

## **Session: Technology**

### **01 Biomolecular archaeology and technological process**

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Recent analytical investigations of amorphous plant and animal products (e.g., plant resins, waxes, dyestuffs) have demonstrated archaeological potential over a considerable chronological and geographical range. The secure identification of specific products from molecular and isotopic patterns can assist in assessing the roles that these substances played and in determining the use of artefacts on which these residues survive. This presentation aims to survey the growing potential of recent work in contributing to the study of technological processes. It is argued that, by placing these studies into a wider context, the full archaeological value can be realised.

### **02 A black art in the Blackland: a systematic approach to Egyptian mummification?**

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Surprisingly little is known about the so-called 'art' of ancient Egyptian mummification, this despite the long held fascination with mummies. This is particularly true of the types of organic preservatives and unguents employed in the process, with relatively little attention paid to their practical or indeed ritual role. The use of these biomaterials is often ignored, being considered unimportant and described dismissively by the generic terms 'resin' and 'bitumen' (though, notably, not 'wax' despite its widespread use!), with little or no consideration for their true biochemical nature, or any role they may have played in the preparation of the deceased. Yet mummification clearly combined the practical considerations of body preservation with ritualistic/symbolic concerns. With the extent of the ancient embalmers' skills and knowledge still uncertain, was the choice of organic materials a reflection of their physical and chemical properties and were there known benefits for cadaver survival? Sequential TD-GC/MS and Py-GC/MS, and GC/MS were employed to characterise and identify these organic materials, with the results revealing notable differences in the use and nature of these agents depending on their proximity to the body. Their choice and application would also seem to reflect the extent of the preservative role they would play, thus suggesting an understanding of properties of the organic materials the Egyptian embalmers chose to employ.

### 03 **The use of bitumen in Egyptian embalming**

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The confusion over the use of bitumen in Egyptian embalming has probably arisen because of the blackened nature of many mummies. The blackening/darkening of aged organic materials can arise through a range of processes other than the application of petroleum bitumen, e.g. chemical changes waxes, fats and oils, which are major components of many balms. Moreover, pyrolytically produced wood tars and pitches, such as the pine resin products commonly used to treat mummies, are black. An early source of confusion appears to stem from the black resins used to prepare mummies being mistaken for mum/mumia (a Persian/Arabic word for bitumen or mineral pitch) in the medicinal trade that grew up around mummies in the 16<sup>th</sup>/17<sup>th</sup> centuries. Recent biomarker studies of mummies have raised questions concerning the ubiquity of petroleum bitumen in their balms (Buckley & Evershed, 2001).

In this paper we report an examination of more than twenty mummies, including some studied previously, and a number of new specimens sampled from the Manchester Mummy Tissue Bank. The extent of bitumen use was determined via solvent extraction and fractionation with gas chromatography-mass spectrometry with selected ion monitoring (GC/MS-SIM) being used to screen for the diagnostic steranes and hopanes (Connan & Dessort, 1989) Quantification was achieved using co-injected standards and generating calibration curves. We conclude, based on the detection of steranes and hopanes, that bitumen was not used to treat all mummies. Where steranes and hopanes were detected their concentrations were in the ppm range of the solvent extractable balm. Using the method of Harrell & Lewan, (2002) the majority of the detectable bitumen appears to derive from a Dead Sea source.

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### 04 **Bulk stable light isotopic ratios in recent and archaeological resins: can we detect the transport of resins in antiquity?**

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Modern and archaeological resin samples have been analysed by bulk stable isotopic measurements ( $d^{13}C$ ,  $dD$  and  $d^{18}O$ ) to establish a methodology for resin isotopic measurement and to establish patterns of variation associated with location, species, altitude, etc. with the aim of detecting transport in archaeological resins. Two case studies were examined: the transport of *Pistacia* resin in Late Bronze Age Canaanite amphorae and *Pinaceae* resin in Roman amphorae.

Different modern species from the same location were found to have different C, H, and O isotopic values.

For the same species from the same location we found that there are isotopic differences between individual trees but that this is lower than that caused by species differences. The range of values for both different altitudes and sampling times was similar or less than the variation exhibited by the same species at the same location – therefore isotopic variation due to season and altitude over the sampled height range cannot be clearly distinguished. The same species at different locations showed (for O and H, less clear for C) the greatest variation in isotopic values – showing that different locations gives the greatest difference in isotopic values. There is an offset between the modern and archaeological resins. For Carbon, the offset of + 1.2 ‰ to correct modern resin for the burning of fossil fuels accounts for the *Pistacia* difference, but not the *Pinacea*. There is an O and H offset between modern and archaeological resins and all data plot away from the meteoric water line, which is possibly due to biosynthetic fractionation.

