Sr}$  and  $\delta^{18}\text{O}$ . This allows a multi-isotopic approach, as well as a comparison of tissues from different formation periods during an individual's life. The research has been undertaken on burials from the north of Scotland down to the south of England, giving results from material from a range of UK geological and environmental backgrounds and providing a database of around 2000 analyses. The aim has been to investigate mobility during the 'Beaker period' in Britain, alongside aspects of diet, environment and subsistence practices.

The analyses for this project are now complete and interpretation and publication is in process. A short conference presentation is not sufficient for a full presentation of the results, but this paper is intended to give a flavour of the overall isotopic data-set, some of the major conclusions coming out of the project from this database, and the ways in which the data will become accessible.

## Illuminating “Dark Age” Northumbria: Mobility, diet and health in the Bowl Hole Early Medieval cemetery, Bamburgh

Sarah Groves<sup>1</sup>, Charlotte Roberts<sup>1</sup>, Graham Pearson<sup>2</sup>, Geoff Nowell<sup>2</sup>, Colin McPherson<sup>2</sup> & Darren Grocke<sup>2</sup>

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The Bowl Hole cemetery is a well-preserved 7<sup>th</sup> to 8<sup>th</sup> century AD burial ground associated with the royal fortress at Bamburgh, the seat of the kings of Northumbria. To date almost 100 individuals have been excavated including males, females and children of all ages, providing the opportunity to examine a population in the context of a well documented royal site. The skeletal sample is the focus of an AHRC funded project using a range of osteological, archaeological, historical and biomolecular analytical techniques to answer questions about cultural contact, population movement, social status, health and religious change in Early Medieval Northern England.

Analysis of strontium and oxygen isotopes from tooth enamel from 84 individuals suggests that a substantial proportion of the people buried in the cemetery did not spend their childhood in Northumbria, and several individuals may have originated outside of the British Isles. Differences in health status were identified between “local” and “non-local” individuals suggesting that childhood origin may have had an impact upon subsequent quality of life. Stable isotope analysis, together with archaeological data provides evidence for a distinctive diet, and variation within the population indicates that access to certain foods was probably influenced by social and cultural factors.

By applying a multidisciplinary approach to the study of the skeletal population from Bamburgh it is possible to enhance our understanding of a region which might be considered to be on the periphery of the Early Medieval world. The findings of this project emphasise the importance of Bamburgh as a regional centre and give an insight into health, diet, burial practice and identity at this unique site.

**Session 5A: 12.10 – 12.30**

**Investigating cattle birth pattern in the late Neolithic in south-eastern France using  $\delta^{18}\text{O}$  analysis of tooth enamel**

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Primitive breeds of cattle raised extensively tend to give birth seasonally, at the time of the year of greatest food availability. In the western Mediterranean area, nowadays, calving takes place mostly between late winter and early summer, with a peak in spring (March-April). It is likely that cattle births were seasonal in the late Neolithic in south-eastern France. However, birth pattern might have changed according to husbandry practices and environmental conditions. The period of births, their spread and distribution can be defined more precisely by sequential analysis of tooth enamel  $\delta^{18}\text{O}$ .

A modern data set was realised from a population of a primitive breed of cattle raised in semi-feral conditions with no control of breeding in Camargue (south-eastern France). The herd was slaughtered in 2005 and skeletons were reserved for osteological and stable isotopes studies. Animals were born in different seasons, mainly between January and June, sometimes in fall. The lower third molar (M3) was sampled from individuals born in spring and in late summer to early fall. This is the first reference data set for cattle with two seasons of birth. The protocol was then applied to cattle teeth from La Citadelle in south-eastern France (Vauvenargues, Bouches-du-Rhône). The site was seasonally occupied in the first half of the third millennium BC. The results indicate that the seven archaeological specimens were born in the same period of the year. However births were not tightly grouped. The modern reference set is then used to determine the season of births.

Birth season is a central parameter of pastoral economies determining the rhythm of breeding activities and the availability of animal resources, including milk. At La Citadelle, cattle milk was heavily exploited, as indicated, in the mortality profile, by a good representation of young (5-9 months) and cows maintained to advanced ages. Defining the seasonality and season of calving helps in assessing the availability of milk for human consumption.

Palaeoclimate, palaeoenvironment and herbivore palaeoecology  
at the Middle Palaeolithic site of Neumark-Nord 2: analysis of  
tooth enamel phosphate and 120,000 year old bone collagen

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Here we present stable isotope data from horse (*Equus* sp.) and other herbivores from the Middle Palaeolithic site of Neumark-Nord 2. With a rich archaeological and palaeofaunal assemblage, this site offers a great opportunity to investigate the local palaeoenvironmental conditions in central Europe during the Eemian (MIS 5e) and early Weichselian. Deposition at the site – a very shallow pool – was rapid, and favourable local taphonomic conditions have resulted in a well-stratified, well-preserved assemblage of anthropogenically-modified faunal remains.

Horse teeth were sampled throughout the sequence and tooth enamel was analysed for its phosphate oxygen isotope composition ( $\delta^{18}\text{O}_{\text{PO}_4}$ ). Data from bulk and intra-tooth samples provides palaeoclimatic and palaeoseasonal information, revealing local terrestrial (early) Eemian and Weichselian conditions and fluctuations. The  $\delta^{18}\text{O}_{\text{PO}_4}$  data will be discussed in the context of the contemporary global climatic record and other local proxy evidence.

In addition to the horse enamel, bone collagen was extracted from a number of large herbivorous mammalian taxa from the Eemian deposits and analysed for its carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope composition, meeting published quality control criteria. The herbivore data indicates clear inter-specific differences, notably between the horses and bovids. The possible reasons for these differences and the implications for our understanding of the palaeoecology of these species will be discussed.

**Session 5A: 12.50 – 13.10**

Variations in the relationship of  $\delta^{18}\text{O}_c$  and  $\delta^{18}\text{O}_p$  values from enamel increments. Mechanisms of mineralization in modern teeth and/or taphonomic processes.

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The comparison between oxygen isotope ratios in the carbonate and phosphate fractions of bioapatite has been proposed and applied as an internal check for diagenesis because oxygen in the two compartments is believed to form simultaneously. It has become clear, however, that this relationship is quite variable. For some time this variability was thought to be induced by “species” effects, although it has never been clear what these might be.

Two studies comparing  $\delta^{18}\text{O}_c$  and  $\delta^{18}\text{O}_p$  in incrementally sampled enamel crowns show that  $\delta^{18}\text{O}_c$  values are attenuated compared to the  $\delta^{18}\text{O}_p$  values in their seasonal profiles. One of these studies used the M1 and still maturing M2 in just one modern animal (a rhino). The other, currently under publication, observed the same phenomenon in enamel crown of eight equids and deer teeth from five different Upper Paleolithic sites in peninsular Italy, encompassing a wide area with different geological units. The observation is surprising if the bioapatite mineral precipitates both fractions from body water simultaneously.

In order to distinguish whether diagenesis is implicated, or whether there are indeed differences in the timing or mode of amelogenesis patterning between phosphate and carbonate, we determined seasonal  $\delta^{18}\text{O}$  variations of both compartments for the incrementally sampled tooth crowns of modern horse and Cape buffalo individuals.

Data suggest that the modern tooth sequences retain an attenuation of  $\delta^{18}\text{O}_c$  through seasonal differences, but the difference is often slighter than in the archaeological specimens. The results may go some way in explaining the size of the envelope amongst different animals in the  $\delta^{18}\text{O}_c/\delta^{18}\text{O}_p$  relationship.

## Oxygen isotopes in enamel as indicators of geographical origin in humans and animals: limitations and potential

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Increasing numbers of publications are using oxygen isotope analysis of archaeological tooth enamel to establish whether the area in which an individual human or animal was buried could also have been where it lived in during the time of its enamel formation. Where this is not the case attempts are sometimes made to infer the alternative geographical location of the individual's early life. However, inferring a locality's characteristic enamel oxygen isotope composition has proved to be far from straightforward. The following assumptions usually have to be made:

- (a) we have a good characterisation of the composition of local precipitation
- (b) the local precipitation is the primary source of drinking water ( $\delta^{18}\text{O}_{\text{dw}}$ ),
- (c) any seasonal effects can be accounted for, and
- (d) there has been no significant change thorough time.

Given these, an empirically-established species-specific calibration can be used to relate  $\delta^{18}\text{O}_{\text{dw}}$  to the composition of the enamel phosphate ( $\delta^{18}\text{O}_{\text{p}}$ ) expected in a particular species at a specific location. If enamel carbonate is analysed, then fractionation between phosphate and carbonate must also be allowed for.

There are many steps in this procedure, each involving the use of quantities which are uncertain. Thus care must be taken not to underestimate the uncertainties and any equations used must reflect current theoretical understanding of the processes. I will consider how these issues limit the precision with which we can compare our measurements on archaeological enamel samples with the oxygen isotope composition of precipitation, using examples from my recent work. Careful statistical analysis is needed of modern datasets relating  $\delta^{18}\text{O}_{\text{dw}}$  to  $\delta^{18}\text{O}_{\text{p}}$  for each species. However the published studies have limited sample sizes, so I will outline the potential of statistical methods for creating a calibration which 'draw strength' by incorporating data from several species or from tissues other than enamel. In the longer term, I will argue, we need to recognise the available datasets are inadequate to answer many archaeological questions about geographical provenance, and so more empirical data needs to be acquired. Our current state of knowledge does suggest that more data could bring significant improvements, but some fundamental limitations will remain.

**Session 5B**

DNA Damage and Contamination

Thursday 9<sup>th</sup> September, 2010

11.30 – 13.30

*Moderator: Ludovic Orlando*

## **American Southwest prehistory through ancient DNA**

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The American Southwest is one of the best archaeologically known areas of the world. It is also one of the most ethnically and linguistically diverse regions inhabited by contemporary Native American groups in North America. To what extent are the early and late prehistoric Southwest occupants associated with the Mesa Verde, Chaco Canyon, Mimbres and Basketmaker cultures related to today's Athapaskan, Puebloan and Uto-Aztecan speakers? Is there genetic evidence for an earlier migration into the Southwest by populations ancestral to today's Southern Athapaskans? Can the spread of farming into the Southwest region by Uto-Aztecan speakers from Mexico be detected in the gene pools of these earlier cultures? How are the former occupants of Chaco Canyon related to other prehistoric and modern inhabitants of this region? Does the current regional diversity reflect the geographical distribution of Southwest cultures prior to European contact? Previous ancient DNA research from the greater Southwest has demonstrated both regional continuity and discontinuity through the study of short-read mtDNA sequences. With the advent of second generation sequencing technology it is now possible to address in finer resolution these micro-continental migrations questions associated with the spread of language families into the American Southwest.



## Session 5B: 11.50 – 12.10

### Exploring collagen and DNA damage in archaeological bone through a non-destructive method in mass spectrometry: the warm-water method

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With collagen established as a remarkably stable protein in bone, radiocarbon dating, stable isotope analysis, and proteomics have become reliable tools for archaeologists. The latter, proteomics, is still a young addition, but due to femtomole sensitivity of contemporary machines a valuable one, likely to change grounds in the archaeological field. Through the legacy of Zooarchaeology by Mass Spectrometry (ZooMS) we have devised a novel method, using buffered warm water to extract collagen without demineralization. This facilitates a non-destructive way to determine animal genera through indexed peptides with the possibility to revisit the same sample. In addition, the elutes of collagen with warm water may give insight into the degradation process of collagen. Our current hypothesis states that collagen in bone does damage over time, though minimally, and it is this ‘damaged fraction’ that becomes soluble and is leached out with our method. Usually, sufficient material is obtained to investigate, but the absence of a soluble fraction can provide us with information on the burial environment of a sample. Especially when these results are compared to the preserved fraction of close-packed collagen after demineralization. A second hypothesis is that this first elute consists of collagen that through deamidation can indicate for chemically similar deamination in DNA. This could provide a simple and efficient screening method that prevents sacrifice of bone artefacts or samples to select for DNA analysis.

**Session 5B: 12.10 – 12.30**

## Ancient DNA research without PCR: the power of single molecule sequencing

Eveline Altena<sup>1</sup>, Michiel van Galen<sup>2</sup>, Matthew Hestand<sup>2</sup>, Johan den Dunnen<sup>2</sup>, Peter de Knijff<sup>1</sup>

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We would like to present the results of the first experiment in which human DNA from archaeological samples was sequenced by means of a PCR-free single molecule sequencing platform with a shotgun approach (whole genome sequencing). Sequencing was performed by Helicos Biosciences with their true Single Molecule Sequencing technique. The samples were taken from skeletal remains of five individuals buried between 1225 and 1850 AD in Eindhoven, The Netherlands. Samples were collected in the field under strict forensic conditions to avoid contamination with exogenous human DNA and sample preparation took place in a lab dedicated to ancient DNA research. Additionally four modern human samples were sequenced as a reference. The modern samples were taken from the four persons that were directly involved in sample collection in the field, DNA extraction and conventional sequencing.

We will discuss sample preparation and sequencing methods, aligning methods, yield, coverage of the genome, method of defining gender, contamination with both exogenous human DNA and microbial DNA and the analysis of degradation. The single molecule sequencing results will also be compared with results from conventional and massive parallel sequencing of the same samples. This will give an impression of the power of single molecule sequencing of ancient DNA.

## Next generation sequencing of enriched libraries and its potential for ancient fixed human remains

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In 1951 the US Department of Defense started repatriating deceased soldiers in the midst of active combat operations for the first time in military history. Today, the U.S. Government is still committed to recovering, identifying and returning to their families, the remains of all military service members. Included among these efforts are over 860 unidentified American soldiers from the Korean War buried in the National Memorial Cemetery of the Pacific in Hawaii. Since 1999, several caskets have been disinterred in hopes of using mtDNA to establish identification. Unfortunately, the Korean War era mortuary procedures that were performed on these remains involved aggressive embalming. The remains were - one after the other – soaked for 5 days in vats of highly concentrated formaldehyde and then liberally sprinkled with a powdered hardening compound rich in paraformaldehyde.

Despite improved DNA extraction protocols and aggressive techniques to amplify mtDNA, we have been unsuccessful in obtaining authentic DNA with our standard amplification protocols that target amplicons as small as 124bp. Preliminary data based on modified methods suggest that the surviving human mtDNA fragments are approximately 70bp or less in length. As a result, they present a challenge for conventional PCR amplification and Sanger sequencing methods, particularly for a laboratory conducting high-volume casework, where cloning is a poor option.

Thanks to high quality publications describing the recovery of entire mtGenomes from very old remains via next generation sequencing technologies (humans, Neanderthals, mammoth, polar bear, cave bear, etc.), we are hopeful that NGS coupled with efficient enrichment will permit the recovery of reliable, authentic mtDNA data that can be used for identification. However, it remains to be seen whether the cross-links present in these fixed, degraded remains will limit successful amplification of the libraries and final data quality. Here we present our recent efforts to obtain authentic mtDNA sequences from these Korean War remains using second and third generation sequencing technologies.

## Analysing ancient human DNA - challenges and possibilities

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Ever since the first retrieval of ancient DNA sequences more than two decades ago, there has been great interest in analyzing prehistoric human remains. Long-standing question relating to Paleolithic humans and Neanderthals, ancient colonization routes and the spread of the Neolithic lifestyle could potentially be addressed using such an approach. The field has, however, been hampered by difficulties relating to modern human DNA contamination. It is especially challenging to authenticate ancient DNA in the cases where sequences may be indistinguishable from modern human DNA. However, in recent years we have achieved a better understanding about the nature of contamination and also about how to monitor and minimize it. Here I will briefly review this area and add some new quantitative data on the efficacy of different decontamination methods. Further, I will present a case where I test the obtained sequence motifs in published synthetic clones from Neolithic hunter-gatherers with the *c* statistic. The clones were retrieved using FLX genome sequencing and authenticated based on the amount of template DNA content as quantified using real-time PCR, on support from two independent DNA extractions from different tissues, on the amount of clonal support, and on the inverse relationship between fragment length and DNA yield. The statistical approach explores the most common type of damage found in clone sequences, cytosine deamination, to identify endogenous sequence motifs.

## **Ancient DNA: from damage characterization to repair**

Ludovic Orlando<sup>1,2</sup>, Marie-Alice Gariel<sup>1</sup>, Sophie Romero<sup>1</sup>, Gianella Garcia-Hughes<sup>2</sup>, Jakob Bredsdorff-Fredriksen<sup>2</sup>, Enrico Cappellini<sup>2</sup>, Tom Gilbert<sup>2</sup>, Catherine Hanni<sup>1</sup> & Eske Willerslev<sup>2</sup>

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DNA molecules from fossils and other ancient materials offer precious sources of information in evolutionary biology. However, ancient DNA (aDNA) research is still hampered by the very nature of aDNA templates that have been chemically modified post-mortem. As a result, PCR amplification success is generally limited and high rates of nucleotide misincorporation are observed in aDNA sequences. We will present some experimental estimates of main aDNA damages and promising prospects for repair. We are convinced that these latter developments could advantageously be used in parallel with Next-Generation Sequencing technologies in order to improve genomic data reliability as well as to reduce analytical costs.

**Session 6A**

Residues

Thursday 9<sup>th</sup> September, 2010

14.30 – 15.50

*Moderator: Oliver Craig*

**Session 6A: 14.30 -14.50**

**Food preparation and consumption among late foragers and early farmers in Northern Europe: molecular and isotopic approaches to evaluating continuity and change**

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The appearance of imported cereals and domestic animals demonstrates that farming was at least practiced in Northern Europe by the start of the 4<sup>th</sup> Millennium BC. However, the speed and completeness of the transition from foraging, hunting and fishing to food production has been much harder to gauge from the fragmented faunal and botanical record and views have oscillated between a rapid and complete change driven by the arrival of migrant farmers and a slow gradual adoption largely mediated by indigenous populations.

This debate has often centred on evidence from the Western Baltic region where there is an excellent record of both Late Mesolithic and Early Neolithic archaeology. Here there was a clear transition in material culture, including a distinct change in pottery styles, which is thought to coincide chronologically with a rapid and major shift in diet and economic practices, directly linked to the arrival/adoption of food production at the onset of the Neolithic at around 4,000 cal BC.

Here, we present the results of the analysis of organic residues from over 200 pottery vessels from northern Germany and Denmark in order to determine whether the types of food prepared rapidly changed with the introduction of agriculture and pastoralism. Uniquely in Europe, the peoples of the circum-Baltic region had a ceramic tradition which predated the arrival of farming by at least 500 years. This allows a direct comparison of pottery use across the transition and at individual sites that were used continuously during this period. Our approach combines data from gas chromatography-mass spectrometry (GC-MS) and GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS) with a detailed microscopic (optical and SEM) investigation aimed at identifying plant and animal tissues embedded in preserved deposits on vessel surfaces.

## Detecting marine product processing in prehistory: recent developments using lipid biomarkers and isotopic signatures

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Reconstructing the ancient exploitation of wild aquatic fauna has proved challenging for archaeologists. Taphonomic biases against the preservation of remains, the likelihood of off-site product processing and lack of sensitivity of bulk collagen isotope analyses of skeletal remains has likely obscured evidence for smaller-scale, or sporadic exploitation of marine fauna in prehistory. A more sensitive approach would be to exploit organic residues preserved in the pottery used to process marine products. Identifying marine lipids in absorbed residues in archaeological pottery requires the analysis of organic residues for potential biomarkers, since the long-chain, highly unsaturated fatty acids that are major, and characteristic, components of marine fats and oils are liable to rapid oxidation and will not survive over archaeological timescales. Our approach has been to search for more stable compounds which may form from these unsaturated precursors. Recently it has been demonstrated that vicinal diols, which form *via* the dihydroxylation of carbon-carbon double bonds have the potential to act as indicators of the original presence and location of double bonds in monounsaturated fatty acids of the same carbon chain length. In addition,  $\omega$ (*o*-alkylphenyl)alkanoic acids form when di-, tri- and polyunsaturated fatty acids are heated in a clay matrix under anoxic conditions. As well as these relatively stable products deriving from unstable precursor fatty acids, isoprenoid fatty acids deriving from phytol in marine algae are passed through the food chain and accumulate in marine fats and oils. Further, the stable carbon isotopic composition of fatty acids is preserved over time, and can be used to separate fats and oils of different origins. Thus, a suite of biomarkers now exists to explore marine product processing in prehistoric pottery. Through the characterisation of the lipid composition of a wide range of reference species and laboratory heating and degradation experiments, the presence and/or formation of these biomarkers has been confirmed. We demonstrate the utility of these biomarkers exploiting the high sensitivity and specificity of GC/MS, operating in selected ion monitoring mode, to detect these biomarkers in archaeological pottery residues from coastal sites. The potential of these biomarkers to offer new information regarding the class of marine fauna *via* these molecular and isotopic signatures is explored.



## Identifying Chemical Markers for Anomalous Radiocarbon Dates from Surface Organic Residues

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Surface residues recovered on archaeological pottery have become a valuable resource for radiocarbon dating. Each char is likely to result from a single cooking event and the age offset of the char is likely to be minimal, meaning these dates can be used to accurately determine when the vessels were used. The use of Bayesian statistics for chronological modelling has highlighted how important it is that items being dated are not residual within the archaeological context from which they are recovered. Pottery residues from groups of refitting sherds are therefore particularly valuable alongside other sources of dates, such as articulating animal bone.

However, it has been discovered during the incorporation of radiocarbon dates from charred residues within Bayesian models that these residues are more likely to return anomalous results than other sample types. Approximately 15% of the measured ages are non-reproducible between laboratories and are statistically inconsistent with the other information included in the models. Dates that are both too old and too young are encountered. There also does not appear to be a correlation between the anomalous ages and laboratories or methods of sample pre-treatment. It appears therefore that there is some form of contamination present in a proportion of the dated residues.

Rigorous chemical characterisation of a range of relatively abundant residues is being undertaken at the bulk and molecular level in order to identify the chemical features of residues that might return an anomalous radiocarbon date. The majority of these residues are from the ongoing radiocarbon dating programme funded by English Heritage and range from the early Neolithic to Anglo-Saxon periods. Analyses focus primarily on the use of pyrolysis-gas chromatography/mass spectrometry (py-GC/MS), alongside other techniques, such as Fourier transform infrared spectroscopy (FTIR), organic elemental analysis (C/H/N) and solid state cross-polarisation magic angle spinning nuclear magnetic resonance spectroscopy (CP/MAS NMR), providing complementary composition information. The results of this study will be presented with a view to identifying a pre-treatment protocol to remove the contamination or an appropriate screening process to eliminate residues likely to return anomalous radiocarbon dates.

## On the Beaker trail: Investigating the function of British Beakers through organic residue analysis

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Beaker pottery is traditionally regarded as a material symbol of social, material and ideological changes that began in the latest Neolithic – these included the appearance of new ceramic technologies, modes of dress and adornment, the introduction of metallurgy and single burial. As far as the pottery goes, meticulous and numerous typological schemes have been produced in the past, but the function of Beakers has never been established on a larger scale from a scientific point of view.

British Beakers are most commonly found with inhumation burials, laid in pits or cists, and often in association with other objects. It has often been supposed that Beakers were produced specifically for grave deposition, since they differ in terms of fabric quality and decoration from those produced for non-funerary use. Due to their elaborate decoration and innovative fine fabric, Beakers have been considered as prestige items. As a consequence of Sherratt's interpretation of Beakers as drinking cups, used to consume alcoholic beverages or narcotic substances at ritual gatherings, these vessels have gained almost a legendary status as prestige drinking equipment that has not been scientifically contested.

The porous fabric of prehistoric pottery has been known to represent a favourable environment for the long term preservation of organic molecules, such as lipids. Beaker potsherds from funerary and non-funerary contexts have been analysed using solvent extraction, followed by gas chromatography, mass spectrometry and isotope ratio mass spectrometry to provide structure identification, biomolecular fingerprints and compound specific  $\delta^{13}\text{C}$  values.

Through analyses of absorbed lipids we can directly address the function and contents of ceramic vessels. Here we present preliminary results of our research project aimed at addressing the function of Beaker pottery through organic residue analyses. Surprisingly, no support is found for the interpretation of Beakers as vessels used in alcohol consumption, and their very status as prestige items might even be questioned.

**Session 6B**

Domestication

Thursday 9<sup>th</sup> September, 2010

14.30 – 15.50

*Moderator: Greger Larson*

**Session 6B: 14.30 – 14.50**

## Nuclear ancient DNA draws picture of wild and early domesticated horses

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Domesticated horses played key roles in the history of mankind providing nutrition and offering unprecedented modes of transportation. If the reasons related to the beginning of horse domestication are still unknown, horses were crucial to the life of nomadic pastoralists on the Eurasian steppe and had always have a particular position among domestic animal (warfare capabilities, symbol of social status, human's nutrition). For these reasons, deciphering the spatial and temporal origin of domestic horses is of key importance for understanding the origin of modern human societies. Due to the high variability of mtDNA among modern and ancient horse populations, the genetic analysis failed to reveal either time or place of horse domestication. In this case, the failure has pushed us to look for other genetic markers and to adapt new sequencing methods to ancient DNA. Thus, we were able for the first time to address the question of horse domestication by analyzing nuclear trait markers directly linked to early breeding practice. Coat color is an easily detectable phenotypic trait, which was likely a major goal of animal breeders since the beginning of domestication. Fortunately, single mutations are often responsible for color variants, which make these mutations very valuable for the analysis of SNP via pyrosequencing. We successfully typed for a dozen nuclear markers in more than 90 horse samples from the Pleistocene to medieval times. Through this example, we will present the advantage and limits of our methodological approach. By comparing mtDNA data and the data for coat color selection of horses, we will open the discussion about the perspective of the analysis of nuclear markers in palaeogenetics.

## The Mediterranean route: analysing early domestic pigs in Southeast Neolithic France by combining Mitochondrial and Nuclear DNA with Geometric Morphometrics

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The Neolithisation of Europe followed two main routes of expansion – the northern so called Danubian or Balkanic route and the southern Mediterranean route. Previous research has shown that the earliest domestic pigs in Europe were of Near Eastern descent, and specifically, that the spatiotemporal occurrence of haplotype Y1-6A is well correlated with the Danubian expansion. Whether domestic pigs along the southern route carried the same or divergent haplotypes remains unknown. A current hypothesis argues that early domestic pigs in the northern Mediterranean basin carried a different haplotype but has up to date lacked sufficient data to test it.

Here we report the results of our analysis of an 80bp d-loop fragment, a MC1R SNP that's causative of dominant black coat colouring, and 2D geometric morphometric (GMM) data from *sus* remains in early to middle Neolithic layers in southeast France. Our results support the current hypothesis that divergent mitochondrial lineages accompanied the different routes of expansion as we find high prevalence of the Near eastern haplotype Y2-5A, but not a single Y1-6A. By applying GMM shape analysis we can show that individuals that carried a European d-loop signature (Aside haplotype) were significantly differentiated from individuals that carried the Y2-5A haplotype. This could imply a diverse origin that might represent local wild boar and imported domestic pigs. However, at least one individual that belonged to a European mitochondrial lineage also carried a derived allele at the 0301 locus in the MC1R gene – an allele that is assumed to have originated in domestic stock. Combined with previously published data, these results indicate that by 4000 BC, introgression with wild boar was widespread in Europe. For future analyses we aim to apply the integrated use of DNA and GMM to archaeological wild and domestic pig remains from locations across Europe and the Near East. As we demonstrated here, different analytical techniques can be used to answer a variety of questions and their combined use will make small case studies like this one more easily incorporated into a larger framework.

**Session 6B: 15.10 – 15.30**

The flying pig, migration or transfer of ideas in prehistory.

Molecular genetic and archaeological investigations of Mesolithic and Neolithic pigs (*Sus scrofa*).

Ben Krause-Kyora, Melanie Röpke, Rebecca Renneberg, Almut Nebel, Nicole von Wurmb-Schwark, Claus von Carnap-Bornheim

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This study shows the reflection of population dynamics, like mobility and migration, in archaeological evidence from pigs. How did the domestication of the pigs take place in Northern Europe? Did domestic pigs of Near Eastern ancestry were definitely introduced into Europe during the Neolithic or did local European wild boar were also domesticated by this time?

First goal of this study was the development and establishment of extraction methods suited for extraction of DNA from historical samples, the selection of suitable genetic markers, and the establishment of sensitive, reliable and reproducible detection methods. PCRs were established to amplify pig-specific DNA with high sensitivity down to single molecules. Different primer pairs were used to amplify and sequence highly variable regions of the mitochondrial DNA like the dloop, cytb, XXX to determined specific mtDNA haplotypes. Further on specific nuclear DNA were analysed to determine the sex and the paternal haplotypes. The sequences finally aligned and compared to those already deposited in databases. A SNP analyse were established to determine the coat colour.

The results of over 300 individuals from 25 neolithic sites shows that around 4800-4000 BC domestic pigs are introduced in the archaeological sites in northern Germany. The study points out that the oldest domestic pig in the sample (4600 BC) has a “Near East” haplotype. All other domestic and wild boars show the same “European” haplotype.

The conclusion leads to the opinion that the domestic pigs with a maternal “Near East” ancestor were introduced into central Europe with the linear pottery (LBK) culture. After a short period the domestic pigs with “European” haplotypes coexist with the “Near East” haplotypes in the LBK and the Chaséen culture. An explanation could be that the people of the Ertebølle culture adapt the idea of domestication and permuted it on the indigenous wild boar population. With the established methods it is possible to determine the sex and the coat colour of ancient individuals. Further on the study shows the important of the coat colour as a marker for the domestication.

## Signs of contrasting selection in North European cattle as revealed by single nucleotide polymorphisms

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New large sequencing and genotyping techniques along with programs developed for analysis of temporal samples offer improved possibilities to resolve long standing questions such as introgression of aurochs and prehistoric breeding in domestic cattle. Here I present an example of how analysis of autosomal SNPs in ancient cattle can reveal signs of selection.

There are close to a 1000 different cattle breeds in the world today. Cattle are the most important of our livestock animals, but the knowledge of how and when this diversity arose is obscured by lack of written documentation prior to the last 200- 300 years. It is more than 10 000 years since the domestication of cattle and the need for specialized animals is likely to have invoked selection for specific traits already early on in history. In Northern Europe the middle Ages was a time of a lot of dynamic change in society, this likely affected also the cattle. Here we genotype a large amount of cattle remains ranging in time from Iron Age to the 18<sup>th</sup> century, and also modern cattle, for 14 coding and neutral nuclear SNPs. We use coalescent simulations to estimate the effect of genetic drift on allele frequency changes over time. We find small changes from the Iron Age to medieval time, but the largest change is that observed in the coat colour gene *MC1R* when all ancient samples are compared to modern cattle. It shows clear signs of having been under selection, and in order to account for the radical change in allele frequency a drastic reduction in effective population size must have occurred, consistent with the formation of breeds.

**Session 7A**

Compound-specific Isotopes

Thursday 9<sup>th</sup> September, 2010

16.20 – 18.00

*Moderator: Colin Smith*



**Session 7A: 16.20 – 16.40**

**Investigation of amino acid  $\delta^{13}\text{C}$  signatures in bone collagen to reconstruct Korean palaeodiets using liquid chromatography-isotope ratio mass spectrometry**

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This research presents the carbon stable isotope ratio analysis ( $\delta^{13}\text{C}$ ) of individual amino acids in bone collagen of humans ( $n = 9$ ) and animals ( $n = 27$ ) from two prehistoric shell midden sites in Korea. We obtained complete baseline separation of 16 of the 18 amino acids found in bone collagen by using liquid chromatography-isotope ratio mass spectrometry (LC-IRMS). The isotopic results revealed that the humans and animals in the two sites had similar patterns in both EAAs and NEAAs, reflecting the similar amino acid biosynthetic pathways found in vertebrates. However, the threonine  $\delta^{13}\text{C}$  values in animals measured in this study were unusual relative to other amino acids. This pattern of  $^{13}\text{C}$ -enrichment in threonine is in contrast with the  $^{15}\text{N}$ -depletion of threonine in previous studies. At both sites, all amino acids in marine animals are  $^{13}\text{C}$ -enriched relative to those of the terrestrial animals. The Tongsamdong human had EAAs and NEAAs from marine food resources, while the Nukdo humans mainly had EAAs from terrestrial food resources but obtained NEAAs from both terrestrial and marine resources. The  $\delta^{13}\text{C}$  isotopic differences in amino acids between marine and terrestrial animals were the largest for glycine (NEAA) and histidine (EAA) and the smallest for tyrosine (NEAA) and phenylalanine (EAA). In addition, threonine among the EAAs also had a large difference ( $\sim 8\text{‰}$ ) in  $\delta^{13}\text{C}$  values between marine and terrestrial animals, and has the potential to be used as a unique isotopic marker in palaeodietary studies. Threonine  $\delta^{13}\text{C}$  values were used in conjunction with the established  $\Delta^{13}\text{C}_{\text{Glycine-phenylalanine}}$  values and produced three distinct dietary groups (terrestrial, omnivorous, and marine). In addition, threonine  $\delta^{13}\text{C}$  values and  $\Delta^{13}\text{C}_{\text{Serine-phenylalanine}}$  values were discovered to separate between two dietary groups (terrestrial vs. marine), and this is a possible new isotopic indicator that can be used to distinguish between marine and terrestrial diets.

## Identifying the Consumption of Marine Resources in Prehistory: An Investigation of Bone Collagen Amino Acid $\delta^{13}\text{C}$ in Archaeological Humans and Fauna

Noah V Honch<sup>1</sup>, Philip J H Dunn<sup>1</sup>, Gunilla Eriksson<sup>2</sup>, Kerstin Lidén<sup>2</sup> & Richard P Evershed<sup>1</sup>

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The identification of marine protein consumption in prehistory has received considerable attention in recent years, particularly with respect to the Mesolithic/Neolithic transition in Europe. Bulk stable isotope analysis is routinely applied to archaeological bone collagen and is capable of distinguishing high marine protein consumers (HMP) from those whose diets are dominated by terrestrial-derived protein. However, it is often difficult to make finer distinctions, for example between populations with terrestrial derived diets and those with minor contributions of marine protein (e.g. <25% of dietary protein).

Attempts have been made to investigate the isotopic variation of single amino acids in modern and archaeological tissues to better understand the underlying factors that contribute to bulk  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures. Resulting data have also been used to develop new methods of identifying marine protein consumption in prehistory, such as using the intra-individual  $\delta^{13}\text{C}$  spacing between glycine and phenylalanine. In the future, it is hoped methods like this will be capable of identifying real dietary differences between and amongst archaeological populations which are effectively “invisible” using bulk collagen stable isotope analysis.

Here, we present collagen amino acid  $\delta^{13}\text{C}$  from archaeological humans (n=32) and fauna (n=49) from three sites in Sweden (Köpingsvik, Rössberga, Visby). We also present amino acid  $\delta^{13}\text{C}$  from muscular tissues and collagen from modern marine reference material collected from various locations in the UK (Orkney, Plymouth, and the Bristol Estuary). We investigate the validity of the  $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$  proxy as an indicator of marine protein consumption, and explore the utility of other analytical benchmarks. We also examine the tissue-collagen offset of amino acid  $\delta^{13}\text{C}$  in modern marine fauna, and discuss the value of using this information to elucidate the consumption of marine resources in prehistory. Finally, we consider the significance of compound specific isotope analysis in terms of clarifying the dynamics of the Mesolithic/Neolithic transition in Europe.

**Session 7A: 17.00 – 17.20**

**Palaeodietary reconstruction of prehistoric human populations from Japan using the  $\delta^{15}\text{N}$  values of individual bone collagen amino acids.**

Yuichi I Naito<sup>1,4</sup>, Noah V Honch<sup>2,3</sup>, Yoshito Chikaraishi<sup>4</sup>, Naohiko Ohkouchi<sup>4</sup>, Minoru Yoneda<sup>1</sup>

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We investigated the stable nitrogen isotopic composition of bone collagen amino acids from a variety of archaeological human and faunal populations from prehistoric Japan. The samples include an inland population dating to the Initial Jomon Period (c. 10,000-6,000 BP), and individuals from a range of coastal sites dating to the Early Jomon Period (c. 6,000-5,000 BP) and Okhotsk Culture Period (c. AD 550-1200).

At the two Jomon sites, we observed characteristic and consistent isotopic values for two amino acids: glutamic acid and phenylalanine. While glutamic acid showed quite a large inter-trophic  $^{15}\text{N}$ -enrichment along the food chain, phenylalanine showed little  $^{15}\text{N}$ -enrichment. These isotopic trends are consistent with results obtained from several ecological studies and modern feeding experiments. However, at the coastal site of the Okhotsk Culture, unexpected  $\delta^{15}\text{N}$  variability of phenylalanine was observed for marine faunal species. Because most of the marine species are migratory, we think that the variability reflects the isotopic differences of nitrogen sources (i.e. nitrates) between oceanic regions. It has been generally suggested that Okhotsk Culture peoples developed specialized maritime adaptation as evidenced by bone tools, animal remains (including a large number of marine animals), and site distributions that are primarily restricted to coastal and island environments. Many factors such as ocean currents and differences in tools and strategies for procuring marine animals might relate to the observed isotopic differences between these periods. With these complicated situations in mind, we have developed methods for quantitatively evaluating the consumption of marine protein using the  $\delta^{15}\text{N}$  values of glutamic acid and phenylalanine. By applying this method to above samples, we estimated that the two coastal populations obtained approximately 70-80% of their dietary protein from marine resources. In contrast, it seems that the inland Jomon population consumed little marine protein. In this case, the  $\delta^{15}\text{N}$  of glutamic acid might indicate the extent of carnivory because it provides wider scope of  $^{15}\text{N}$ -enrichment between trophic levels ( $+8.0 \pm 1.1\text{‰}$ ) than those usually observed for bulk bone collagen ( $+2\text{--}5\text{‰}$ ).

## Bulk and compound-specific isotope analysis of pathological bone collagen: Preliminary results

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Carbon- and nitrogen-isotope compositions ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) of human bone collagen are traditionally used to interpret dietary variability in the past but the likely influence and extent of disease processes on isotopic ratios, particularly  $^{15}\text{N}/^{14}\text{N}$ , has not yet been determined. Archaeological bone from medieval Europe (12-16th century) is used to explore the effects of pathological conditions on both carbon- and nitrogen-isotope ratios. Samples were chosen to represent the major categories of disease and include trauma (fracture), infection (osteomyelitis and periostitis), nutritional disease (rickets), and joint disease (osteoarthritis and rheumatoid arthritis) (n=46). For each individual, bulk collagen extracted from an area of bone that exhibited pathological reactions was analysed and compared to an unaffected area of bone. No differences were detected in carbon-isotope compositions but pathological bone had consistently higher  $\delta^{15}\text{N}$  values than unaffected areas of bone for all categories of disease, except nutritional (rickets). The differences may result from metabolic and immune changes, which affect nitrogen balance during periods of stress and/or amino acid composition differences between reactive and normal bone. Compound-specific isotope analysis of amino acids derived from the collagen samples is being undertaken to investigate the observed differences at the molecular level and to provide a better understanding of the biochemical and physiological processes involved.

**Session 7A: 17.40 – 18.00**

Can  $\delta^{15}\text{N}$  values of individual amino acids in bone collagen be used as a proxy for manuring in the past?

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The advent of manuring practices was a huge breakthrough for ancient farmers since adding extra nitrogen to the soil by the addition of manure increases crop yields and ultimately allows more people to be sustained on the same area of land. Identifying the first instances of manuring is therefore of great interest to archaeologists investigating the socio-economic changes synonymous with the Neolithic transition from hunting and gathering to farming.

The  $\delta^{15}\text{N}$  values of individual amino acids within hydrolysates of human bone collagen have been found to differ significantly. Since the  $\delta^{15}\text{N}$  value of the essential amino acid phenylalanine ( $\delta^{15}\text{N}_{\text{Phe}}$ ) changes little with increased trophic level, it is believed to reflect the  $\delta^{15}\text{N}_{\text{Phe}}$  value at the base of the food chain, i.e. that of the plants. Since the  $\delta^{15}\text{N}$  values of manured cereal grains are more enriched in  $^{15}\text{N}$  than unmanured cereal grains, the  $\delta^{15}\text{N}_{\text{Phe}}$  value of manured cereal grains is also expected to be enriched.

Amino acids have been extracted from cereal grains and pulses grown on manured and unmanured experimental farm plots. The amino acid  $\delta^{15}\text{N}$  values have been determined by GC-C-IRMS as N-acetyl iso-propyl (NAIP) esters. The development of this internal isotope proxy for the identification of manuring has important implications not only for the study of the Neolithic transition but also for understanding the different contributions to bulk collagen  $\delta^{15}\text{N}$  values.

**Session 7B**

Ecology

Thursday 9<sup>th</sup> September, 2010

16.20 – 18.00

*Moderator: Cristina Valdiosera*

## Genetic change in the last remaining population of woolly mammoth

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At the end of the last Ice Age, the woolly mammoth (*Mammuthus primigenius*) experienced a series of local extinctions across Eurasia and North America. On Wrangel Island, however, a population of woolly mammoth became isolated as sea levels rose and subsequently survived there in isolation for another 6000 years. One possible explanation for why this population finally disappeared some 3500 years ago is that the small population size led to a gradual loss of genetic diversity, which together with inbreeding depression eventually may have caused the extinction. To investigate this, we have used a set of different genetic markers, both from the mitochondrial and nuclear genomes, to examine the genetic changes that occurred in northeast Siberia and on Wrangel Island throughout the last 50,000 years. The results suggest that the isolation on Wrangel Island led to both a shift in genetic structure and a significant loss in genetic diversity. However, genetic variation seems to have increased again during the Holocene, or at least remained relatively stable. This suggests that the extinction was caused by extrinsic factors and happened relatively abruptly, rather than having been caused by a gradual deterioration in genetic variability.

## aDNA phylogeography of the wild asses: the demise of a species?

Sophie Champlot<sup>1</sup>, Mathieu Gautier<sup>2</sup>, Benjamin Arbuckle<sup>3</sup>, Adrian Balasescu<sup>4</sup>, Simon Davis<sup>5</sup>, Vera Eisenmann<sup>6</sup>, Mietje Germonpré<sup>7</sup>, Marjan Mashkour<sup>8</sup>, Arturo Morales Muniz<sup>9</sup>, Joris Peter<sup>10</sup>, Jean-François Tournepiche<sup>11</sup>, Hans-Peter Uerpmann<sup>12</sup>, Thierry Grange<sup>1</sup> & Eva-Maria Geigl<sup>1</sup>

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Despite intense palaeontological research, some phylogenies of the *Equidae* remain unresolved. Recently, Orlando et al. used aDNA techniques to revisit equid phylogeny, which lead to a reduction of the number of palaeontological species. The situation is still particularly unclear for the wild asses, whose geographical distribution in the Pleistocene and the early Holocene stretched from Northern Africa to Eurasia before they became endangered or extinct. We engaged in a phylogeographic study based on the mitochondrial DNA preserved in Pleistocene and Holocene archaeological ass remains covering their former geographic range. Our data resolves the population structure of the Asiatic wild asses formed by five significantly distinct populations or subspecies, which could assist in devising conservation strategies. Moreover, bone remains attributed to the extinct European wild ass based on morphological characters show different genetic signatures depending on their geographic origin and the time period they lived in. The relationships of these genetic signatures with those of other equids indicate that morphological criteria alone can be an unreliable index in inferring various equid species. The diversity of the genetic signatures in populations with similar morphology along with genetic signatures shared between morphologically distinct animals reveal a significant morphological plasticity among *Equus* species. This feature may have allowed local morphological adaptations to various environmental conditions, creating ecomorphotypes without speciation events.



**Session 7B: 17.00 – 17.20**

## **Ancient plant DNA as trace fossils in Pleistocene and Holocene sediments**

Tina Jørgensen<sup>1</sup> & Eske Willerslev<sup>1</sup>

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Sedimentary ancient DNA of plants is a promising complementary tool allowing palaeobotanical reconstructions. By comparison with pollen and macrofossils the value and use of ancient DNA as a trace fossil is analysed from different sediments, from a variety of spatial and temporal environments. These include; permafrost sediments from Taymyr Peninsula in Siberia covering the end of Pleistocene (45.000-10.000 C14 years bp), lake sediments from Andøya in northern Norway (from the transition between Pleistocene and Holocene) and from Nunataks on the ice sheet in South Greenland.

## Phylogeography of Ice Age Beringian Beetles: an Ancient DNA Approach

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The Beringian region consists of northeast Asia, northwest North America and the now inundated land bridge between the two. During ice ages, much of this region remained ice free and, together with its variety of habitats and large size, provided a refuge for populations of animals that were escaping the advancing ice sheets. At the end of an ice age, these populations could then recolonise the newly ice free continents. Beetles, which are important for ice age climatic reconstruction, were one such group that took refuge in Beringia and subsequently recolonised Asia and North America, although the exact mode of this recolonisation is unknown. Additionally, at the end of the last ice age, there was widespread extinction of the large mammalian fauna; beetles seem to have passed through this extinction event unscathed, although the effects of this event are not known at the population level. A phylogeographic approach, using both ancient and modern DNA of beetles from the Eastern Beringian region, is proposed here in order to address these two important questions. Initial work is focusing on two ground beetle species (*Amara alpina* and *Pterostichus brevicornis*) and a preliminary phylogeny reveals that *A. alpina* displays some phylogeographic patterning, although much work still needs to be done.

## **Biodiversity in the dirt: reliable (mini)barcodes for DNA-based paleo-ecology**

Laura S Epp<sup>1</sup>, Sanne Boessenkool<sup>1</sup>, Eva Bellemain<sup>1</sup>, Eske Willerslev<sup>2</sup>, Pierre Taberlet<sup>3</sup>, Eric Coissac<sup>3</sup> & Christian Brochmann<sup>1</sup>

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The retrieval of ancient DNA from environmental samples, such as soil and sediments, offers the possibility to identify species and reconstruct past biotic assemblages even in the absence of identifiable macrofossils. PCR-based amplification combined with deep sequencing can potentially reveal a wide array of sequences from single samples. In order for the information generated to be meaningful and as representative as possible, the primers employed must fulfil certain criteria. They must be optimised both for their ability to amplify DNA of (preferably) all members of a target taxon without amplifying other taxa, and for amplifying a sequence stretch that offers the best possible species identification. These markers also have to be short enough to amplify from severely degraded DNA.

We are systematically designing such DNA barcoding markers for several groups of organisms (bryophytes, fungi, insects, springtails, vertebrates) with newly developed bioinformatic tools. Our approach is based on screening all available complete mitochondrial genomes for target taxa and for a set of non-target taxa. We identify conserved regions suitable as primers that span short, taxonomically informative regions. After establishing optimal priming sites, we evaluate candidate primers in an in-silico PCR, using all sequences in public databases as templates. These primers are then tested empirically, and results for amplifications from permafrost samples are very promising.

We are currently applying these markers to reconstruct past biodiversity from permafrost soil samples collected throughout the Arctic and ranging in age from recent to several 100 000 years before present.

## **Session 8**

New Directions

Friday 10<sup>th</sup> September, 2010

9.00 – 11.00

*Moderator: Matthew Collins*

## Ancient Transcriptomics from Archaeological Maize Kernels

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The characterization of biomolecules from ancient samples can shed otherwise unobtainable insights into the past. However, despite the fundamental role of transcriptomal change in evolution, the potential of ancient RNA remains unexploited – perhaps due to dogma associated with the fragility of RNA. We hypothesize however that seeds offer a plausible refuge for long-term RNA survival, due to the fundamental role of RNA during seed germination. Using RNA-Seq on cDNA synthesized from nucleic acid extracts, we validate this hypothesis through demonstration of partial transcriptomal recovery from two sources of ancient maize kernels. The results suggest that ancient seed transcriptomics may offer a powerful new tool with which to study plant domestication.

## Molecular preservation in Mammoth and Mastodon

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Recovery of original ancient biomolecules occurs primarily from archaeological material of relatively recent (e.g. <100,000yrs) origin, but recent controversial reports suggest that in some cases, informative biomolecules may persist beyond this limit. Demineralization of mammoth (MOR 604) and mastodon (MOR 605) bones, recovered near the base of 125,000- to 600,000-year old gravel deposits near Miles City, Montana, USA, revealed fibrous bone matrix, with apparent cells and blood vessels. Mass spectrometry recovered abundant peptide sequences from chemical extracts of these bones. The large grain size and porosity of entombing gravel deposits are considered detrimental to molecular preservation, but current and previous work does not support this idea. New peptide sequence data recovered from each of these specimens and a relatively recent (~12,000-year old) Colombian mammoth (MOR 501) from the Blacktail Caves, Montana, USA, was obtained using a high-resolution Thermo LTQ-FT Ultra equipped with a 7T superconducting magnet. Bone extracts have yielded almost 100% sequence coverage of the predominant bone protein, collagen type I. The RAW data files (6 per species-2 sample prep replicates with 3 LC-MS runs each) were processed using MASCOT Distiller before being searched against the NCBI non-redundant database using MASCOT. RAW data files were also processed and searched using SEQUEST to search the same database. Searches were carried out with a 5 ppm peptide mass tolerance and a 0.6 Da fragment ion tolerance. Sequence data consistent with the preservation of collagen in these bones are confirmed using several antibodies to bone matrix proteins, including mammalian collagen I. Positive binding is visualized both on bone extracts and *in situ*, on demineralised bone fragments. Our data provide evidence that the temporal limit on molecular preservation is arbitrary, and suggest that original biomolecules may persist well beyond the proposed 100,000 year limit.

**Session 8: 9.40 – 10.00**

## **Scurvy bones: a collagen biomarker to detect vitamin C deficiency in archaeological bone**

Hannah Koon<sup>1</sup>

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How is it possible to infer health from archaeological populations when only their bones remain? This much-debated osteological paradox is well illustrated by scurvy. Despite extensive historical documentation of this common metabolic disease, there is little supporting archaeological evidence because – similar lesions, extreme before see changes to bone.

Scurvy arises when levels of dietary vitamin C are low and results in lethargy and greater risk of haemorrhaging caused by weakened connective tissue – and bone. Vitamin C (ascorbic acid) is essential for the hydroxylation of the amino acids lysine and proline, key structural components of collagen. Sub-clinical disease must have been common in populations over-wintering on stored produce, especially amongst the poor.

Here we report a novel biomolecular assessment of scurvy, based upon a series of collagen peptides which exhibit hitherto unexpected variation in proline hydroxylation. Mass spectrometric analysis has revealed sites which are less hydroxylated in scorbutic individuals. The utility of the method was tested on skeletons of 17<sup>th</sup> and 18<sup>th</sup> C whalers which displayed manifest signs of scurvy.

Tested on remains from the Smithsonian institute naval hospital.

## Session 8: 10.00 – 10.20

### Ancient bone proteomics

Enrico Cappellini<sup>1</sup>, Jesper Velgaard Olsen<sup>2</sup>, Lars Juhl Jensen<sup>2</sup>, Damian Szklarczyk<sup>2</sup>, Vibha Raghavan<sup>1</sup>, Thomas W Stafford Jr.<sup>3</sup>, Eske Willerslev<sup>1</sup>, M Thomas P Gilbert<sup>1</sup>

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Ancient DNA investigation is the most common way to retrieve genetic information from the past. Nevertheless the study of proteinaceous remains in some cases can represent an alternative approach. In the past, most of this work was based on immunological methods, but the recent development of high throughput mass spectrometry-based protein techniques allowed an increase in sensitivity and specificity. Although previous studies reveal that proteins appear prone to degradation in archaeological contexts, it has been demonstrated that mass spectrometry can be fruitfully used to recover ancient protein sequences. However, methodological improvements to optimise each analytical step are still required, in particular with focus upon (i) the extraction method, (ii) reliability criteria, (iii) data analysis.

Preliminary results, obtained analysing total protein extracts from ancient bones collected in archaeological and geological contexts, clearly show the possibility to retrieve extended portions of ancient collagen sequences and to characterise peptides attributed to other bone non collagenous proteins.



## Session 8: 10.20 – 10.40

### Smashing the egg timer: investigating the amino acid clock

Beatrice Demarchi<sup>1</sup>, Enrico Cappellini<sup>1,2</sup>, Molly Crisp<sup>1</sup>, Mike Buckley<sup>1,3</sup>, Matthew Collins<sup>1</sup>, Jane Thomas-Oates<sup>1</sup>, Julia Lee-Thorpe<sup>4</sup>, Curtis Marean<sup>5</sup> & Kirsty Penkman<sup>1</sup>

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A secure chronological framework is vital for framing any archaeological debate. Recent advances in amino acid racemisation (AAR) geochronology provide a reliable biomolecular clock by measuring the extent of diagenesis within a closed system of proteins trapped in biominerals. This clock is able to span the last 2-3 Ma and is applicable to a range of biominerals which can be directly related to the human occupation of an archaeological site.

Our research has focused on building chronological frameworks on a wide spatial and temporal scale. Beginning from the Lower Palaeolithic in Northern Europe, we are now exploring a wider range of sites with different opportunities and challenges, including Gibraltar, Morocco and Southern Africa, where some of the earliest evidence for modern human behaviour has been found. In Africa, ostrich eggshell (OES) is one of the most common biominerals found in archaeological contexts: early humans used them as water containers, as personal ornaments and engraved them as a form of symbolic communication. OES is also known to be one of the best substrates for AAR dating – the ultimate egg timer.

But how does the egg timer work? The answer lies in the patterns of diagenesis displayed by different proteins at different environmental conditions. Heating the proteins at high temperatures was traditionally thought to mimic the diagenetic pathways occurring at burial temperatures, but we show that this is not always the case. Therefore, high temperature data, used routinely in archaeological science to simulate degradation, must be treated with caution.

However, by coupling bulk chiral amino acid analysis with protein mass spectrometry for the first time, we are now able to study the degradation patterns of protein in detail. We are focusing on struthiocalcin (the main protein within OES), to unpick the breakdown pathways of proteins from the individual residue to the protein chain. We intend to answer one of the fundamental questions of AAR studies: is hydrolysis driving racemisation, or is it the contrary? This study has important implications not only for its use as a geochronometer, but also for the advance of protein diagenesis studies.

## Session 8: 10.40 – 11.00

### ZooMS: What is it and what is it good for?

Michael Buckley<sup>1,2</sup>, Matthew Collins<sup>2</sup>, & Jane Thomas-Oates<sup>3</sup>

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‘Zooarchaeology by Mass Spectrometry’, or ZooMS, is a recently-developed technique of using protein mass spectrometry in the analysis of zooarchaeological remains. Branching from earlier studies targeting the small mineral-binding protein osteocalcin as a species biomarker which failed to be detected in samples yielding ancient DNA, ZooMS is predominantly based on using peptides from the fibrous and thus highly-insoluble protein collagen. Initially the method used isolated collagen peptides that were highly-informative, however, now the approach can yield much more information through the fingerprinting of the entire molecule. The first case studies to appropriately use ZooMS were the analyses of sheep/goat remains at Epipalaeolithic and Neolithic sites of the Near East, to discriminate these morphologically-similar species. More recently the technique has been applied to a much wider geographical range of samples including Europe, Australia and the Americas, and wider temporal range from relatively recent to the Early Pleistocene, and perhaps earlier. As well as identifying bone fragments that lack diagnostic criteria for morphological identification, ZooMS has also been recently used for the analysis of bone tools and samples of mummified skin. The method is now maturing and its hope in the future is to go beyond genus-level (and in some instances species-level) information to obtain further information about the bones themselves.

## **Session 9**

Friday 10<sup>th</sup> September, 2010

11.30 – 13.30

*Moderator: Terence Brown*

**Session 9: 11.30 – 11.50**

**Recent cotton evolution tracked through archaeogenomics**

Robin G Allaby<sup>1</sup>, Sarah A Palmer<sup>1</sup>, Pamela Rose<sup>3</sup>, Fábio O Freitas<sup>4</sup>, Bruce D Owen<sup>5</sup>, David Beresford-Jones<sup>3</sup>, Jonathan D Moore<sup>6</sup>, James L Kitchen<sup>1</sup>, Alan J Clapham<sup>2</sup>

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We sequenced 36 million base pairs of DNA from archaeological cotton (*Gossypium* L.) up to 3750 years old from low latitude sites. We found that up to 97.3% of the DNA sequenced was endogenous to the samples. Identification of *G. herbaceum* and *G. barbadense* confirms indigenous origins of cotton in both the Old and New Worlds respectively. Genomic composition was remarkably similar in lineages of *G. barbadense* from archaeological sites in South America separated by over 2000 miles and 3000 years, but differed in overall repetitive element content. The archaeological *G. herbaceum* differed dramatically in composition from modern cotton and was closer to the A genome of the New World tetraploids. The evidence supports a recent rapid and punctuated evolution of the genome in *Gossypium*.

**Session 9: 11.50 – 12.10**

**Re-evaluating the history of the wheat domestication gene *NAM-B1* using historical plant material**

Linnéa Asplund<sup>1</sup>, Jenny Hagenblad<sup>2</sup> & Matti W Leino<sup>3</sup>

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The development of agriculture is closely associated with the domestication of wheat, one of the earliest crop species. During domestication key genes underlying traits important to Neolithic agriculture were targeted by selection. One gene believed to be such a domestication gene is *NAM-B1*, affecting both nutritional quality and yield but with opposite effects. A null mutation, first arisen in emmer wheat, decreases the nutritional quality but delays maturity and increases grain size; previously the ancestral allele was believed lost during the domestication of durum and bread wheat by indirect selection for larger grain. By genotyping 63 historical seed samples originating from the 1862 International Exhibition in London, we found that the ancestral allele was present in two spelt wheat and two bread wheat cultivars widely cultivated at the time. This suggests that fixation of the mutated allele of *NAM-B1* in bread wheat, if at all, occurred during modern crop improvement rather than during domestication. We also discuss the value of using historical plant material, especially 19<sup>th</sup> century seed collections, to further the understanding of the development of agriculture.

## Session 9: 12.10 – 12.30

# Using ancient DNA to contextualise the evolution of maize within the cultural and ritual framework of pre-Columbian South America

Terry Brown<sup>1</sup> & Claudia Grimaldo<sup>1</sup>

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The diffusion of maize cultivation throughout South America has been largely interpreted within a modern Western paradigm in which the primary use of the plant is as a source of grain. This view has increasingly been questioned in recent years. Cobs are rare in older archaeological contexts, and stable isotope evidence suggests that maize was present in several parts of South America for 1000–2000 years before it became a significant component of the diet. It also seems inevitable that the early maize domesticates inherited the hard cupulate fruit cases of teosinte, which would make the kernels inedible. Cultural records suggest that maize had an important ritual significance in many societies, possibly linked with its use as a source of fermentable sugar rather than grain. In previous work, based on typing a single locus in archaeological specimens and primitive landraces, we showed that pre-Columbian maize from South America falls into three genetic groups. Initially we interpreted this, in our modern Western way, as evidence for more than one trajectory for the spread of maize cultivation into South America. We have now carried out a more comprehensive microsatellite study with a greater number of specimens. The data confirm the existence of three populations, but the phylogeography of this larger dataset is not consistent with the multiple trajectories that we previously proposed. An alternative is that the populations, rather than originating from geographical separation, reflect cultural and/or ritual specialisation. According to this hypothesis, the types of maize grown for different purposes were genetically distinct and reproductively isolated. To test this hypothesis we are studying the same set of microsatellites in archaeological specimens, making use of the excellent preservation of DNA in many desiccated maize remains. As these specimens come from known archaeological contexts their usages can be inferred, enabling us to identify the extent to which the genetic populations map onto the cultural and ritual framework.

**Session 9: 12.30 – 12.50**

## Insights into the Analyses of Human and Animal Hairs in Archaeological, Paleontological and Forensic Contexts

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Spanning four centuries the microscopical study of hairs is a venerable one. Studying the morphological structure of hairs assists in addressing the ‘who, what, when and how’ questions in relation to a sample discovery. These questions, and the applications to answer them through light microscopy, are equally applicable to whether one is attempting to derive answers from the examination of hairs that are centuries old, or those that are more contemporaneous in nature. In relation to human hairs it is possible, for example, to determine the differences between people belonging to different populations and how individuals differ; in relation to animal hairs it is possible to affect family or species levels of identification and infer the environment the animal inhabited. Whether hairs are human or animal in origin, the microscopical study of human and animal hairs may also yield information in relation to *pre-* and *post mortem* morphological changes and attribute the manner in which hairs become damaged. This presentation will showcase a number of studies to illustrate the practical applications, and eclectic nature, of microscopical morphological structure of hairs. Case studies, that range from homicides to customs’ seizures to ancient hairs. The presentation will also discuss the utility of hair as a privileged site of DNA preservation in the *post-mortem* environment and the impacts high throughput sequencing and metagenomics will exert on the discipline.

## Session 9: 12.50 – 13.10

# SYNTHESYS II: Improving access to DNA from museum material

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SYNTHESYS (<http://www.synthesys.info/>) is a four-year European Union-funded Integrated Activities grant which began in September 2009 and comprises 19 European natural history museums, Universities and botanic gardens. It aims to create an integrated European infrastructure for researchers in the natural sciences. SYNTHESYS is split into three activities: Access, Networking and Joint Research Activities (JRA).

The JRA will assist in meeting researcher demands for usable DNA from museum specimens by enhancing and improving extraction technology and protocols. It will develop non-invasive tools for estimating the presence of ancient DNA and optimal extraction protocols in both ancient specimens, through five activities, three of which we would like to bring to the attention of ISBA4:

- JRA 1: PrediCtoR: A novel decision-making software tool will be developed to help collections managers and users to quantify the risks associated with destructive analysis of specimens.
- JRA 2: Screening: Development of a new screening protocol for non- or minimally-destructive analyses to complement PrediCtoR, enabling users to estimate the ancient DNA preservation status of rare or unique bone specimens.
- JRA 3: MORDOR: (Methods for Optimal Recovery of DNA from Osteological Remains) Development of a system for ancient DNA extraction of very small samples from museum bone specimens.

SYNTHESYS Project funding is available to provide scientists based in European Member and Associated States to undertake short visits to utilize the infrastructure (namely the collections, staff expertise and analytical facilities) at one of the partner institutions for the purposes of their research.



## Session 9: 13.10 – 13.30

### Will ancient DNA analysis ever become routine in archaeology?

Keri A Brown<sup>1</sup>

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For archaeologists, ancient DNA is an exciting scientific development, but it is still perceived by many as a costly, difficult technique of little relevance to the concerns of archaeology. Ancient DNA is extracted and analysed in specialist laboratories, and most of their projects are concerned with the ‘big questions’, such as the domestication of plants and animals, the evolution of diseases in the past and the genome reconstruction of extinct species (including Neandertals). What about the archaeologists’ questions? The demand for ancient DNA analysis of human remains is rising, with questions about sex identification, kinship amongst burial groups and incidence of diseases such as tuberculosis being the most frequent. The cost of ancient DNA analysis has come down, at least for materials – however the salary costs are still expensive (even scientists have to eat). I have recently started an ancient DNA consultancy service for archaeologists to meet this demand and will discuss case studies and costing of ancient DNA analysis. The prospects for ancient DNA becoming routine in archaeology are just beginning, but archaeologists must also play their part, both in the field, with appropriate DNA sampling strategies, and by asking the right questions.

**Session 10**

Friday 10<sup>th</sup> September, 2010

14.30 – 16.30

*Moderator: Tom Gilbert*

## **Pig domestication, migration, replacement, and hybridization:**

### **Preliminary results from the analysis of 60k SNPs**

Greger Larson<sup>1</sup>, Hendrik-Jan Megens<sup>2</sup>, Richard Crooijmans<sup>2</sup>, Alan L. Archibald<sup>3</sup>, Martien Groenen<sup>2</sup> & Larry Schook<sup>4</sup>

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Investigating the patterns and processes of animal domestication from a genetics perspective has thus far been carried out primarily (though not exclusively) by analyses of mitochondrial sequences. The greater resolution offered by DNA as compared with more traditional morphological approaches to within species questions has led to a great deal of novel results including the recognition that many more discrete populations have been involved in domestication across the Old World than previously suspected. The maternal inheritance pattern of mtDNA, however, has necessarily limited the DNA perspective. The advent of high throughput DNA sequencing technology has allowed the rapid identification of sequence variation between and within species and the subsequent development of chips for high throughput genotyping of several thousand single nucleotide polymorphisms (SNPs) and thus rendering the nuclear genome increasingly accessible for a range of studies, including domestication.

Here we report the preliminary results of an effort to characterise more than 2,400 *Sus* specimens (both wild boar and domestic pigs across the Old and New Worlds) for about 60k SNPs. Most of the samples are derived from modern tissue, though by developing a novel technique, numerous museum samples have also been successfully typed, thus allowing a greater range of variation found in *Sus* samples to be obtained and analyzed. In addition, the sequencing effort also typed ~700bp of mtDNA in order to contrast the nuclear and mitochondrial signatures of the same specimens. Preliminary analyses of the data suggest that by investigating mtDNA and nuclear DNA in tandem, numerous insights can be gleaned into both the role humans have played in the domestication of pigs, and the convoluted genomic histories of many modern pig populations.

**Session 10: 15.30 – 15.50**

## Ice Core Genetics – Reveals Biodiversity from Ancient Ecosystems in Greenland and Antarctica

Astrid M Z Schmidt<sup>1&2</sup>, Eske Willerslev<sup>1</sup> & Jørgen Peder Steffensen<sup>2</sup>

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One of the major difficulties in paleontology, paleoecology, and paleoclimatology is the acquisition of fossil data from the ~10% of Earth's terrestrial surface that is covered by thick glaciers and ice sheets. This is especially true for Greenland and Antarctica where the basal sections of deep ice cores can act as archives for ancient biomolecules. Ancient DNA from ice cores may reveal new biological information allowing reconstructions of past climate, flora and fauna leading to inferences about past climates and ecosystems in arctic regions, which is relevant for understanding the effects of climate change.

Preliminary DNA sequence amplified from *rbcL* and *trnL* regions in the chloroplast genome from North Western Greenland and Antarctica show a variety of plant taxa, e.g. at least 5 orders, 7 families and 6 genera.

The identified plant taxa groups provide evidence for forest vegetation indicative of a climate much milder than what we see in these ice-covered areas today.

Genetic analysis was carried out by means of a new rigorous statistical Bayesian approach that assign the sequences to taxonomic level by calculating the probability that each sequence belongs to a particular taxonomic group by considering its position in a phylogenetic tree based on similar GenBank sequences. The composition of taxa found is compared to vegetation maps of extant plant communities as well as vegetation studies of extinct plant communities. Further aims for this study is to reconstruct the paleoenvironments including ecosystem diversity and climate by obtaining diverse plant, animal and insect DNA from the basal silty part of the ice cores from Greenland and Antarctica with the DNA sequences assigned to their taxonomic levels (family, genus, and species).

**Session 10: 15.50 – 16.10**

**Combining aDNA and climate niche modeling to elucidate the relative impacts of humans and climate on the demographic trajectories of three Late Pleistocene megafauna taxa**

Eline D Lorenzen<sup>1</sup>, David Nogués-Bravo<sup>2</sup>, Jonas Bin Laden<sup>1</sup>, Ludovic Orlando<sup>1</sup>, Michael Borregaard<sup>2</sup>, Jesper T Stenderup<sup>1</sup>, Andrei Sher<sup>3</sup>, Simon Ho<sup>4</sup>, Beth Shapiro<sup>5</sup>, Carsten Rahbek<sup>2</sup> & Eske Willerslev<sup>1</sup>

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Two thirds of the world's genera of megafauna were lost during the Late Quaternary extinctions. Pinpointing the driving forces behind these extinctions has not proven easy, and the relative roles of climate change versus human impact are still heavily debated.

By combining ancient population genetic data, the megafauna fossil record, palaeoclimatic data, climate envelope modeling, and the human fossil record, we present a novel, interdisciplinary approach to elucidating the relative roles of climate change and humans in shaping the demographic histories of three Late Pleistocene megafauna species.

The three species—woolly rhino, musk ox, and reindeer—were all widespread during the Late Pleistocene. Yet they represent three very different evolutionary trajectories and are now either extinct, restricted, or widespread. The woolly rhino was one of the iconic ice age megafauna species, and went extinct c. 12 thousand years ago. The musk ox is severely restricted in distribution and found only in the Canadian Arctic Archipelago and on Greenland. The reindeer numbers in the millions and is today widely distributed throughout the Holarctic.

**Session 10: 16.10 – 16.30**

## Investigating the impact of Late Pleistocene environmental change using ancient DNA from small mammals

Ian Barnes<sup>1</sup>, John Stewart<sup>2</sup> & Selina Brace<sup>1</sup>

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The Late Pleistocene was a period of climatic and environmental change with cycles of glacial advance and retreat throughout. Habitats suitable for arctic fauna expanded south during glacial periods and contracted north during interglacials. There is substantial interest in the process by which faunal populations reacted to these changes, with increasingly detailed reconstructions based on the combination of palaeoecological reconstruction, high resolution radiocarbon dating, and ancient DNA analysis. The primary focus of this work has been large mammal species, the megafauna, in part driven by an interest in the relative roles of human overpredation and climate change in the global extinction of many members of this group. However in order to better determine the mechanisms underpinning the megafaunal extinction, analyses of a range of small mammal taxa are required. Our recent analyses have focused on an ecologically critical arctic prey species: *Dicrostonyx torquatus* (collared lemming). Today the species' distribution stretches across north Eurasia, but during glacials would have spread south into European mid-latitudes. Our work on material from the Belgian Ardennes dating to the last glaciation suggests that *D. torquatus* in northern Europe experienced complete population extinction and replacement around the last glacial maximum (LGM; ~21 ka). In this talk we will outline these results, and place the extinction and recolonisation history of this arctic species in Europe within the broader faunal and environmental setting to establish a more thorough understanding of species response to environmental change.

# Poster Presentations

## **Poster Session**

Wednesday 8<sup>th</sup> September, 2010

16.10 – 18.00



## **Poster #01**

# **Kinship analysis and sex identification of skeletons from two archaeological sites in Greece**

Naglaa Abu-Mandil<sup>1</sup> & Terry Brown<sup>1</sup>

<sup>1</sup>Faculty of Life Sciences, University of Manchester

Ancient DNA offers unprecedented opportunities for anthropologists and bio-archaeologists to assess the biological relationships of ancient populations. This study is designed to assess the family relationship among skeletons from two different archaeological sites in Greece which can help in shedding light on the ritual practice in Aegean prehistory. Another aim is to identify the sex of these skeletons genetically to confirm the conventional sexing methods. These sites are called Kouphovouno and Bostani. Kouphovouno is an important Neolithic and Bronze Age archaeological site near Sparta in Lakonia, while Bostani is dated back to the Early Helladic period in Ancient Greek history. In both cases the sites are recently excavated and DNA samples from all people who have handled the skeletons are available. Both mtDNA and nuclear DNA markers are being studied in order to identify maternal relationships and to reveal the sex of the skeletons.

## Poster #02

### Analysis of mercury levels in historical bone material from syphilitic subjects – pilot studies

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Mercury was used mainly in the Middle Ages and in the early modern period as a means of treating individuals suffering from syphilis (lues), other venereal diseases as well as leprosy. The diseased were recommended to rub mercurial ointment into the body inside a tightly shut, warm room. This procedure was accompanied by mercury vapour inhalation. Such treatment resulted in hypersalivation already during the exposure, and the ultimate results of this medication included damage to kidneys, loss of teeth and hair, brain tissue lesions or even death. The mechanism whereby mercury accumulates in the body is not completely clear. It is assumed that the element may bond with carbonates included in bones or incorporated into hydroxyapatite by substituting calcium ions.

The aim of the present work is to study the remains of four individuals with typical symptoms of tertiary syphilis in terms of mercury content in bones and teeth, thereby verifying whether they were subjected to treatment and, if they were, how long their organisms were exposed to toxic fumes of mercury. Skeletons in which morphological symptoms of syphilis were observed (e.g. caries sicca, bone lesions caused by gummas, articular lesions, general inflammatory reactions of periosteum) used in the present study originate from historical burial grounds in southern and north-central Poland. One of the studied skeletons was unearthed at the burial ground located in Szczepański Square in Krakow (Southern Poland) and belongs to a young woman, deceased at the age of 20–23. The remaining skeletons come from several archaeological sites in north-central Poland (the Pomerania region). They include: a skeleton of an adult (adultus/maturus) woman, discovered in St. Anna's crypt in St. Nicholas Church in Gniew; another skeleton – the remains of a woman deceased at 50–60 – unearthed at a Mennonite burial ground in Pień. Other objects include: an isolated skull of an adult found at a burial ground next to a church in Płonków, an isolated skull with traces of caries sicca belonging to a woman deceased at the age of adultus discovered in a church in Pieranie, as well as the skull of a woman deceased at adultus/early maturus age with similar pathological characteristics found in Strzelno during excavations on the premises of Norbertine monastery.

As far as possible, due to the condition in which the skeletons were preserved, two samples were collected: a tooth and a piece of a long bone. Each tooth was bisected along the bucco-lingual axis with a diamond saw. Tooth halves and bone pieces were rinsed in a spectrally clean water with the application of ultrasound, then dried in a laboratory thermostat at 60°C. The control group included bones of individuals without characteristics typical of syphilis. As an additional background for the analyses, soil samples from the grave environment were used in order to exclude the possibility of contamination of the environment with mercury. The analyses were performed by LA ICP MS technique, with the use of laser Nd: YAG, Macro, 266nm, New Wave, USA, coupled with Spectrometer Elan DRC-e Perkin Elmer, USA. For quantitative analysis standards prepared on the base of calcium phosphate, supplemented with appropriate elements, pressed in pastille, were used. The method was tested using Standard Reference Materials 120c (Florida Phosphate Rock) and 1400 (Bone Ash) both pressed in pastille form.

**Poster #03**

Analyzing the origins of the Spanish wild goat *Capra pyrenaica*  
based on mitochondrial DNA of fossil remains

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The Spanish wild goat *Capra pyrenaica* has been subdivided in four subspecies: *Capra pyrenaica lusitanica*, *Capra pyrenaica victoriae*, *Capra pyrenaica hispanica* and *Capra pyrenaica pyrenaica*. The species was abundant during the Pleistocene in all mountain ranges throughout the Iberian Peninsula. The *Capra pyrenaica pyrenaica* became a threatened species at the beginning of the 20<sup>th</sup> century due to heavy hunting pressure and habitat fragmentation and went extinct during the year 2000. Two main hypotheses have been suggested for the origin of this species *i*) it derived from the *Capra caucasica* and *ii*) it derived from the *Capra ibex*. In this study we intend to test these two hypotheses by analyzing a segment of the cytochrome b of fossil remains of the four subspecies spanning its current and historical geographic distribution. Moreover, further analysis will be done to confirm the relationship of the 4 determined species. In order to this, ancient samples from these subspecies from throughout the whole peninsula will be collected for further DNA analyses. So far, 46 remains have been collected from the Spanish Pyrenean region and the Basque Country, all of them potentially belonging to the subspecies *Capra pyrenaica pyrenaica* based on morphological traits.

## Poster #04

# Palaeogenetics of a critically endangered felid: evolutionary history of the Iberian lynx

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The Iberian lynx, *Lynx pardinus*, classified as Critically Endangered species (IUCN, 2002), is the most endangered species of carnivore in Europe and the most threatened of the 36 existing species of the family Felidae in the world. The Iberian lynx was widely distributed all throughout the Iberian Peninsula until the 20th century, when it became rare and very scarce in the north. Its reduction in distribution and population size began about 200 years ago with a dramatic decrease in the last 50 years. Today there are only 2 isolated populations of Iberian lynx with less than 200 individuals. Currently only one haplotype has been found in the Iberian lynx, based on a small fragment of the D-Loop in the mitochondrial DNA (mtDNA). In order to investigate when the reduction of mtDNA variability occurred and the potential bottlenecks leading to it, we have analysed a fragment of the control region from a series of Iberian lynx fossil and subfossil remains. The samples belong to different geographic points within the peninsula, spanning a time range of approximately 50000 years to the present. The DNA sequences were analysed as serial data by simulating different population sizes and different mutation rates. Only one haplotype was detected in all 19 samples. This haplotype corresponds to the one present in the current populations. The simulations performed in this study show that the lack of mitochondrial DNA variation through time, i.e. one single haplotype in 50000 years, can only be explained by a low mutation rate and/or a small female effective population size.

## Poster #05

# Bangers 'n Mash or Pasta Puttanesca? Insights into the Romanisation of South-West Britain from a dietary perspective.

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This study uses burial data and stable isotope analyses ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of human bone collagen to investigate social differentiation in three Romano-British (1<sup>st</sup> to early 5<sup>th</sup> century AD) cemeteries in South-West Britain. Food is much more than a mere necessity for survival. What we consume is not only being dictated by what is available but also what we can afford. Moreover, religious beliefs or differences in status can influence what we are allowed to eat. Dietary differences, as revealed by stable isotope analysis, can thus yield fascinating insights into past societies and provide an indication of social differentiation, whether due to gender, religion, economic or social status. We compared burial practices and dietary habits in four Romano-British cemetery populations from the south-west of England. In total, we compared bone collagen stable isotope values from 87 individuals, including 41 from Roman Gloucester, and 46 from two rural cemeteries at Horcott Quarry and Cotswold Community respectively. Five individuals from urban Gloucester were buried in a mass grave; all others were buried in single inhumations. Results show significant differences in stable isotope ratios between the urban and the two rural populations and reveal that burial practice (e.g. burial orientation and burial position) does correlate with diet, suggesting that differences in diet were indeed related to social differentiations. These patterns are placed in the wider context of other Romano-British settlements in the region, and although a general pattern is not observed, it is clear that diet is a very sensitive, if settlement-specific, indicator of social differentiation.

**Poster #06**

**Studying killer whale ecology over evolutionary time scales  
using aDNA and stable isotopes**

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Population structuring and speciation can be driven by variation in ecology. Studying this process in long-lived species has typically relied upon phylogenetic analyses that make assumptions about the ecology of ancestral populations. Here we use analysis of isotopic ratios and aDNA sequencing of the mtDNA control region of killer whale (*Orcinus orca*) tooth and bone samples of both contemporary and ancient sub-fossil samples from the North and Norwegian seas, to investigate diet and niche width over evolutionary timescales in this long-lived top marine predator. Our results indicate a broad niche width due to individual specialisation within one lineage in both the contemporary and ancient samples.

## Poster #07

### Detection of Epigenesis in Archaeogenetic Systems

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Epigenetics is a process occurring at the forefront of evolutionary change. It refers to the action and effects of gene expression / regulatory mechanisms which are not caused by changes to the organism's genetic code. Instead, various processes such as histone modification, chromatin remodeling, DNA methylation and post-transcriptional silencing cause changes in gene expression that can result in significant phenotypic changes. Epigenetic changes, and thus phenotype, can be unique to individuals but can also be inherited transgenerationally, making them significant drivers in evolutionary change.

A major area of epigenetics currently under investigation is that of small RNAs (sRNA) - sequences of around 19 – 25nt in length that act as gene regulators. By acting as 'targeter' molecules for various protein complexes such as RISC (RNA-induced silencing complex), small RNAs can induce post-transcriptional silencing and also induce histone modification / DNA methylation via RNA-induced transcriptional silencing (RITS). Small RNAs, due to their size, are stochastically less likely to be broken than larger molecules and are hence ideal candidates for ancient RNA study, where depositional environmental conditions have been suitable. The exceptional preservation of genetic material in samples of archaeological barley (*Hordeum vulgare*) from Qasr Ibrim makes them ideal candidates for ancient sRNA analysis, which will take place using Illumina Solexa sequencing technology. This poster represents research being undertaken on RNA-mediated epigenesis within the archaeogenome; by specifically selecting for and isolating small RNA, we intend to build an expression profile from an apparently drought adapted variety of ancient Egyptian barley.

Once we have built up an expression profile of sRNA, we intend look for epigenetic activity across the genome that may be involved in adaptation to drought. To confirm any evidence of epigenetic drought adaptation in the Qasr Ibrim barley, its sRNA expression profile will be compared to a modern, cultivated crop from the same geographic region showing no such adaptation.



**Poster #08**

To move or not to move: The fate of glacial European *Lagopus* *sp.* after the Ice Age

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During the last glaciation, midlatitude Europe was dominated by tundra-steppe vegetation, harbouring widespread populations of cold-adapted species. By the Pleistocene-Holocene transition, these habitat types became greatly reduced and can today only be found in high latitude and alpine areas. In this study we investigate how cold-adapted species responded to these severe climatic changes, and evaluate the hypothesis of habitat tracking. The *Lagopus* genus, now restricted to northern and alpine regions, had an expanded distribution during the Late Pleistocene period covering more southern and lowland ranges. Here, we use ancient DNA techniques to examine the fate of the Ice age population by analysing genetic variation within the mitochondrial control region in *Lagopus sp.* fossil remains, sampled throughout the genus' former large European range. By comparing these results with sequence data from extant European populations of *L. muta* and *L. lagopus* we test the hypothesis that the widespread glacial population managed to track its habitat as it shifted to northern and alpine regions, subsequently founding our contemporary European *Lagopus sp.* populations. A contrasting hypothesis is that the glacial fossil remains represent lineages that went extinct during the onset of Holocene, and that our contemporary *Lagopus sp.* populations have been established by post-glacial immigrants from the Beringian population in Siberia. The results from this study demonstrates that DNA can be retrieved from Pleistocene bird remains, and based on genetic similarities between glacial and extant European *Lagopus sp.* populations suggests that both *L. muta* and *L. lagopus* were able to track their shifting habitat during Holocene climate warming.

**Poster #09**

## Coalescent-based Simulations of Population Ancestry of the two-humped Camel (*Camelus bactrianus*)

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The domestication of the two-humped camel (*Camelus bactrianus*) has promoted unprecedented progress in cultural and economic development for ancient human civilisations in the cold deserts of Eurasia. However, time and place of the origin of domestication of these animals remain unresolved, as only few references are available in this field of research.

Therefore a 460bp hypervariable fragment of the mitochondrial DNA (*mtDNA*) control region was analysed in 12 bone samples of *Camelus bactrianus* from Late Bronze and Early Iron Age sites of Uzbekistan and Siberia. A subsequent comparison with sequences of 122 recent domesticated bactrian camels from China and Mongolia as well as with 20 recent wild camels (*Camelus ferus*) from Mongolia by Arlequin showed that the extant wild camels are neither the ancestors of the aDNA-samples, nor modern domesticated two-humped camels. A kinship between domesticated Bronze/Iron Age camels and modern ones was supposed, but  $F_{st}$ -values showed no significance.

Furthermore, different ancestry scenarios were modeled and tested by serial coalescent simulations, whether the differences of genetic diversity observed within and between the populations can be explained under the null hypothesis of population continuity alone.

## Poster #10

# A Swedish whale fall from the late Pleistocene – on shore displacement, paleoecology and the identity of the Swedenborg whale (*Balaena swedenborgi*)

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The Swedenborg whale *Balaena swedenborgi* is a baleen whale species believed to have existed in the North Sea from the period when the inland ice melted around 13,000 BP until about 8,000 years ago, when it died out. The first bones attributed to this species were found in Sweden in 1705. Gigantology was an established science at this time and it was commonly believed that whale bones were the remains of giants that once walked the earth. However, the Swedish scientist Emanuel Swedenborg identified the bones as that of an extinct species of whale, and later it was described under the name *Balaena swedenborgi* Lilljenborg, 1868. However, in more recent times it has been suggested that the bones should be identified as belonging to other species, such as the Greenland whale, and that the Swedenborg whale never existed.

In June 2009 a whale fall was discovered outside Strömstad on the west coast of Sweden during the work on the extension of the E6 motorway. It was speculated immediately that this could be one of the extinct Swedenborg whales. The bones were found 72 meters above present day sea level. Surrounded by oxygen-free fine clay, the bones showed excellent preservation and samples were taken for genetic analysis and radiocarbon dating. Shelly remains of marine organisms were present in the clay deposit surrounding the whale fall and sediment with associated specimens were therefore collected for further analyses. Here we will give a preliminary report of the results from our multidisciplinary project: radiocarbon dating, species identification, palaeoecology, shore displacement and the importance of whale falls in marine environments.

## Poster #11

# Application of animal manure in prehistoric farming revealed by analysis of nitrogen isotope ratios

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The use of animal manure has experimentally been shown to be reflected in plant isotopic signature ( $^{15}\text{N}/^{14}\text{N}$ ). Isotopic analysis of archaeologically excavated ancient grains is potentially a promising new methodological tool to reveal the beginning and onset of prehistoric manuring intensity in a more direct way than archaeologically possible. Evidence of manure effecting plant isotopic signature is, however, based on studies of fresh plant material. As prehistoric grains generally are found in a charred stage it has been tested whether the isotope ratio is affected by charring. Preliminary results indicate that the degree of distortion caused by charring is negligible. Charring does not seem to disturb the isotopic signature in cereal grains significantly, which holds good promise for the application of the method in order to answer agroarchaeological problems, such as the introduction of systematic human usage of animal manure as fertilizer to enhance yield and productivity in crop production.

The effect of manuring in plant isotopic signature ( $^{15}\text{N}/^{14}\text{N}$ ) most probably also induces changes in the overall food chain. This give rise to further implications for the reconstruction of prehistoric diet based on isotope analysis. New and ongoing research aims at incorporating this methodological approach when addressing archaeological problems concerning dietary and agricultural reconstruction. The wider implications of the method still remain to be fully understood and appreciated for which reason further work is needed to fine-tune and disseminate this new approach.

## Poster #12

### Viking Age Diet. Variations within and between individuals.

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This poster presents results from the first and so far only systematic Danish conducted investigation of the stable isotopes C and N in skeletons from a single Viking Age grave site. The site Galgedil, on North Funen is size-wise moderately with more than 50 burials and offers extremely well-preserved bones.

Isotopic analysis in archaeology is often first and foremost aimed at reconstructing prehistoric diet. This objective is challenged by limitations in our understanding and interpretation of the 'mantra': 'You are what you eat'. More exact isotope mapping of likely food sources is also still needed. New research has demonstrated that animal manure effect the <sup>15</sup>N/<sup>14</sup>N isotope rate and results in elevated values. This difference is expected to influence the very food chain in general and thus add on yet another query to the discipline of diet reconstruction.

The method of isotope analysis is in addition also a potential tool in studying social and ethnic identities. The Vikings at Galgedil was examined due to assess whether the buried Vikings at Galgedil diet-wise constituted a homogenous population. Isotopic data was examined on three different levels:

- 1) intra-individually (life history)
- 2) inter-individually (in relation to sex, height and status)
- 3) Population wise.

This provides a more qualified understanding of Viking Age food ways. The poster deals primarily with level 1) and 2) results. Men and women had slightly but significantly different diet. Some individuals had very interesting diet life histories. Some slaves had a distinctly different diet.

Besides presenting results from a specific case study the poster also aims to emphasize that dietary studies are more than just about the actual menu, ingredients, recipe and meal. Dietary studies deals with the norms and variations within the entire sphere of food and the cultural aspects of food and eating.

## Poster #13

# Strontium isotope evidence of the mobility patterns of Eastern Iberian Neanderthals

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Zooarchaeological and spatial analyses studies from Middle Palaeolithic Eastern Iberia sites show a high level of mobility in Neanderthal populations. Additional evidence for this mobility pattern (which is also observed in other Iberian Neanderthal populations) is provided by short and spontaneous occupation periods of caves and rockshelters as well as by studies of alternating carnivore-human occupations in the sequences of the sites for this period. To provide additional evidence to evaluate the territorial range for Neanderthal mobility patterns in this area we undertook laser-ablation strontium isotope analyses of enamel and dentine from eight teeth from eight different individuals from three Valencian Middle Palaeolithic sites in the Valencia Region. This data was compared with baseline local strontium isotope values determined through the measurement of solution strontium isotope values of modern plants and shells from the region. The value of the  $[87\text{Sr}/86\text{Sr}]$  was measured using a ThermoFinnigan Neptune multi-collector inductively coupled plasma spectrometer (MC-ICP-MS) coupled with the NewWave UP213 laser-ablation system.

**Poster #14**

**Isotope evidence for human diets in the Mesolithic and Neolithic periods of Eastern coastal Iberia (València)**

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We report here on the results of carbon and nitrogen stable isotope analysis of humans and fauna from twelve Mesolithic and Neolithic sites from the Valencia region of Iberia. In other parts of Europe there is isotopic evidence for an abrupt change in diet between these periods, especially in coastal regions of Northern Europe. To date, there are few isotopic studies for these periods from the Mediterranean. This poster reports new isotopic data from the eastern Iberian Peninsula, from sites dating to the Mesolithic, and early, middle and late Neolithic. The results show no major diet shift between these two periods, and confirms the consumption of marine protein resources during the Neolithic.

## Poster #15

# The Maltese through time: A comparison of prehistoric, Roman and modern Maltese mitochondrial DNA haplotypes

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The Maltese islands are a small archipelago situated in the middle of the Mediterranean Sea. Throughout history these islands have been dominated by the Mediterranean power of the era due to their strategic importance in controlling the shipping lanes between the eastern and western Mediterranean Sea. This study compares ancient DNA amplifications from a prehistoric site situated on the island of Gozo, two Roman burial sites in Malta, one of which is found in an urban context whilst the other in a rural context, and a sample group from the modern Maltese population. By analysing mitochondrial DNA Hypervariable Region 1, due to its higher copy number and survivability, this project aimed to study if any changes to the population of the islands can be observed through time. Another aim of the study was to see if any unique haplotypes might have survived these colonisations, and might still be present in the modern population. The modern Maltese population was also compared with other modern populations in the region in order to ascertain who it is most closely related to, and thus, which neighbouring influence most closely affected the matrilineal line of the Maltese population.



## Poster #16

# Tuberculosis in ancient Roman skeletal remains: two different ancient DNA approaches

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Ancient DNA (aDNA) analyses of the causative agents of tuberculosis, namely the *Mycobacterium tuberculosis* complex (MTBC), have successfully supported the palaeopathological diagnosis of tuberculosis in a number of cases. These studies have mainly applied standard PCR procedures, targeting a fragment of the MTBC-specific insertion sequence IS6110, followed by agarose gel electrophoresis and direct sequencing of the products obtained. Further targets, such as parts of IS1081 and the *rpoB* gene have been used to additionally support some of these results. Sequencing of the complete genome of some members of the MTBC now allows insights into species and strain variability and has, recently, even enabled lineage attribution of members of the MTBC detected in archaeological samples. However, specificity of the results obtained in aDNA studies on tuberculosis might sometimes be questioned as standards in ancient DNA procedures are not followed rigorously. New methodological approaches using probe-based real-time PCR can enhance specificity of the results and can replace the laborious authentication step of cloning. Here, the preliminary results of screening for IS6110 in Roman skeletal samples from the UK and Continental Europe are presented. The results obtained by targeting a 123bp product of IS6110 will be compared to those obtained with a probe-based real-time PCR approach. The outcome of this initial screening process will determine whether the skeletal remains investigated are suitable for further examination with regard to strain variability between the members of the MTBC.

## Poster #17

# Isolation of single amino acids from soluble collagen for radiocarbon dating

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Bones from archaeological sites have been radiocarbon dated successfully for the past several decades. Isolation and analysis of bone-specific molecules continues to be of interest, however, as a way of eliminating contamination from soil and museum treatment. Hydroxyproline (Hyp) serves as a good biomarker for collagen, consisting of about 10% of bone collagen but not found in significant amounts elsewhere in nature.

It has been reported that bones with little or no insoluble collagen left may still contain indigenous soluble amino acids, probably originating from collagen leaching. If sufficient amounts of hydroxyproline could be isolated from non collagenous bones, then those bones that would normally fail the requirements for dating could be radiocarbon dated.

In order to characterize the soluble collagen fraction (SF), bones were ‘cooked’ for different lengths of time prior to collagen extraction, as a way of mimicking diagenesis. We will present data showing that a distinct soluble collagen fraction exists, and its amino acid content.

The protocol adopted for purification of single amino acids from the SF starts with bone protein demineralization in a dialysis tube, to release the SF from the hydroxyapatite, and at the same time remove the minerals. The fraction is then further separated from non collagenous proteins (NCPs) and humic acids using a cation exchange resin. Finally, single amino acids are obtained using a mixed mode semi-preparative HPLC, and graphites are produced to be dated on the accelerator mass spectrometer. Results of the amino acid content of soluble collagen extracted this way will be presented.

## Poster #18

### Ancient mitochondrial DNA from domestic pigs (*Sus scrofa*): tracking human dispersals in ancient Anatolia

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In the last few years, archaeological and biomolecular studies of pigs have become an important proxy to understand the human dispersals. Particularly regarding genetics, the strong phylogenetic structure that has been observed at the level of mtDNA in pigs has proved to be decisive for tracing back the origin of pig lineages, and for reconstructing the domestication processes. Recent studies highlighted that pig domestication occurred independently in several diverse geographic locations across Eurasia. For the Near East, archeological evidence points to complete domestication processes by at least 8,000-7,500 years BP (uncalibrated). As attested by ancient DNA data, domestic pigs of Near Eastern ancestry were introduced into Europe in the 6<sup>th</sup> millennium BC, and later replaced by the pigs which were independently domesticated in Europe. By at the least the 7<sup>th</sup> century BC domestic pigs of European ancestries spread eastward to Armenia.

The main goal of our study is to reconstruct the human migratory trajectories which took place in the eastern Anatolian peninsula from the Bronze Age. For this purpose, a genetic survey of 62 archaeological pig samples from the site of Lidar Höyük is being carried out. The relevance of this study stands in the long chronological sequence of the pig samples, spanning from the Bronze Age to the Medieval period, and the fairly good state of preservation of ancient DNA. This allows reconstructing a timeframe within which to document the main population movements in this Anatolian region. In this context we present here the preliminary results of the research.

## Poster #19

# A new specialised laboratory for genetic analyses of faunal remains from archaeological sites at the Centre for Baltic and Scandinavian Archaeology

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Although many questions of ancient DNA analyses are ecological, cultural and historical, most aDNA-laboratories, however, are part of forensic, anthropological or molecular biological institutions, in which archaeological, archaeozoological, and palaeoecological questions are often subordinated. Against this background the Centre for Baltic and Scandinavian Archaeology in Schleswig (Schleswig-Holstein, Germany) established a specialised laboratory for archaeologically relevant genetic research. Because we are first and foremost line interested in the study of the prehistory of the mentioned area, the laboratory concentrates on the research of faunal remains with main regards to research topics of regional character. One reason of this focussing on northern Central Europe is that we will be able to explore our comprehensive collection with some millions of subfossil animal remains from the Late Glacial period up to Medieval times. Most of these bones have been studied with morphological methods, and the results of these investigations are published.

Evidently, such investigation of animal bones from northern Germany is of broader interest, because the Jutland peninsula served since the deglaciation as a land bridge between Central Europe and Scandinavia as well as between the Baltic and the North Sea. Cultural processes that emerge here are decisive for understanding the cultural development in other regions of northern Europe, not only in Denmark but also in Norway and Sweden. Accordingly, we are interested in an intensive cooperation with other colleagues working in this area.

## Poster #20

# Diet and Health in the Prehistoric Pacific Islands: New Insights from Stable Isotope and Dental Analyses

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It is well recognised that the reconstruction of prehistoric diet can shed light on ancient subsistence practices and, in the case of stable isotope analysis, can illuminate sex and age-related trends in diet that are potentially a response to cultural processes. The scarcity of prehistoric Pacific Island human remains has resulted in relatively few studies focused on either stable isotope analysis or dental assessments in this area of the world. In this study, two prehistoric Pacific Island skeletal samples from Nebira, Papua New Guinea and Taumako, Solomon Islands (both dated around 400-600 BP), are assessed by both chemical and dental analyses to investigate patterns of diet and health in Near Oceanic communities that lived in environments biogeographically distinct from one another. This study aims to reconstruct the diet to further understand subsistence practices and possible sex-related differences in diet within these communities. The  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  values of bone collagen were used in conjunction with dental wear and dental health indicators (caries, calculus, periapical cavities and periodontal disease) to address these aims. When assessed in relation to a dietary baseline of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  values of Pacific Island plants and animals from previous studies, the  $\delta$  values suggested these populations practiced very different subsistence regimes from one another. The  $\delta$  values indicated the population from Nebira relied heavily on terrestrial-based protein sources from lower trophic levels with some type of  $\text{C}_4$  plant component or the consumption of animals that ate  $\text{C}_4$  plants, such as wallabies. On the other hand, the  $\delta$  values of the population from Taumako suggested that the major dietary protein sources were from the marine environment, most likely originating from both pelagic and reef ecosystems. The positive correlation between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  suggested terrestrial protein (probably root vegetables) also played a role in the diet at Taumako. The dental wear and health indicators generally supported the dietary interpretations, although the potential consumption of the cariostatic betel nut (an intoxicant) at these sites could possibly have influenced the dental health results. No significant sex related differences in diet were observed in either sample suggesting that, at this time in prehistory, males and females had access to similar foods.

**Poster #21**

**Parallel tagged amplicon sequencing of highly degraded Y-chromosomal DNA from archaeological skeletons**

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The intent of the study was to develop a Y-SNP multiplex-PCR suitable for genetic analysis of ancient human remains. Therefore 37 SNPs characterizing Eurasian haplogroups, with a focus on Europe and Central Asia, were selected in order to get a high phylogeographic resolution.

The 37 SNPs, using amplicon lengths between 64 and 107bp, were co-amplified within 2 multiplex PCRs followed by parallel tagged sequencing on the 454 platform. After testing on 3 recent male and 2 recent female individuals it was applied to 8 male prehistoric samples from Central Asia and Europe. One sample was too poorly preserved for haplogroup identification. Another individual could be narrowed down to Q or R\*. The haplogroups of the remaining 6 samples could unambiguously be defined. This shows that this approach is adequate for Y-chromosomal typing of highly degraded ancient human remains.

## Poster #22

# The historical ecology of the 19th century East African ivory trade: Preliminary results and ongoing challenges of using isotope analysis to provenance historic ivories

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During the 19th century, East Africa became a major source of elephant ivory for a range of rapidly expanding industries, including cutlery, comb, piano and billiard-ball manufacturers. The scale of extraction was enormous: between 1840 and 1875, British demand alone rose from 200,000 kg per annum to over 800,000 kg per annum, and even the more conservative estimates based on historical trade records suggest that as many as 12,000 elephants a year were being killed. However, there is a problem with historical data in that they are patchy and record only the point of export rather than the area of extraction. Knowing the area of extraction in further detail could shed light on historical arguments about which areas of East Africa were considerably depleted of elephant populations, which would have had severe impacts on trade patterns and the ecology of specific regions.

Given that the varying geology and climate of the East African region produce distinguishable isotope signatures in the ivory (and other tissues) of elephants, it is becoming possible to provenance ivory using a combination of isotopes including  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$ , and  $^{87/86}\text{Sr}$ . These results include isotope analyses from museum samples of historic elephant bone, tooth, ivory, and tail hair as well as archaeological ivory from excavations in Kenya and modern reference samples collected in Kenya and Tanzania. The results not only support the use of isotope analysis for provenancing historic and archaeological ivory, but also highlight the use of isotope analysis as an ecological tool for understanding diet and habitat changes between historic and modern elephant populations living in the same regions.

## Poster #23

### Intra-tooth trace element analysis for hypsodont mammal species: innovation and first archaeozoological example at *tell Halula* site.

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Biogeochemical analyses had been increased and broaden during the last decades in archaeology with success results. Trace element analysis had been recently rethought and more accurate qualitative information is now available. This work shows our latest advances for its integration in archaeozoology. More exactly, our purpose is to contribute to the better understanding of information provided by alkaline-earth elements in intra-tooth variation studies from hypsodont mammal species. Dental remains of goats (*Capra hircus*) and sheep (*Ovis aries*) recovered from Pre-Pottery Neolithic-B site of tell Halula (Middle Euphrates Valley, Arab Republic of Syria) were analyzed. Enamel samples obtained perpendicularly to the growth axis (i.e. sequential analysis) exhibit a trend encompassing more than one seasonal cycle in agreement with tooth development and period of the enamel formation. Results show that elemental concentrations are amazingly preserved and cycle through the growth axis of tooth. Absolute data and sequential Sr/Ca & Ba/Ca concentrations show enormous inter-specific differences while intra-specific differences shows distinct individual histories. Since observed patterns could be likely explained in terms of seasonal consumption of plants, the results could additionally suggest different herd management strategies (human control of animal food, foddering herds during periods of less available resources without a seasonally mobile pattern) during entire annual cycles. These data contain significant information for our effort to understand the kind of management strategies developed with the first herds of domesticated animals.



## Poster #24

# Stable Isotope Analysis of Human Diet from the Proto-Shang Cemetery at Nancheng, China: Implications for Diet and Social Status

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Carbon and nitrogen isotope values were measured from human and non-human faunal bones from the early historic cemetery site at Nancheng, Ci County, Hebei province, China. Grave goods and gender-related differences in burial style are apparent at this site. 75 humans and 2 animals' samples had suitable collagen for isotopic analysis. The  $\delta^{13}\text{C}$  values obtained from human ranging from -7.8‰ to -5.8‰ (average  $-6.8 \pm 0.4\text{‰}$ ) indicated that human diet almost completely relied on C4 food (presumably broom millet or foxtail millet) in that time period. Carbon isotope value for one individual (M70,  $\delta^{13}\text{C} = -14.9\text{‰}$ ) was significantly different from other humans, suggesting that its diet included a mix of C3 and C4 plant-derived components. Comparing to those pure C4 consumers, the lower C4 component (estimated at 47%) reflected that M70 might be an immigrant. The faunal isotope data also indicated a mixed diet of C3 and C4 plants. The large range of  $\delta^{15}\text{N}$  values measured in both human and faunal samples suggests that there was a wide range of dietary protein sources. The differences in the human dietary patterns might be associated with social status-related differences, such as sex, age, grave goods, burial style etc. This data will provide evidence for dietary divisions relating to regional hierarchical organization of settlements and social stratification in this early state society.

**Poster #25**

**Detection of *Yersinia pestis* *pla* and *pst* DNA sequences in late medieval skeletal finds from Manching-Pichl (Upper Bavaria)**

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*Yersinia pestis*, the causative agent of plague, is held responsible for three human pandemics: The Justinian plague, the Black Death and the modern plague.

In 1894, the plague bacterium was discovered during an epidemic of plague in Hong Kong. However, whether *Y. pestis* was indeed responsible for the medieval epidemics is still discussed controversially. Against this background, our research group investigated a mass burial which was discovered underneath the sacristy of the Catholic church St. Leonhard in Manching-Pichl, near Ingolstadt in Bavaria.

Up to now thirty-three skeletons have been analysed in search of the presence of *Y. pestis* DNA. At no time of these investigations modern *Y. pestis* DNA was introduced into the institute to avoid a contamination of the ancient samples.

The molecular genetic analyses were directed to DNA sequences of the *Y. pestis* high copy number plasmid pPCP1, particularly on the plasminogen activator gene (*pla*) and the pesticin gene (*pst*). With regard to the skeletons under study *Y. pestis* DNA was detectable in ten skeletons. We therefore could confirm the hypothesis that the mass burial below the vestry of the St. Leonhard church contained human remains who presumably died of plague.

## Poster #26

### Different techniques for screening ancient DNA in bone

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The application of biomolecular approaches to Neolithic sites has been recognised as a particularly exciting prospect for assessing the socio-economic impact of agriculture and pastoralism and for determining the socio-biological processes of domestication. As ancient DNA (aDNA) analysis is expensive and extremely time-consuming, methods which are useful for predicting bone samples likely to yield aDNA are warranted. Here we used a range of different geochemical techniques to determine the state of organic and inorganic preservation of human bones from Mora Cavorso cave site in Lazio (Early Neolithic 5,472-5,314 BC).

In this study, we present the preliminary aDNA investigation of human samples from this Italian prehistoric cave site. We extracted and analyzed mitochondrial DNA (mtDNA) from four individuals, in particular we studied the D-loop mtDNA region, both the hypervariable regions I (HVS-I) and II (HVS-II) and informative single nucleotide polymorphisms (SNPs) within the coding region.

For the organic component, the amount of collagen was measured gravimetrically and the rate of racemization of both whole bone and the acid insoluble fraction was determined by gas chromatography. For the inorganic component, the infrared splitting factor (SF), the ratio of carbonate to phosphate (C/P), the presence of francolite (F-apatite) and amounts of CaCO<sub>3</sub> were determined by Fourier Transform Infrared Spectroscopy (FT-IR). In addition the surface area and the pore distribution parameters were also evaluated by nitrogen adsorption-desorption isotherms at 77 K, following the BET (Brunauer, Emmet, Taller) and BJH (Barret, Joyner, Halenda) methods. The results of this analysis show that the bone from Mora Cavorso had undergone little diagenetic alteration i.e. observing the SF and the amino acid racemization results, and this is commensurate with the finding of DNA preserved in this material.

## Poster #27

### Direct linking of diet and consumer amino acid $\delta^{13}\text{C}$ values

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Detection of marine food consumption by ancient humans is an area of much debate, with particular regard to the Mesolithic-Neolithic transition where some have suggested that marine resources were abandoned with the advent of farming. The most common method to determine marine consumption by archaeological populations is the use of bulk collagen  $\delta^{13}\text{C}$  values, whereby more enriched  $\delta^{13}\text{C}$  values result from marine consumption while more depleted values represent terrestrial  $\text{C}_3$  consumers. Bulk  $\delta^{13}\text{C}$  can only separate marine and  $\text{C}_3$  terrestrial consumers when the proportion of marine food is above ~20% of the diet. Some individuals with bulk collagen  $\delta^{13}\text{C}$  values that appear to be terrestrial could therefore be consuming significant proportions of marine food. We contend that the ability to separate low level marine protein consumers from purely terrestrial consumers will be improved by undertaking compound-specific stable carbon isotope analyses of the individual amino acids that comprise bone collagen.

Controlled feeding studies allow direct linking of dietary isotopic composition to consumer tissues. Previous experiments have included studying the effects of  $\text{C}_3$ ,  $\text{C}_4$  and marine diets on the carbon isotopic composition of rat tissues. A new study has been designed and is in progress which further expands the relationship between isotopic composition of diet and consumer tissues with particular focus on the marine component: Pigs, metabolically robust analogues for humans, are being raised on a range of controlled diets containing constant amounts of crude protein (20% of the whole diet) but with five different percentages of marine and  $\text{C}_3$  terrestrial proteins: Diet 1: 0% marine protein, 2: 12.5%, 3: 25%, 4: 50% and 5: 100%. These diets were formulated to provide adequate nutrients for the pigs throughout their lifetimes. The experiment is running over two generations and the results from tissue analyses will provide new biochemical and metabolic insights to the marine isotope signal of bone collagen and the degree to which compound-specific and bulk stable isotope signals can reveal the consumption of marine diets in ancient humans. This paper discusses the experimental design and preliminary  $\delta^{13}\text{C}$  analyses of the diets and pig tissues by LC-IRMS.

## Poster #28

# Provenance studies of archaeological textiles applying a new method based on the Strontium Isotopic System: The “Huldremose” case study

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In the last two decades, measurements of strontium isotopes in archaeological bone tissue / skeletons have shown to be an effective technique for the characterization of human and animal mobility in prehistory. Recently a new method, also based on the strontium isotopic system, has been developed in order to address questions regarding the provenance of the raw material of archaeological textiles. The focus is mainly on wool fibers, as hair can be considered a minor excretory organ, and has therefore been often used as biopsy material for proxies of the body. Presently, this method is being applied to Danish pre-Roman Iron Age (500 BC- 1AD) garments, which are part of one of the best-preserved textile collections from European prehistory. Several textiles from the peat bog site of “Huldremose” located on the northeastern part of Jutland were analyzed with this novel method. Textiles from the “Huldremose” site have been recovered in two occasions. 1) The first textiles were unearthed from the site together with a woman's bog body and consisted of several wool garments and skin capes (on display at the National Museum of Denmark). In addition, the authors discovered threads made of plant fibers during the course of this study which were also analyzed. 2) Later, and independently, unusually large garment, which had been deposited in the bog as a single deposition, was also recovered. The several strontium isotopic analyses of two wool garments, one plant fiber threads, and skin from the bog body as well as peat bog samples from the site are presented and discussed in this poster. The strontium isotopic results from the “Huldremose” site show that wool trade in pre-Roman Iron Age has been larger than previously anticipated. The growing interest in the study of archaeological textiles and their potential to elucidate not only historic and prehistoric textile technology, but also socio-economic and even religious purposes, makes this method an important new tool.

## Stable isotope evidence of Early Neolithic subsistence strategies in South-eastern Italy

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As with other parts of Europe, the transition from foraging to food production in Southern Europe marks a fundamental and irreversible change in the social and economic trajectory of this region. Unlike other parts of Europe, especially the north, palaeodietary investigations aimed at determining the speed and completeness of this change have only recently begun in earnest. Here we have completed carbon, nitrogen and sulphur stable isotope analysis of some of the earliest Neolithic human remains and fauna from South-eastern Italy, dating to the 6<sup>th</sup> Millennium BC, in order to reconstruct human subsistence strategies. This region is often considered to be one of the first to be ‘settled’ by farmers, arriving from Eastern Mediterranean and as such we may postulate that their diets would have been relatively homogenous and based largely on cultivated cereals and the meat and milk from domestic sheep and goat. To examine this scenario, we consider the isotope data in association with geographical location, i.e. coastal or inland, to see if there is any evidence for variability in the consumption of marine, terrestrial herbivore and plant resources during this period.

**Poster #30**

**“Try new XXX Ultra: nothing analyses your fossils faster...”**

Molly Crisp<sup>1</sup> & Kirsty Penkman<sup>1</sup>

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The development of analytical technologies can provide powerful new tools for biomolecular archaeology, particularly as they tend to increase both sensitivity and resolution, vital for the analysis of the low biomolecule concentrations within fossil material. However, the updating of old methods to suit the new technologies presents its own challenges: manufacturers don't focus on the small (but very important!) world of ancient biomolecules during development, so each new advance has to be adapted for fossil material. In liquid chromatography, the last few years have seen the rapid development of “Ultra” High Pressure LC, enabling much faster run times and better separation. In this poster we present the trials, tribulations and triumphs of converting established HPLC methods for fossil samples to suit the better, faster world of UPLC.

## Poster #31

### Blubber lamps in the Ertebølle?

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Seventy-five years ago, the Danish archaeologist and ethnographer Therkel Mathiassen proposed that the small oval ceramic bowls of the Ertebølle culture of northern Europe were used as oil-burning lamps for heating and illumination. Introduced in the early 5<sup>th</sup> millennium BC, late Mesolithic Ertebølle pottery is found in two distinctive styles – pointed-based vessels with an S-profile and oval bowls with a curved base. In a paper entitled ‘Blubber lamps in the Ertebølle culture?’ Mathiassen described a number of vessels in the collections of the National Museum in Copenhagen drawing on the analogy of the use of soapstone or ceramic lamps among the Inuit and suggested that oil from seal or whale was the most likely fuel. Although other uses have been suggested these distinctive Ertebølle vessels have become known as ‘blubber lamps’.

As part of a wider investigation into pottery use among late foragers and early farmers in the Baltic, we have conducted lipid biomarker, bulk and single-compound isotope analysis on eight ‘blubber lamps’ from Denmark and Germany in order to identify organic residues associated with the use of these vessels. Specifically we aim to confirm whether the analytical data is consistent with the use of these vessels as lamps. This prompts wider consideration of the context of use of these vessels in the Ertebølle and necessitates evaluation of their disappearance at or soon after the introduction of farming in the region.



## Poster #32

### Ancient *Yersinia pestis* sequences retrieved from 14<sup>th</sup> century plague victims

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In the Middle Ages, the second plague pandemic ravaged all over Europe killing millions of people in a very short time. Plague pits associated with either the Black Death or its resurgences were excavated from North, Central and South European sites. Biological material from 76 individuals from five putative plague mass graves dated to 14<sup>th</sup> – 17<sup>th</sup> century were analysed in the ancient DNA laboratories at the University of Mainz. Our goal was to genetically establish whether the causative agent of the second plague pandemic was *Yersinia pestis*, as shown by several previous aDNA studies, or if other agents have to be considered as suspected by some other scholars. *Yersinia pestis* DNA was successfully amplified in ten individuals and for those collectives, which gave no aDNA results, an immunochromatographic test helped to investigate the protein content in skeletons. After genetic confirmation of the nature of the bacterium, our major goal remains the determination of its genetic relationship with extant bacteria from all over the world and its phylogeographic origin.

### Poster #33

## Using ancestral and contemporary DNA in Peary caribou to examine the relative loss of microsatellite and Mhc variation following population bottlenecks

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Scientists generally use neutral sequences of DNA to estimate genetic variation in endangered species. However, neutral sequences may not reliably indicate adaptability because they are unrelated to fitness by definition and may lose variation more rapidly than functional genes. This study uses threatened Peary caribou (*Rangifer tarandus pearyi*) to compare loss of genetic diversity at neutral and functional loci by comparing historical and contemporary DNA. Specifically, it: 1) tests if current levels of genetic variation in Peary caribou have been similarly or differentially reduced at neutral versus fitness loci as a consequence of population bottlenecks; and 2) provides data to recovery planning by identifying accurate methods to assess and manage genetic variation for threatened species generally. We report preliminary data comparing genetic variation at 8 microsatellite and one Mhc locus between Ellesmere Island historical samples (n~30 samples) collected c. 1905 prior to population bottlenecks and contemporary samples (n~50 samples) collected c. 2001 from post-bottleneck populations.

## Poster #34

# Ancient DNA Applications to Environmental Archaeology

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In a world increasingly concerned with the effects of climate change, eroding biodiversity and human-induced environmental degradation, researchers are seeking concrete information about past ecosystems and baseline environmental data. Archaeologists have the capacity not only to offer unique insights into the diversity and abundance of ancient plant and animal communities, but also to elucidate the dynamic interrelationship between humans their ecosystems. Ancient DNA analysis of archaeological remains can provide an additional level of detail through unambiguous species, population and sex identifications, as well as by characterizing the size, structure and diversity of ancient populations. We have termed this emerging field of study ‘Molecular Environmental Archaeology’. Fruitful collaborations between ancient DNA researchers and archaeologists can reveal how anthropogenic pressures such as hunting, harvesting and controlled breeding have affected the distribution and diversity of past faunal species. Moreover, this interdisciplinary research can highlight how past human populations responded to natural shifts in species’ diversity, abundance and behaviour. Through a series of case studies, this poster will highlight how Molecular Environmental Archaeology has contributed to our understanding of: 1) past human responses to climate-induced fluctuations in resource availability; 2) the long-term effects of over-harvesting on species distributions and genetic diversity; and 3) the effects of imported domestic animals on the distribution and diversity local wild populations through time.

## Poster #35

### Spatial variations in biosphere $^{87}\text{Sr}/^{86}\text{Sr}$ in Britain

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The potential of  $^{87}\text{Sr}/^{86}\text{Sr}$  isotope composition to the tracking and origin of material, whether it is humans, animals, water or wine can only be fully realised if high-quality reference data sets are available against which to compare the unknowns. Until recently, in the UK, such data were not available. Despite the rapid increase in  $^{87}\text{Sr}/^{86}\text{Sr}$ -based provenance studies of biosphere materials there were no well documented maps available. Most researchers resort to generating reference maps from a variety of geological datasets and the analysis of sparse “environmental” samples. A major reason for this lack of reference material is the investment needed to undertake systematic sampling and analysis over large areas. A further problem is associated with choice of proxy material, as organisms differ in their habitat and exploitation of resources. We present a map of strontium isotope variations across Britain for use in archaeological and other studies. It is produced by direct measurement of strontium isotope compositions of biosphere components, predominantly plants. The map characterizes the biosphere signature of individual lithologies: a technique that is applicable worldwide. Marine derived strontium, from both rainwater and coastal sea-splash and spray, is an important contribution to the British biosphere particularly on the western seaboard which is subject to high rainfall and prevailing westerly winds.

**Poster #36**

**Reconstructing early Neolithic diet and animal husbandry through stable isotope analysis of humans and fauna from three LBK sites in Central Germany**

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The first appearance of the Neolithic (Linear Pottery Culture (LBK)) in Central Germany occurred around 5,500 BC. However, though LBK sites are abundant in the German loess areas there are only a few studies that reconstruct the diet of these first farmers using biochemical methods. Here we present the largest study undertaken to date on LBK material using stable isotope analysis of carbon and nitrogen to reconstruct human diet and animal husbandry strategies. We analyzed the bone collagen of 97 human individuals and 45 associated animals from the sites of Derenburg, Halberstadt and Karsdorf in the Elbe-Saale region of Central Germany. Mean adult human values are  $-19.9 \pm 0.4$  ‰ for  $\delta^{13}\text{C}$  and  $8.7 \pm 0.8$  ‰ for  $\delta^{15}\text{N}$ . The  $\delta^{13}\text{C}$  values are typical for terrestrial, temperate European regions, whereas the  $\delta^{15}\text{N}$  values fall within an expected range for farming societies with a mixed diet consisting of both meat/milk from domestic animals and domesticated plants. There were no indications of dietary differences in sex. Young children under 3 years of age are enriched in  $\delta^{15}\text{N}$  due to breastfeeding indicating that weaning likely occurred around the age of 3 years. The fauna exhibit mean  $\delta^{13}\text{C}$  values of  $-20.9 \pm 0.8$  ‰ and mean  $\delta^{15}\text{N}$  values  $7.0 \pm 0.9$  ‰ respectively. Variation in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the domestic animals is likely to be caused by different livestock managements.

**Poster #37**

## Early Bronze Age couch potatoes – Is there evidence for mobility at the Bronze Age site of Singen?

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The Early Bronze Age necropolis of Singen (Hohentwiel) located near Lake Constance represents a population from a period of technological transition in southwestern Germany. The site contains several graves with metal artifacts which originated in other parts of Europe, and therefore these could be interpreted as being the graves of non-local individuals. The purpose of this study is to investigate this possibility, as well as the dietary habits of these people using isotopic analysis. We measured the carbon and nitrogen stable isotope ratios in samples of collagen from human bone to reconstruct past diets, particularly the trophic level of protein sources consumed and investigated if mainly terrestrial, marine or freshwater resources were explored. We also analyzed the ratios of strontium and oxygen isotopes in human enamel since this reflects the geological origin of food and drinking water consumed during enamel formation in early childhood. Additionally the ratio of sulphur isotopes from bone collagen is used as geological tracer reflecting the origin of food sources consumed during the last decade of life of an individual. With the combination of different isotope systems we can differ between local and non-local individuals. This gives us new perspectives on the life history of people involved in the early metal industry or transfer in Central Europe.

## Poster #38

# Application of Stable Isotope Analysis to the Studies of Human and Animal Diets in the ‘Rice-Millet Blended Zone’ of central China

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In this study, we present the results of stable carbon ( $\delta^{13}\text{C}$ ), nitrogen ( $\delta^{15}\text{N}$ ), and sulphur ( $\delta^{34}\text{S}$ ) isotope analyses on human and animal bones from the Neolithic site of Qinglongquan, China, including materials during the Yangshao (5500-5000 B.P.), Qujialing (5000-4600 B.P.) and Shijiahe (4600-4200 B.P.) periods. The site lies in a region between two agricultural centers, also called ‘Rice-Millet Blended Zone’, with rice ( $\text{C}_3$ ) production to the south and millet ( $\text{C}_4$ ) to the north. Therefore, this site presented a unique opportunity to explore not only the development of ancient agriculture in China, but also the production of these two different crops, with respect to the palaeoenvironment and context of cultural interactions between the north and the south. The  $\delta^{13}\text{C}$  values of the ancient humans and pigs showed that although both rice and millet were consumed, rice was the primary food resource in the Neolithic period. As the mean  $\delta^{13}\text{C}$  value of the domestic pigs was almost the same as that of humans, and the mean  $\delta^{15}\text{N}$  value of pigs was only 1.7‰ less than that of the humans, it was likely that the domestic pigs and humans consumed similar food resources, including human refuse. The human  $\delta^{15}\text{N}$  values (mean=9.0±1.2‰) showed that animal protein also made a contribution to the diet. The intra-population variation indicated some differences in the amount or type of animal protein consumed. In the later Shijiahe period (4600-4200 B.P.), human and pig  $\delta^{13}\text{C}$  values were more positive, which, based on a simple mixing model, suggested that millet contributed 10% more to the human and pig diets during this period. This apparent dietary shift matched the change to a more cool and arid climate. It also suggested the spread of millet agriculture to the South. Despite these changes, there was no significant change in human or animal  $\delta^{34}\text{S}$  values throughout these different periods at the site and the total range of sulphur values was small, which suggested that humans consumed mainly local foods.

**Poster #39**

**DNA analysis of an artificial deformed skull of an early medieval cemetery in Burgweinting, Regensburg.**

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In the context of the long-time excavation campaign in Burgweinting (Regensburg), five early medieval burial groups had been discovered. In one of these cemeteries the morphological examination revealed one female with an artificially, circularly deformed skull. According to the burial offerings this cemetery is dated to the end of the 5<sup>th</sup> century. In comparison to two other early medieval burial sites from this excavation it had been partly robbed and available grave goods were of poorer quality.

From this time, intentionally deformed skulls are rather known from nomads of the Eurasian steppe, the Huns, while graves with such deformations are rare in Europe. In only a few cases, deformed skulls were found in early medieval cemeteries in Central Europe. This coincides temporally with numerous archaeological pieces of evidence for the strong cultural and fashion-related influence the Huns had on Germanic tribes. Regarding the artificial cranial deformations it is not sure whether a foreign custom was taken over by Germanic tribes or whether the individuals were members or descendants of Eurasian nomads.

To identify the possible foreign origin of skeletons with alleged “Asian” skull deformations, we sequenced the HVRI region of the mitochondrial DNA and additionally typed 33 SNPs of the mitochondrial coding region by parallel tagged amplicon sequencing. Additionally, we determined a series of phenotypic markers, such as skin pigmentation and the lactase persistence allele 13.910 C/T to substantiate the phylogeographic inference made on the mitochondrial DNA data.



## Poster #40

# Reconstructing dietary patterns and social structures in the post-medieval Carmelite friary of Aalst (Flanders, Belgium). An integrated anthropological and isotopic study

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The isotopic data from the Carmelite friary of Aalst (province East Flanders, Belgium) are the first to be presented as part of a new large-scale stable isotope study ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of excavated human remains in order to reconstruct diet and its diversity in late and post medieval populations from Flanders and Brussels, Belgium. Previous isotopic studies from Flanders have been limited in size and scope and no isotopic research has until present been carried out on skeletal samples from the Brussels region.

The focus of this project is twofold. The first goal is to examine diet composition and diversity by comparing isotopic data from a number of contemporaneous but geographically different sites, representing a variety of social groups. In particular, this study will examine variation in diet between monastic and lay communities. Furthermore, social stratification within a site will not be neglected. At Aalst, the more or less complete skeletons of 238 individuals, all dated between the 16<sup>th</sup> and the 18<sup>th</sup> century, were recovered and studied. The human remains were excavated in three different burial locations: the church, the cloister alley and the cloister garth. Based on historical sources (and corroborated by the physical anthropological research), it is clear that diverse social groups are present in the different burial locations.

The second goal of this research is the isotopic analysis of skeletons that display 'diffuse idiopathic skeletal hyperostosis' (DISH), a condition characterized by the ossification of the anterior longitudinal ligament of the spine and large enthesophytes throughout the remainder of the skeleton. Despite it being easy to diagnose this disease, very little is known about its aetiology. The most prevalent theory states that DISH is caused by a diet rich in animal protein. In the osteoarchaeological literature the condition is often recorded in monastic populations, which is also the case at Aalst. Stable isotope analysis will be used to identify monastic and non-monastic dietary components and to test whether a high trophic level diet is indeed linked to the prevalence of DISH in individuals. This research will contribute to a better understanding of a disease attested in many archaeological populations but still poorly understood.

## Poster #41

# Strategies to improve identification of species using peptide mass fingerprinting of robust extracellular proteins

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Proteomics methods have been used to identify species using peptide mass fingerprints of the extracellular proteins such as collagen and keratin. Collagen is particularly useful in bioarchaeological applications as it has been shown to survive for over one million years in bone. In addition to collagen the robust bird feather protein keratin is an excellent candidate for species identification. Keratins from fur have been used in identification from archaeological samples; the use of feather keratin extends its use to other applications such as fur and feather imports, birdstrikes and bioarchaeology. The use of these proteins to identify samples to species level depends on an in-house database of known protein sequences. Currently this database is limited, having been populated using *de novo* peptide sequencing, which can be slow, inaccurate and expensive. To improve identification of species using collagen and keratin more sequence data is needed. The recent development of new protocols for automated DNA library creation and methods in next generation sequencing means the sequencing of small regions of DNA from a large number of samples is now a fast, accurate and cost-effective option. We aim to develop a pipeline using automated DNA library creation followed by selective exon capture and sequencing of keratin and collagen genes to provide sequence data for a large number of species in a relatively short amount of time. These sequences will populate our collagen and keratin databases allowing for more accurate identification of species from archaeological samples. Subsequently we will develop the informatics tools for assignment of species from peptide mass fingerprint data for collagen and keratin.

## BioDNA: a new Research Centre on Biodiversity and Ancient DNA

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The newborn facility of the Università del Sacro Cuore of Piacenza, BioDNA the Research Centre on Biodiversity and Ancient DNA (<http://centridiricerca.unicatt.it/biodna>) gathers the expertise of seven Institutes of the Faculty of Agriculture (Institute of Agronomy, Genetics and Crop breeding; Agricultural and Environmental Chemistry; Entomology and Plant Pathology; Fruit-growing and Viticulture; Microbiology; Food and Nutrition Sciences; Zootechnics) and is coordinated by a Scientific Committee with expertise in the fields of archaeology, archaeobiology, human genetics, wildlife genetics and conservation. The Centre takes advantage of a modern laboratory dedicated to ancient DNA (aDNA) analysis, and focuses on studying past and present biodiversity through the most innovative and up-to-date technologies for the analysis of nucleic acids in plants, animals and microorganisms. Among the main goals of the Centre there are: i) the study of the evolutionary history of living species, in particular those that were tightly linked to human history by domestication events; ii) the characterization of present-day biodiversity, to understand its origin and define adequate programs for its conservation, management and exploitation; iii) the comprehension of the dynamics of interaction within microbial communities of different matrixes (e.g. soils) and between these and organisms of higher levels of biological organization. Here we briefly present the research activities the Centre focused on so far: a) the analysis of domestic donkeys aDNA to compare the present-day levels of mitochondrial diversity to sequence data obtained from fossil/sub-fossil Italian samples; b) the morphological and molecular identification of the bone remains of livestock and other domestic species excavated from an archaeological site close to Flero (province of Brescia, Lombardy, Northern Italy) and dating back to the Roman age, III-IV century A.D.. Both these studies were carried out in collaboration with the Laboratory of Anthropology of the University of Firenze (Italy); c) the chemical (e.g., lipids) and biomolecular (DNA barcoding approach) analyses of natural archives such as peat samples of different origins to assess past ecological changes. This last study is still ongoing and will take advantage of collaboration with the Universities of Bari and Foggia (Italy).

## Poster #43

# Molecular analysis of the *Bos taurus* samples from the archaeological site of Terreni Freddi (Northern Italy): preliminary results

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We describe the preliminary results obtained from the molecular analysis of livestock bone remains excavated from the archaeological site of Terreni Freddi, which was discovered in 2007 during the construction of a new highway close to the municipality of Flero (Province of Brescia, Lombardy, Northern Italy). The site returned almost complete skeletal remains of several individuals belonging to livestock and other domestic species, identified as cattle *Bos taurus*, sheep *Ovis aries*, goat *Capra hircus*, pig *Sus scrofa* and dog *Canis familiaris* through morphological analyses. The samples were radiocarbon dated to the III-IV century A.D., thus dating back to the late Roman age.

To confirm the identification of the species and possibly the number of different individuals, a molecular study was undertaken to amplify and sequence a short fragment of the mitochondrial control region (d-loop). The first analyses were performed on 10 cattle bones attributed to 9 different individuals. The adopted amplification strategy included 3 different primer pairs corresponding to overlapping fragments of about 100 bp and covering the first hyper variable region (HVRI) of the d-loop. PCR products were cloned, 10 amplicons per sample were sequenced and aligned to reconstruct a consensus. Despite repeated efforts to get positive PCR results, sequence data could only be obtained for 3 individuals with the third primer pair, covering positions 16258-00034 of the Bovine Reference Sequence (BRS). This was probably due to some problems in the preservation of the samples, which remained exposed to adverse weather conditions before being collected from the excavation site. All the three individuals were confirmed to belong to the species *B. taurus*; in particular, they possessed control region motifs identical to the BRS and could therefore be attributed to mitochondrial haplogroup T3, the group of mtDNA sequences most widespread in Europe and Northern Italy at present. This result is concordant to previous ancient DNA evidence which suggested the wide diffusion of T3 haplogroup already in Neolithic and post-Neolithic Europe. Molecular analyses on sheep and goat remains are still undergoing.

## Poster #44

# Kinship analysis of three individuals from a 1000 CE Christian cemetery

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A Christian cemetery with no indications of continuity from pagan periods is located in Broby (Täby) in the Stockholm area of Sweden. Within this cemetery the remains of an older woman and two adolescents were buried in a close proximity that implies a family relation. The woman is believed to be Estrid, the matron of a wealthy and powerful Viking age kin, with a rich legacy consisting of several rune-stones. They are believed to have founded the cemetery. The history of their family, as well as their status at the cemetery makes the family relations of the individuals buried of archaeological interest.

In order to establish a possible biological kinship between the two adolescents and the woman HVR1 (mtDNA) was PCR amplified with tagged primers. The fragment was 341 bp long and amplified in 7 overlapping fragments of 85-127 bp. Subsequently the PCR products were sequenced through high throughput 454 FLX/Roche sequencing. Using the unique tag-primer combinations the sequences of each individual were identified and assembled. Authentic ancient DNA (aDNA) sequences were found through PhyloNet analysis and a conservative sequence was assembled based on the authentic sequences identified by PhyloNet. The comparison of the sequences with the Cambridge reference sequence (CRS) and each other showed 3 different haplotypes in the above mentioned individuals.

## Poster #45

# Ancient DNA Analysis of Northeast Pacific Humpback Whale (*Megaptera novaeangliae*): Implications for Archaeology and Conservation Biology

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Archaeological research can produce important insights into the natural history of ancient faunal species, and their interactions with humans. Using ancient DNA techniques, such data can reveal genetic diversity changes over time that can be invaluable not only to archaeological interpretation but also to modern conservation and resource management. Otherwise invisible in the palaeontological record, ancient marine fauna such as whales can be accessed through the archaeological record, providing a unique resource through which to examine the natural history of whale populations. This research illustrates how ancient DNA analysis of humpback whale bones recovered from archaeological sites (200-5000 BP) in Barkley Sound on the west coast of Vancouver Island can not only provide unambiguous species identifications, but valuable information regarding the genetic diversity of past populations. Current humpback whale populations have been severely depleted by commercial hunting; ancient DNA analysis of archaeological remains can therefore provide a more accurate baseline of historic genetic diversity and population size to aid conservation management in making informed decisions concerning the future management of this species.

Of 222 ancient whale remains positively identified through DNA analysis (success rate 85%), the majority (78%) were humpback whale, followed by grey whale (13%) – results which are consistent with species preference by indigenous whalers. Additional cetacean species did not appear in the assemblage until later times periods (<2000 BP). Detailed sequence analyses of 105 Humpback whale samples revealed several haplotypes currently unknown in the North Pacific today, supporting a loss of genetic diversity likely caused by unsustainable commercial hunting in the 19<sup>th</sup> and early 20<sup>th</sup> centuries. However, statistical analyses of past and present whale population diversity also suggest a change in population size prior to industrial whaling in the 19th century. The study demonstrates that ancient DNA analysis, in conjunction with other archaeological and ethnographic data, can provide relevant data for sustainable conservation management.

## Poster #46

### A chemical and biomolecular approach to study ombrotrophic peat bogs as natural archives of past ecological changes

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Among natural archives, ombrotrophic bogs are considered unique records of past environmental conditions because their genesis is directly linked to the atmospheric conditions occurring during peat formation. Although several studies have been carried out in the last decades using ombrotrophic bogs in order to reconstruct the historical trends of inorganic and organic pollutants due to anthropogenic activities, scientific literature is still rather controversial about the role of ombrotrophic bogs as reliable record of past climatic and environmental changes. To answer such a nodal point, it is extremely important to develop and apply multidisciplinary approaches to characterize peat samples at both chemical and biomolecular level, and better understand the process of decomposition/humification in these ecosystems.

For this study, a peat core was collected from a Swiss bog (Etang de la Gruère). The whole core, corresponding to ca. 2,100 years of peat formation, was characterized using several molecular spectroscopic methods (Ft-IR, UV-Vis, DSC, Molecular Fluorescence). Furthermore, peat samples were analyzed by XRF and ICP-OES (for major and trace elements), Low Background  $\gamma$ -spectrometry (for <sup>137</sup>Cs and <sup>241</sup>Am), Isotopic Ratio Mass Spectrometer coupled with an Elemental Analyser (for isotope ratios,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$ ), and GC-MS (for PCBs and PAHs). Biomolecular analyses are now undergoing: lipids (e.g., alkanes, sterols, membrane lipids) are analyzed as markers of climatic changes and shift in the structures of microbial communities. DNA is extracted, amplified with primers specific to different taxa (bacteria, plants and animals) and analysed to gain insights into ancient biological residues. Data will be statistically analyzed in order to correlate the chemical features of samples at different depths with changes at the biological level.

## Poster #47

# Stable isotope analysis of individuals from the Iron Age cemetery at Triberga, Öland, Sweden

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Partial excavation of an Iron Age cemetery on the island of Öland, Sweden, uncovered the remains of 24 individuals. The majority of these individuals were infants, with 18 having died within the first 6-7 months of life, and a further two being between 15 and 18 months old at death. Osteological analysis of these individuals suggested that these individuals may have been under nutritional and environmental stress during their lives. Given the age of the individuals it was hypothesised that this stress may have arisen from poor quality infant feeding practices, and stable light isotopic analysis was conducted in order to investigate if these infants were being breastfed and how long for. There was a large diversity in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of both the infants and adults, and as a result it was not possible to determine whether or not there was a standard weaning pattern across the population. Sulphur isotope analysis was also conducted on collagen from a limited number of individuals, and  $\delta^{34}\text{S}$  values were also varied. These results, whilst only from a small number of individuals, support the interpretation that diversity in isotopic composition between individuals arose from the consumption of differing amounts of marine foods. It is not clear, however, whether the diversity in the infant values was simply the result of consuming breast milk of varied isotopic composition, or whether infant diets also differed from one another. Many of the infants, including one aged approximately 15 months, had  $\delta^{15}\text{N}$  values sufficiently higher than all the adult values that it can be said with some confidence that they were probably still breastfeeding shortly before death. In contrast, none of the infants can conclusively be said to have not been breastfeeding at death.



## Poster #48

### Detection of fish remains using ZooMS

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We report on the application of ZooMS (Zooarchaeology by Mass Spectrometry) for the identification of fish remains. ZooMS uses peptide mass fingerprints of collagen to discriminate between samples, differences in peptide masses representing differences in sequence. Identification of specific collagen peptides is a particular problem for fish, in which there is very little sequence coverage within a highly diverse group.

We present the results from our investigation of the analysis of archaeological remains, with a particular focus upon fish scales, which are often difficult to identify to species when recovered from archaeological sites. Using the wet-water method it was possible to obtain adequate spectra from modern and archaeological material. One surprise was the difficulty of recovering peptide mass fingerprints from many modern fish bone samples held in reference collections, which we suspect may be due to the harsh methods used to prepare the bones. Finally we will discuss our attempts to apply ZooMS to tiny fragments of bone recovered from coring of monastic and secular medieval English fish ponds, which should aid our understanding of medieval fish 'farming' practices.

## Poster #49

# Bioarchaeological investigation of a unique prone burial from Castricum, The Netherlands

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In 1995-1996 seven supine burials dated to the 2<sup>nd</sup>-3<sup>th</sup> century AD were discovered in Castricum, in the north-western part of The Netherlands. In addition, a 24-30 year old female, dated to the 3<sup>rd</sup>-4<sup>th</sup> century AD, was buried facedown in a shallow grave 800 meters west of these burials. She wore a rare gold foil glass bead necklace, but also suffered a Parry fracture of the right ulna with surfaces remodelled into a pseudo-articulation, induced by lack of immobilization during callus formation. This type of fracture may suggest a direct trauma that could have resulted from a object breaking the bone in a defensive movement of the arm. The presence of the prone burial raised some questions concerning the provenance of the young lady. Was she a local inhabitant who was made to undergo a disrespectful and humiliating burial ritual, or could she have been of non-local residence, or even an unwanted stranger? To answer this question, the skeletal remains were analyzed for information on diet and migration by means of stable and radiogenic isotopes.

Carbon and nitrogen analyses of bone collagen showed that the diet of the investigated individuals did not differ from each other and were consistent with archaeozoological data. Both reflect a terrestrial diet. Strontium analysis were performed on three individuals, including the prone burial. The analyses were carried out on both bone and enamel samples due to the absence of bio-available strontium. The strontium values between the two supine burials and the prone burial differ significantly. The results strongly suggest that the individuals from the supine burials lived their whole live in similar geographical conditions. On the contrary, the strontium values of the young lady from the prone burial indicate a different place of origin then where she was buried. Based these results, as well as on available archaeological evidence, the rather exciting conclusion was made that the woman buried in the shallow prone burial is likely to be non-local.

## Poster #50

### Dating - an uncertain business

Joanne Powell, Matthew Collins, Kirsty Penkman, James Cussens & Norman MacLeod

The preservation of biomolecular material is influenced by many factors and intrinsically linked to the thermal history of the specimen and determines the extent to which extractable and analysable material has endured. Palaeoclimate models can assist in evaluating the likelihood of successful aDNA amplification but detailed information of local climate conditions are usually lacking. However, within the sediments surrounding organic specimens are often found remains of shells from marine or terrestrial gastropods. Uniquely, trapped within the biomineral crystalline matrix of such shells, amino acids of original proteins persist and can be extracted and quantified. Amino acids are chiral molecules and exist in two forms; the L orientation when the organism is alive and the D orientation after death. This conversion process known as racemisation, is a chemical reaction dependant on temperature, thus, determination of D/L ratios, provide us with a direct measure of the thermal history of the sample enabling us to predict the extent of preservation of aDNA more precisely, permitting better informed decision making and avoiding unnecessary destructive sampling of rare organic remains. In addition, when combined with appropriate kinetic and palaeoclimate models, will enable us to derive more accurate age estimates for archaeological sites and key events.

The aim of the project is to improve the reliability of amino acid geochronology by identifying and evaluating sources of error and uncertainty in an extensive record of AAR data. This is being achieved through the evaluation of intra-laboratory precision data and reproducibility values derived from an international inter-laboratory collaborative trial. It is hoped to be able to develop a weighted probability model using Bayesian statistics to integrate the differential rates of racemisation for the various amino acids with precision data, in order to calculate age dependant confidence limits and associated uncertainties.

## Poster #51

# Isotopic Webs (C, N, O, S) in Viking Haithabu and Medieval Schleswig

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Viking Haithabu (9<sup>th</sup> to 10<sup>th</sup> century AD) and medieval Schleswig (11<sup>th</sup> to 12<sup>th</sup> century AD) form a settlement continuum in an extraordinary ecological system, the brackish water system of the river Schlei. Performing an economic rise from a small sailor's village to an important trade centre followed by a descent to meaninglessness, both settlements represent interesting research material. Profound changes in available food supplies caused either by gain or loss in food sources should be reflected in a population's nutritional status.

Research on food supplies, food chains or foraging represents an important element of human ecology and culture. Food webs show the composition of food resources for consumers based on stable isotopes of light elements ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$ ,  $\delta^{34}\text{S}$ ). The base for the Schlei food web are 373 data sets from animals found in Haithabu and Schleswig like cattle, pig, horse, sheep, deer, dog, chicken, goose, several salt and freshwater fish and some migratory birds. A total of 302 human data sets were measured. Isotopic data from bone collagen give evidence of the origin of plant and animal protein as, for example, marine habitats are distinguishable from terrestrial ones.

The mean  $\delta^{15}\text{N}$ -value ( $\sim 12\text{‰}$ ) indicates a high level nutrition based on meat or fish. A linear mixing model for carbon and nitrogen isotopes shows that terrestrial, marine and freshwater sources contributed nearly equally to the human diet, with a  $\frac{1}{2}$  to  $\frac{2}{3}$  fraction of fish in total. But  $\delta^{13}\text{C}$ -values ( $\sim -19.7\text{‰}$ ) indicate a nearly exclusive nutrition on terrestrial sources. Thus, sulphur isotopes can offer the answer to that question.

Preliminary results show that no economic or chronological influence is detectable in the nutritional status or habits of the inhabitants of Haithabu or Schleswig. Apparently, neither the economic situation nor the course of time had any influence on the nutritional status of the human population. Thus, people appear to have maintained the same subsistence strategy well adapted to their close environment without relying on trade goods.

## Poster #52

### Methodology for ancient DNA from prehistoric wool

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Sheep and man have a long history of interaction, and due to selective breeding since its initial domestication, the species now contains more than 1400 recognised breeds worldwide. To help investigate questions about the history of sheep, such as, what breed did an individual come from, were they selectively bred for particular genetic traits, and when did these traits develop, we have verified a methodology that enables the recovery of DNA from wool.

Preliminary studies were performed on mitochondrial DNA (mtDNA) from modern untreated, dyed and artificially buried wool. The data showed that while both mtDNA and nuclear DNA were present in wool, at levels that could successfully be extracted and PCR amplified, some techniques involved in staining of the wool were detrimental to DNA quality.

We subsequently applied the methodology to 15 ancient samples, provided by the National Museum of Copenhagen. The samples date from 1300BC to 1600AD, and originate from different environments including earth graves, tombs, bogs and unburied material. From these mitochondrial DNA was successfully extracted from ten of the ancient samples.

In summary, we verify that PCR amplifiable DNA is preserved in natural sheep hair, and can be extracted from ancient wool should preservation be suitable. The environment and treatments of the wool seem to be the factors determining the preservation level.

## Poster #53

# Extinctions of South American camelid species and populations in the late Pleistocene followed by early Holocene recolonizations revealed by Ancient DNA

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*Lama guanicoe* (guanaco) is a large herbivorous artiodactyl inhabiting the semi-arid steppes of South America, and one of the few megafauna species to survive the end of the Pleistocene on this continent. The effect of the Pleistocene/Holocene transition on its genetic diversity will likely provide insights into South American megafauna extinctions. To investigate the genetic diversity of *Lama guanicoe*, we sampled teeth and bone fragments collected from cave sites in the Patagonia region of Chile and Argentina as well as the highlands of Peru for ancient DNA analysis and radiocarbon dating. Between 288-700 bp of the mitochondrial control region and cytochrome b gene were recovered from 85 samples ranging in age from 360 to 13,915 <sup>14</sup>C years BP. Surprisingly, we discovered that until the end of the Pleistocene a now extinct species of camelid, likely *Lama gracilis*, occupied Patagonia. In addition, a now extinct and divergent clade of *Lama guanicoe* also occupied the region. Both groups appear to have become extinct almost simultaneously, ca. 10,600 <sup>14</sup>C years ago. Less than 1000 years later, a new group of *Lama guanicoe* established themselves in Patagonia and are the ancestors of all modern-day *Lama guanicoe* in this region. We demonstrate that the loss of genetic diversity in South American camelids at the end of the Pleistocene was much greater than previously thought, and that a continental-scale metapopulation dynamic was likely key to its survival.

## Poster #54

### Leaching and adsorption of dirt DNA in soil

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The extent of DNA leaching in a temperate climate regime was investigated by extraction of bulk soil DNA, and high-throughput sequencing, of mammalian mtDNA derived from zoo animals. From Asian elephants (*Elephas maximus*) leaching of a 70bp fragment of mtDNA was detected in a sand textured soil down to 70cm depth, after passing through an overlying sandy loam textured soil. Leaching from tiger (*Panthera tigris*) urine was also detected, of a 70bp and a 76bp fragment of mtDNA, into a sandy clay loam textured soil. However at this site DNA leaching did not penetrate into the underlying alluvial clay horizon in 30cm depth. Furthermore, on a clay soil holding ostrich (*Struthio camelus*) no leaching was detected, but a 78bp mtDNA fragment was detected in the surface soil, using species-specific primers. Amplification was successful in the full pH range of the investigated soils (range, 6.2±0.2 pH to 8.3±0.2 pH), but inhibition in extracts from soil of high organic content was detected. The quantitative data, obtained from high-throughput sequencing, was investigated by using background contamination as an internal standard. The amount of elephant mtDNA was found to be influenced by soil texture, and the amounts of DNA recovered were larger from samples with larger total soil particle surface area.

## Poster #55

# Deep-Sequencing – Extracting Signal From The Dirt

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This study investigates the variation in ‘dirt’ DNA deposition, from large mammals and birds in temperate regions. Results obtained by high-throughput-sequencing of bulk mammal ‘dirt’ DNA, extracted from surface soil, sampled by a quadrat sampling approach, was compared to known numbers of species and individuals of captive exotic herbivores on a 5ha area. A predator enclosure was also monitored for the presence of both predator and prey in a 1ha area – and detected. Qualitative and quantitative data obtained by high-throughput sequencing, was found to reflect the proportional distribution of biomass of ruminant species present in the area. Furthermore, the Shannon-Weaver index was calculated based on the results of deep sequenced surface soil. Interestingly, it was demonstrated that including the spatial scale in sampling, clearly had a positive effect on reproducibility of the measured biodiversity. Sampling approaches of extracting ‘dirt’ DNA from precisely measured pooled soil amounts, including soil from sub-samples of a regular dispersed point pattern of a 400m<sup>2</sup> area, had a higher biodiversity and lower standard deviation ( $H' = 1.04 \pm 0.07$ ), that obtained by individual samples of this quadrat ( $H' = 0.51 \pm 0.36$ ). However, species of low abundance, or which had recently been introduced, was not detected. Furthermore, territorial behaviour of blesbuck (*Damaliscus pygargus*), caused this species to be overrepresented in the investigated 400m<sup>2</sup> area.



## Poster #56

# Faecal biomarker and archaeobotanical analyses shed new lights on ruralisation in Byzantine Sagalassos, Turkey

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Ruralisation in Byzantine Pisidia was marked by an increased migration from the countryside to abandoned urban areas with cities becoming centres of agricultural activities. As a case study, the fillings from a public latrine in the Roman baths of Sagalassos (Turkey) were examined through a multi-analytical approach.

The stratigraphy of the latrine sediments revealed a succession of black organic layers and white layers containing minerals such as lime and calcite. This suggests that organic refuse was deposited intentionally for the production of manure as the addition of calcium improves the quality of the fertilizer. In the studied sample sets, an increase in calcium concentrations was noticed for the top soil layers next to the drainage gutters and for the sediments in a refuse heap of the same room.

The same sample sets were also examined for macro- and microbotanical remains. The few plant macrofossils, preserved mainly in charred state, are typical for settlement refuse deposits. Palynological analyses showed high proportions of non arboreal pollen with few pollen types originating from food plants. Furthermore regular flooding of the room in later periods has probably led to the intrusion of wetland and arboreal pollen (pine, cedar) from the surrounding.

Lipid analyses of faecal biomarkers (5 $\beta$ -stanols and bile acids) enable to distinguish certain types of animal dung. Using gas chromatography mass spectrometry (GC-MS) in single ion monitoring (SIM) mode, it was possible to determine the relative abundances of 5 $\beta$ -stanols. The soil profiles next to the drainage gutters exhibited an accumulation of human faecal markers, such as coprostanol and epicoprostanol, in the deep soil layers. These markers are representative for the former use of this room as a public latrine. Interestingly, ruminant faecal markers, such as 5 $\beta$ -sitostanol and epi-5 $\beta$ -sitostanol, predominated in the topsoil as well as in samples from the refuse heap, indicating a contribution from livestock excrements. The concomitant elevated calcium concentrations in these samples clearly demonstrate that after its abandonment as a latrine, the room was reused for producing manure. Apart from improving fertilizer quality, lime might also have been added to reduce the odours of the faecal material.

**Poster #57**

## An ancient DNA survey of archaeological tuberculosis in Europe

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Funded by the NERC, a detailed and extensive survey of archaeological European tuberculosis (TB) is being undertaken, comparing genetic differences in the pathogen causing TB both geographically and temporally. Using macroscopic skeletal analysis, individuals with specific and non-specific indicators of TB have been identified and screened for the presence of MTBC DNA. Over 500 samples have now been processed from 150 sites across Europe dating from the Neolithic until the end of the 19<sup>th</sup> Century AD.

In the field of ancient DNA research, much has been noted about modern contamination issues, for example human handling of samples that leads to human DNA contamination, or the source of reagents causing domestic DNA contamination. As with animal aDNA studies, microbial aDNA studies create the same issues seen with modern contamination; thus, DNA from microbes in the host and burial environment may be cross amplified. Non-specific sequences have been found in ancient samples with conventional PCR and so the use of a probe based real-time PCR system for screening of DNA has been used in this project.

Clinical PCR systems exist to distinguish between different strains and lineages of *M. tuberculosis*, but they also simultaneously amplify environmental bacterial DNA. We are currently re-designing them to be more specific, and with these systems we will acquire a clearer idea of the evolution and past distribution of the TB pathogen in Europe, and potentially, across the world. In this paper the initial results of this ongoing study will be presented.

## Poster #58

# Archaeogenetics of flax from temperate sites in Denmark and Germany

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The potential of archaeogenetic approaches to identify geographical origins and evolution in flax are being studied. Flax presents two sets of challenges. Firstly, despite its global importance, the genetic coverage of flax is still very poor, making it difficult to develop phylogenetically informative systems. Earlier phylogenetic evidence has supported the scenario of the domestication of flax for its oil rather than its fibre, but phylogeographic markers remain a problem; we are developing an approach to address this. The second problem with flax relates to the small size of the seeds and to the preservation conditions, which are often waterlogged and or associated with practices of retting in fibre preparation which both make biomolecular preservation appear to be less likely. However, techniques for the detection of ancient DNA have become more sensitive in recent years, and next generation sequencing has given valuable insight into ancient DNA fragmentation profiles.

We began by examining 1600-year-old flax remains from ancient Egypt where we have found good preservation in other plant remains. Results here were promising, so we tried samples from a more temperate region (Denmark), which were up to 2000 years old. At the Iron Age site of Fuglsøgårds Mose pits were excavated that contained beside of archaeological finds, flax offerings, and records from more than 150 different plant species. The pits were made for peat winning, and later used for flax retting, which indicates the early use of flax for textile production. We found in this case that very small amplicons could be produced from ancient DNA, fitting with extensive DNA degradation. Encouraged by these results we proceeded to examine flax remains up to 6000 years old from waterlogged Neolithic sites in Southern Germany. In 8 of the 12 sites, we were able to produce the smallest amplicon attempted.

These results are surprisingly promising. The potential for archaeogenetic flax studies across Europe appears to be ripe. We can now look for evidence of changing usage of flax from a food source to a textile source over time at various temperate sites in Europe.

**Poster #59**

**Regional and chronological trends in milk use in prehistoric Europe traced through molecular and stable carbon isotope signatures of fatty acyl lipids preserved in pottery vessels**

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The pottery excavated from archaeological sites contains information relating to food processing and storage in the form of absorbed organic residues, most commonly lipids. Such information can be accessed using chromatographic, spectrometric and, more recently, isotopic methods, to infer the source of the degraded fats that dominate lipid assemblages. Important distinctions can be made between different animal fats, such as non-ruminant and ruminant adipose fats and milk fats. Thus, the study of potsherds can provide insights into the economy and the management of animals within a site or region.

One particularly productive avenue of investigation has been the emergence of dairying and the testing of the theory of the Secondary Products Revolution. As a result it has recently been shown that the dairying practices were strongly dependent on the region and the presence of cattle in the South Eastern Europe and Near East by the 7<sup>th</sup> mil. BC and was a component of agriculture in Britain from the late 5<sup>th</sup> mil. BC.

This new project is investigating the trends in milk use in less well-studied areas in Central, Western and Eastern Europe, and Scandinavia, addressing questions raised by the latter studies, e.g. how is milk processing linked to the frequency of the lactase persistence gene? What spatial and temporal trends were there in milk use in Western Europe? Can milk fats be detected at high frequencies at sites where ovicaprids are dominant in bone assemblages?

**Poster #60**

**'The End of the World?' Famine, Plague and Climate change in  
14<sup>th</sup> Century London**

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Increasingly unstable and extreme weather conditions are alerting us to the dangers of global warming to settled human populations. Researchers are looking to historical events to improve our understanding of current trends and how they might affect our own lives. The site studied in this project, the Royal Mint site at East Smithfield, contains skeletal remains of individuals who survived what was arguably the worst episode of extreme rainfall and flooding in English history, occurring as erratic weather episodes in an overall climatic shift from the medieval warm period to the little ice age. These events resulted in nearly a decade of severe and widespread famine across Europe, one of the most notable events of medieval European history. The Royal Mint site itself was established in AD 1348 during the first outbreak of the 'Black Death' in London and contains hundreds of individuals buried in a period of only 2 years. Given this unusual availability of a known date of death for these medieval individuals, and the well constrained age at which individual teeth mineralize, these Royal Mint plague pits provide us with the unique opportunity to reconstruct individual "life histories" in a period of well documented famine and climatic deterioration, using high resolution stable light isotope data from the teeth and bones of adult individuals of different age groups.

**Poster #61**

## $\delta^{13}\text{C}$ in Southern Scandinavia: “Fishing Stone Age” or the effect of the Littorina Transgressions

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There are four factors behind the marine values of  $\delta^{13}\text{C}$ , the amount of marine intake in the diet, the time and place of the person in question, and finally, the question of whether the same person had been sedentary or not.

Until now, only the first factor has been considered for the Middle and Late Mesolithic coastal occupation of Southern Scandinavia, 7 000 to 5 000 uncal BP. Behind the last factors lies the change in the palaeogeographical situation after the last glaciation, epitomized by the Littoria transgressions. These ensure an increasing influx of enriched saltwater into the sea of Kattegat and finally into the Baltic waters, although the thresholds of the Danish waterways constitute a certain hindrance. There is thus a time and space gradient from North to South for the marine values of  $\delta^{13}\text{C}$ .

Values between -19 and -11‰ of  $\delta^{13}\text{C}$  has been taken as evidence for a “Fishing Stone Age” though the same dwelling sites also show abundant remains of Red deer, Roe deer and wild Boar. It has even been suggested, that individuals with different  $\delta^{13}\text{C}$  values also have consumed different amount of marine food, but as these values are also time and site dependant, the overall picture is different from what has been suggested so far.

## Poster #62

### Eels – carbon and nitrogen isotope signals in eel bone collagen from Mesolithic and Neolithic sites in northern Europe

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The European eel (*Anguilla anguilla*) is considered to be catadromous, spending the majority of its life cycle in fresh water or estuaries, but returning to the sea to spawn. Despite some instructive data emerging from studies of modern eel populations, no systematic study of the carbon and nitrogen isotope ratios in eel bone recovered from archaeological sites has been undertaken. In this study we have sampled eel bone from a number of Mesolithic and Neolithic sites in northern Europe and compared the data with other archaeological faunal remains and modern eel. We assess whether it is possible to discriminate freshwater, brackish and marine signals in archaeological eel bone and we attempt to correlate these data with the size of the eel bone. The implications for archaeology are discussed.

## Poster #63

# The experiment on extraction of starch grains from sediments in subtropical China: preliminary results

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Various procedures have been used to extract ancient starch from the sediments. Variety in the various procedures suggests the methods will require adjustment according to different sediments. Here we report preliminary experiment on extraction of starch grains from sediments in subtropical China and try to establish the protocol of extraction in such an environment condition. Most microfossil extraction procedures use the same basic methodology including sample preparation, disaggregating and deflocculating to break up the constituents of the sample into single particles, removing undesired particles and isolating microfossils from unwanted soil particles with heavy-liquid flotation and so on. The experiment designed here focuses on the extraction efficiency with heavy-liquid flotation in different specific gravity including 1.6, 1.7, 1.73 and 1.79. No starch grains are extracted in 1.6 and 1.79, but in 1.7 and 1.73 some grains are extracted. Furthermore, the number of grains extracted in 1.7 is larger than in 1.73. The results indicate that the extraction efficiency should be best in 1.7. Nevertheless, it is worth noticing starch grains extracted in 1.7 and 1.73 have different sizes, shapes and morphological characteristics (e.g., position and form of the hilum and fissure, presence or absence of lamellae) and should derive from different kinds of plants. This result suggests the extraction methods of starch grains from sediments in different specific gravities may result in extraction of different kinds of starch grains.



## Poster #64

# Mitochondrial DNA (mtDNA) temporal variation in wild and domestic pigs from a North-Eastern Italian site

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Ancient DNA analysis of faunal remains is a useful tool to reconstruct past migration events and different aspects of a domestication process. In the last five years, particular attention has been addressed using both modern and ancient DNA data to clarify the complex domestication processes responsible of the transition between the wild board and the domestic pig (*Sus scrofa*). In this study we focused our attention on a single site in Northern Italy, Biarzo Shelter (Udine). This choice is justified by at least three characteristics of this site. First of all, *Sus scrofa* is the most represented species at all stratigraphic levels, with large number of specimens available. Second, Biarzo Shelter represents the only Northern Italy site with continuous stratigraphic records of the Pleistocene-Olocene transition. This continuity provides an opportunity to directly monitor modifications in both archaeozoological parameters and genetic traits in a  $\approx 6000$  year time frame from the Upper Palaeolithic to the Neolithic. Third, the geographic area of the site represents a likely connection region between the Balkans, Italy, and Central Europe, both for animal migrations and for cultural exchanges. We selected 28 remains recovered from five different stratigraphic units assigned to three different archeological contexts (Epigravettian, Mesolithic and Neolithic). Following standard ancient DNA procedures, we analyzed a small fragment of the mitochondrial DNA ( $\approx 80$ bp) that, despite the short length, has been showed to be highly informative. Our preliminary results suggest that a variation of the frequency of different haplotypes occurred through time, possibly related to the early stages of the pig domestication process. In addition, we recovered in two Mesolithic samples a sequence motif observed today only in the Near East and which was previously associated to the Neolithic diffusion of herding and farming lifestyles.

**Poster #65**

## **An Anatolian Trilogy: Arrival of nomadic Turks with their sheep and shepherd dogs**

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Because of its geographical location, Anatolia was subject to migrations from multiple different regions throughout time. The last, well-known migration was the movement of Turkic speaking, pastoral nomadic group from Central Asia. They invaded Anatolia and then the language of the region was gradually replaced by the Turkic language. Central Asian genetic contribution to Anatolia with respect to the Balkans was estimated as 13% by an admixture analysis implemented in LEA. This estimate was obtained by employing nuclear genetic markers. MtDNA and Y-chromosome estimates confirmed this admixture proportion. Based on the population size estimation for Anatolia in 12<sup>th</sup> century, it can be calculated that at least 1.5 million nomads might have arrived to Anatolia. History tells us that they have arrived to Central and Eastern Anatolia first and only 150 years later they invaded Western Anatolia. Distributions of genetic diversity of domestic sheep and shepherd dogs in Turkey support that as well the language spoken in Anatolia these nomads have changed the genetic landscape of these two domestic species within Turkey. These observations have implications on conservation strategies of domestic sheep in Anatolia which is known to be the cradle of sheep domestication. Results must be confirmed by ancient DNA studies.

## Poster #66

# The Celtic princely seat of Glauberg (Hesse, Germany) – a bio-archaeological interdisciplinary approach

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The Iron Age site of Glauberg (Hesse/Germany) is one of the most important La Tène A period centers in Europe, yielding both settlement features and burials. The results of an interdisciplinary study involving archaeology (artefacts, features), physical anthropology (age, sex, and pathologies), and bioarchaeometry (aDNA and stable isotopes of C, N, and Sr) draw a highly differentiated picture of the former community. At Glauberg, regular inhumations and cremations are rare exceptions. Instead, most skeletons were found in deep settlement pits and lack any semblance of order. Two so called “truncated conical pits” were used as mass graves for six, resp. five individuals. Altogether, 24 individuals were identified, among them men, women, and children of all age classes. Individual postcranial bones exhibit gnaw marks. Indication of diseases such as chronic infections, fractures, caries, degenerative alterations, and stress markers repeatedly attest to poor health conditions. The exceptional preservation of the skeletal material allowed us to retrieve aDNA and reproduce unambiguous mtDNA profiles of the hypervariable segment I (HVS I) of 14 out of 18 individuals. The identified haplotypes and their proportions within the total sample resemble haplotype distributions in extant Central Europeans. The individuals in the mass graves do not share the same haplotypes so that maternal kinship can be excluded. The Glauberg foodweb is consistent with a C3 ecosystem in which the “prince”, an exceptionally rich burial, stands out regarding a better access to animal derived protein. A diet rich in carbohydrates correlates with the high frequency of caries. Although the area is highly variable geologically, strontium isotopes imply that only about 25% of the investigated individuals were locals. The large proportion of non-local individuals concurs with the aDNA results and indicates intense social relationships with other communities.

## Poster #67

# Birth seasonality and feeding practices of domestic pigs: contributions of the first isotopic modern reference set of suidae

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Pigs were part of the Neolithic “ecological package” spread in Europe between the 7<sup>th</sup> and the 4<sup>th</sup> millennium BC, where it became a key resource for meat supply. This success may be ascribed to its prolificacy, rapid growth and ability to digest a wide range of food. Nowadays pig husbandry goes from free-ranging in forests to farmyard or urban-rearing, inducing different practices in human economy. How did the herders manage pig birth and diet on a seasonal scale, and how did they use landscape? Was slaughtering practiced on a seasonal basis? Such issues can be addressed through the analysis of stable oxygen and carbon isotopic composition of tooth enamel. Indeed, the former is linked to the oxygen isotope composition of ingested water, which in turn is linked to those of meteoric water, known to vary according to local temperature; whereas the latter is linked to the carbon isotope composition of plant ingested. The prerequisite for this approach rely on a good understanding of how the isotopic signal is recorded during crown formation in suidae, and on a sampling protocol fitted to archaeological assemblages. In order to fulfil these requirements, we performed a pilot study on a set of modern domestic and wild pigs of known life history including Corsican traditional free ranging pigs and Corsican feral pigs. Sequential sampling of enamel was conducted on lower incisors (I1, I2), canines and molars (M2, M3). The results allowed us to identify the most suited teeth for that kind of sampling: birth seasonality can be investigated on the incisors, whereas male ever-growing canines provide an interesting record of feeding practices. Besides, the amount of C<sub>4</sub> plants in the diet and the seasonal variation in the  $\delta^{13}\text{C}$  pattern in feeding habits between domestic and feral pigs as well as within populations can be deciphered. Two distinct patterns of cyclic variation in birth seasonality have been observed between domestic and feral pigs. Moreover, higher amplitude of  $\delta^{18}\text{O}$  intra-tooth variation has been observed on feral pigs compared to domestic pigs. These results are promising for the studies of archaeological assemblages.

**Poster #68**

## The Evolution of Scandinavian Barley

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Despite its importance as a crop species, little is known about the population genetics of barley and the effects of bottlenecks, adaptation, and gene flow on genetic diversity within and between landrace populations. In areas with highly developed agriculture, such as Northern Europe, these types of genetic studies are hampered by lack of landraces preserved in situ or ex situ. Instead we have used 113-year-old seed samples to study the evolution of barley in Scandinavia. While neutral markers suggests different introduction routes into Sweden, genotyping for known phenotypic traits shows the underlying genetic causes of adaptation to a climate widely different from that of the species' origin. Taken together we can make inferences about the adaptive and non-adaptive processes contributing to the evolution of Scandinavian barley.

**Poster #69**

## Characterising food residues from Neolithic Impressed Ware ceramics in the Western Mediterranean

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Recent research has attributed the introduction of agriculture in the Western Mediterranean to several rapid waves of maritime pioneer colonisation, followed by indigenous adoption. Impressed Ware (IW) ceramics are thought to have spread simultaneously with domesticates through this region, and is hypothesised to have been used to process domestic plants and animal products. To test this, we have applied Organic Residue Analysis (ORA) to determine the content and function of IW ceramics from Early and Middle Neolithic sites in the Western Mediterranean, and hereby present the results obtained for over 300 pottery fragments from 12 sites. IW ceramics retrieved from open air sites in Southern Italy and Malta showed negligible recovery of residual lipids. In sharp contrast, lipids were abundant on IW pottery from the cave site of Can Sadurni, in Catalonia, and their analysis by GCMS and GC-c-IRMS revealed the exploitation of ruminant products. We offer the following interpretations: i) pottery may have been used for storage or preparation of foods with relatively low fat content (e.g. plant products) in the early stage of the Neolithic or, ii) lipids were poorly preserved on ceramics from open sites in this region.

**Poster #70**

## Isotopic evidence for migration and mobility in two Iron Age communities

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Both the historical and archaeological records provide evidence for Celtic migrations but their extent is hitherto unknown. In an ongoing research project, sixteen burial sites from the core and the expansion areas of the Celts are investigated using both archaeological and bioarchaeometric data in order to quantify the extent of Celtic migrations. In this presentation, the initial strontium analyses of early La Tène burial grounds in the core area and in the expansion area are presented. Gäufelden-Nebringen (Germany) probably represents a small community or several small homesteads in the core area. Initial results have detected a number of mobile individuals, among them both warriors and women, most likely from Southwestern Germany. Monte Bibele (Italy) in the expansion area features both graves with exclusively Etruscan and with a mixture of Celtic and Etruscan grave goods. Here, the majority of individuals with either set of grave furnishings were locals, with only few young adult males and females presumably coming from other areas. The combined archaeological and bioarchaeometric data will allow for new insights in the real processes behind mobility and migration in the Iron Age.

## Poster #71

### Discovery of ancient pathological biomarkers

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The *M. tuberculosis* complex (MTC) is an ancient pathogen, its emergence dates several thousands of years. It has widely accepted view that diagnosis of tuberculosis from archaeological human skeletal remains is not an easy task by using of classic morphological methods. A biomolecular approach to diagnosis is probably more reliable than Gross osteological examination of archaeological skeletal remains. The survival of pathological biomarkers in ancient skeletal remains is a major prerequisite for any molecular analysis and is thus essential for the pathological examination of archaeological bones. The use of DNA techniques to detect pathogenic agents in archaeological remains has exponentially increased recently. Papers include detection of aDNA for tuberculosis, leprosy, malaria, plague and syphilis. However, many of these studies have been criticised and doubts have been cast authenticity of their results. Recent advances in bioanalytical techniques especially in modern mass spectrometry (MS) have made the possibility to obtain sequence information from subpicomolar quantities of fragmented ancient proteins and peptides. In this study, ancient mycobacterial proteins were successfully extracted and identified by matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry (MALDI TOF/TOF MS) for the first time from archaeological human bones. The identification and sequencing of ancient mycobacterial proteins with further biomolecular techniques (aDNA, determination of lipid biomarkers) have the potential to expand our understanding of ancient epidemiology and evolution of these pathogens.



**Poster #72**

**Automated classification of starch granules using supervised pattern recognition of morphological properties**

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Image analysis techniques have been used to investigate the likelihood of being able to classify and assign a probability regarding the plant origin of individual starch granules in a collection of granules. Quantifiable variables were used to characterize the granules, and the assignments and probabilities were calculated objectively. We consider the classification of images containing granules of a single species and of mixed species and the possibility of assigning a class to granules of unknown species in an image of a slide obtained from the dental calculus of chimpanzees.

**Poster #73**

## **Dogs as Dietary Analogs: Assessing the Cross-Contextual Validity of the Canine Surrogacy Approach**

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For over 30 years researchers have recognized that domestic dog (*Canis familiaris*) remains may be suitable proxies for humans in stable isotope-based paleodietary reconstructions. While this 'canine surrogacy approach' (CSA) has found wide-ranging applicability among various cultural, geographical, and temporal contexts, its use has been on an *ad hoc* basis and no systematic analysis of its cross-contextual applicability, or cohesive summary of its unique interpretive issues, have been attempted. Here we present a controlled assessment of the CSA's suitability using precontact Canadian human and dog remains from the Maritime Archaic Indian cemetery site (c. 4600-4100 BP) of Port au Choix-3 (EeBi-2), Newfoundland. Previously obtained carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope data from human (n=29) bone collagen at Port au Choix-3 indicated their dietary protein was predominately derived from marine resources, likely focusing on coastal exploitation of marine mammals, fish and birds. As such, this site provides an excellent case study for the CSA by comparing  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from dog (n=4) bone collagen with the well-associated and contemporaneous human remains. Additionally, new  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from faunal remains incorporated into human graves at Port au Choix-3 are also presented here to further assess human-dog dietary relations. This work is part of a larger project that will attempt to survey the CSA's suitability for use across Canadian Arctic contexts.

**Poster #74**

**Re-investigating fish consumption in Greek antiquity: results from fish collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analyses.**

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One of the most important recurrent questions in ancient Greek dietary reconstructions through isotope analyses was the apparent absence of fish protein. The importance of this absence was amplified by the abundant ichthyofaunal remains, iconographic evidence and literary information on fish and fishing, pertaining to almost all sites and time periods of Greek antiquity. Within this project, we analysed for the first time an extensive number of fish bones from Greek sites dating from the Mesolithic to the Classical times, aiming to investigate whether this absence was an artefact of the methodology or whether it reflected a reality of restricted fish consumption. Results show both temporal and spatial trends in fish isotope values and emphasize the importance of intra-ecosystem comparisons. Contrary to expectations, marine fish do not show the most elevated  $\delta^{15}\text{N}$ , making  $\delta^{15}\text{N}$  values alone a less reliable indicator of fish consumption in the Aegean. The highest  $\delta^{15}\text{N}$  values are exhibited by certain euryhaline species in certain sites only, however this is not accompanied by high  $\delta^{13}\text{C}$  values for the same organisms. The variability of results proposes a reconsideration of the amount of fish in ancient Greek diets specifically for each site and amplifies the importance of interdisciplinary studies, especially for regions with variable ecological resources.

## Characterising Isotopic and Compositional Changes in Cereal Grains with Charring and Diagenesis: Implications for Palaeodietary Reconstruction

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Carbonised or charred cereal grains are often found in archaeological deposits and are valuable palaeoecological and palaeoeconomic indicators of plant exploitation and agriculture. Their potential for use in palaeoenvironmental and palaeodietary reconstruction is enhanced through stable isotope analysis. However, robust application of stable C or N isotope analysis calls for thorough investigations of charred grain chemistry, as heating and diagenesis may alter their stable isotopic compositions compared to the fresh seeds, which in turn would complicate wider interpretations.

Einkorn grains grown at Sutton Bonington Plant and Crop Sciences Division were charred at 230°C for times ranging from 2 to 24 h. Solid state <sup>13</sup>C NMR, FT-IR spectroscopy and elemental analysis techniques were employed to provide a general overview of their composition. Parallel investigations of the lipid, amino acid and monosaccharide contents using GC and GC/MS were also undertaken. The compositions of these experimentally charred grains were compared with those of archaeological einkorn grains from a range of archaeological sites across Europe. Our results show that charring results in dehydration of the cereal grains; primarily through loss initially of free water and after 4 h charring, of bound water. Not unexpectedly, Maillard-type reactions take place between the protein and carbohydrate components of the cereal grains, resulting in melanoidin-like polymers. This is reflected in a reduction of the intensity of the starch absorption maximum in the FT-IR spectra and a corresponding increase in aromatic carbon resonances in the NMR spectra. GC analyses have shown that volatiles including: protein, polysaccharide and lignin pyrolysis products, are released during the charring process. NMR and FT-IR spectra indicate also that archaeological einkorn is highly oxidised compared to modern charred einkorn and contains less than 1% of its original lipids, hydrolysable amino acids and carbohydrate components. The NMR spectra shows that the carbonaceous components of the archaeological grains contains both highly aromatic and aliphatic moieties.

These results illustrate the complicated changes that take place both during charring and the subsequent burial of cereal grains. It is essential to understand these changes fully if isotopic analysis of archaeological cereal grains is to inform palaeodietary and palaeoenvironmental reconstructions.

**Poster #76**

## Face to face and hand in hand - A Stone Age Tragedy

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In 2005, four multiple burials of the Corded Ware Culture were discovered near Eulau, Saxony-Anhalt, Germany, with groups of adults and children laid down to face each other. The unique situation raises numerous questions about these individuals buried 4,600 years ago. Proceeding from an integrative concept, archaeological, anthropological, biogeochemical (stable isotopes) and molecular (aDNA) methods were applied to the Eulau burials. Using mitochondrial, autosomal and Y-STR markers, we identified direct child-parent relationships as well as individuals belonging to the same matri- or patriline among the 13 inhumations. Strontium isotope analysis by TIMS revealed different isotope ranges for women, men and children. The proximity of the males' and children's Sr values point at a local origin for both, whereas the females appear to have come from outside, indicating exogamy and patrilocality in the Eulau Corded Ware population. Skeletal evidence for inter human violence as well as the simultaneous interment of the individuals and the grouping of the graves possibly attest to a single violent event the families fell victim to.

**Poster #77**

Oxygen stable isotope analysis and life – history strategies reconstruction of medieval individuals from Kraków (southern Poland) – a preliminary study

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The present, preliminary study was aimed to examine archaeological human bone and teeth from cemetery in Cracow, dating on 10<sup>th</sup>-11<sup>th</sup> century, using stable oxygen isotope analysis. Stable oxygen isotope ratio of phosphate was measured in enamel, dentine and various post-cranial skeletal sections from 5 individuals (2-female, 1-male, 2-children). In addition, the bones and teeth were examined using FTIR (Fourier Transform Infrared Spectrometry) and EDS (Energy Dispersive X – ray Spectroscopy) to describe diagenetic process. Diagenetic condition of skeletal material estimated based on Ca/P, CI and C/P parameters seems to be normal with the exception of a few samples in which on absorbance diagrams there was a peak connected with silicates or exogenous carbonates in the vicinity of phosphate groups. Analyses were carried out on intra – and inter – individual interpretative levels to reconstruct life history in aspect of migration and weaning process.

**Poster #78**

## Recent Advances in DNA-extraction and PCR from historic herbarium specimens

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Investigations in obligate biotrophic pathogens and rare plants are often limited by the amount of recent specimens available for investigation. However, there is often a much higher amount of historic specimens deposited in international herbaria. Through the inclusion of these specimens in molecular phylogenetic investigations, the taxon sampling can be greatly increased and also cryptic genetic changes can be explored through time. The main limitations of these investigations is that usually only limited amount of tissue can be taken from the specimens without doing significant damage, often much less than the 40 to 100 mg routinely used for herbarium specimens. Here report methods for DNA-extraction and PCR amplification from pathogens in more than 120 years old historic herbarium specimens using about 2 mg of infected tissue. Depending on conservation, our method enabled the amplification of up to 1200 bp long fragments of the nuclear ribosomal DNA of specimens from the early 20<sup>th</sup> century. These advances constitute an important step towards the routine use of herbarium specimens, and the inclusion of type specimens in molecular phylogenetic studies.

## Crossing scales and disciplines: the implications of regional variations in biosphere strontium isotope composition for bioarchaeological studies

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The isotopic analysis of skeletal remains has made it possible to assess traditional archaeological data against direct biological evidence for the geographic origins of people and animals. Strontium isotope analysis has given archaeologists an unprecedented opportunity to study geographic mobility, based on the place that individuals obtained their food and water in life. However, it is increasingly clear that the geographic landscape encompasses a level of regional isotopic detail greater than that immediately suggested by small scale geological maps. Accordingly, there is a pressing need for higher resolution environmental baseline data, gathered at a suitable spatial scale, using appropriate biosphere proxies. Archaeologists seek to compare skeletal isotope data with the composition of biologically available strontium associated with different rock types. In conjunction with other isotope systems, strontium has been used as an exclusive technique to identify individuals who originated from geographic locations remote to the archaeological site under investigation. However, a lack of environmental baseline data has seriously restricted the full potential of the analytical method - there is simply very little biosphere data available against which to compare measured skeletal sample values. This means that, across wide areas of the United Kingdom local strontium isotope values have to be extrapolated solely from our understanding of geology.

Although geological maps are pivotal to our understanding of archaeological data, we also need to better understand the relationship between the geosphere and the biosphere: The isotope composition of bioavailable strontium, passed on to the teeth and bones of humans and other animals, is not free to vary independently of other landscape characteristics that are also related to underlying geology. Thus it is possible to show, through biosphere mapping, that strontium isotope analysis may have the potential to address a range of bioarchaeological questions related to human-environment interaction.

Combining existing geological mapping with empirical data acquired from direct biosphere samples (waters, soils and vegetation) our current research represents the first systematic attempt to map biosphere strontium isotope variation within the United Kingdom. We can demonstrate that within a 30 km transect, the strontium isotope composition of vegetation is significantly correlated with the composition of co-located stream water samples. Our data are obtained from material collected by the British Geological Survey's Geochemical Baseline Survey of the Environment (G-BASE).



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