Distinctive isotopic values were found for many of the archaeological samples although at present there is no obvious pattern relating the different fabrics or suggested manufacturing sites.

## Session: Domestication

### 05 Haplotype analyses and cattle origins

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Haplotypes are information-rich groups of markers which segregate together because they are sited closely together on a chromosome or on a special non-recombining unit (mitochondrial DNA or the Y chromosome). Their phylogeography can inform on past domestications, migrations and admixtures. Mitochondrial DNA sequence has highlighted the *Bos taurus* - *Bos indicus* split and the distinctiveness of Western European aurochs. Modern Y chromosome haplotypes distributions roughly follow the same pattern but also give a greater contrast between regions. Both African mtDNA and Y chromosome haplotypes are somewhat distinctive, but the continent has both *Bos indicus* and Near Eastern secondary influences. A recent investigation of X chromosome markers has enabled the close examination of the *Bos taurus* - *Bos indicus* hybridisation process which has been a feature of most African herds. This may have occurred from 3,000 years ago, and from increased mosaicism of haplotypes, seems to have been an older process in the mixed breeds of East Africa and the Sahel than in Southern Africa.

### 06 The origin of European cattle – ancient DNA analyses on Neolithic bones

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The first domestication of plants and animals came up in the Near East during the Neolithics. From there the Neolithic Transition found its way to Europe. It still remains unclear whether farming was spread due to migration or if rather a cultural transfer occurred. The different theories are often referred to as the demic and cultural diffusion model. Concerning the domestic cattle (*Bos taurus*) the question arises whether these animals were imported from the Near East or if there was a secondary, independent domestication of the European wild oxen (*Bos primigenius*).

We analyzed ancient DNA Data from archaeological bone material from several Neolithic sites in Central Europe and the Balkans. The mitochondrial data show different lines between the domestic cattle and the aurochs. These results clearly support the import of cattle as no continuity between the matrilineal lines from wild oxen and cattle can be found. This method both presents the possibilities and limits of ancient DNA analysis as a remarkable tool to reconstruct the prehistory.

## 07 Cattle husbandry and ancient DNA SNPs

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Ancient DNA studies have generally been limited to mtDNA due to a compromise between fragmentation and information. If nuclear DNA can be retrieved, it is usually such small fragments so that very little, or no information can be gained from it. By turning to SNPs, and thereby providing the possibility to amplify minimal fragments without losing information, we have been able to retrieve information about cattle domestication as well as Scandinavian cattle breeding. In this particular case, we have relied on informative sites from the pigmentation gene *Mc1R*, as well as other systems. The material has been aurochs from Italy, Austria, Germany, and Sweden, as well as west Swedish medieval cattle remains. The results provide information about domestication and breeding, and demonstrate that SNP studies on ancient remains may be of great help in a wide range of future investigations.

## 08 Ancient DNA and domestication: new samples, new genes, new techniques, same old questions

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Though ancient DNA has added to our understanding of domestication, the overall contribution of this technically challenging field has been significantly less grandiose than originally hoped. The reasons for this stem not only from the difficulty in amplifying degraded DNA from ancient specimens, but also from theoretical impediments such as our inability to precisely phrase testable hypotheses related to the timing, location, and processes of domestication. This paper will focus primarily on three new approaches to the interrelated categories of sample suitability, locus selection, and molecular techniques.

Given the relatively high ambient temperature of the regions where animal domestication first took place, no ancient specimens dated to the origins of domestication retain a genetic signature. This lack of data has impeded an understanding of domestication of both pigs - because the presence of feral populations means finding truly 'wild' DNA is difficult - and cows - because the wild progenitor of cows is now extinct. Bones recently excavated from the North Sea have yielded up to 300bp of mitochondrial DNA. Faunal material from waterlogged sites is also well preserved, thus providing a source of definitive 'wild' DNA.

Previous studies of the origin of domestic animals have focussed primarily on neutral markers such as mitochondrial DNA (mtDNA). Recent advances in the identification of genes directly involved in the process of domestication provide an opportunity to watch allele frequencies change through time as a direct result of selective pressures associated with domestication. Other non-neutral markers such as immune genes will also provide insights.

Lastly, new molecular techniques such as gap-fill padlock probes allow us to identify SNPs in multiple non-neutral genes simultaneously. This technique also circumvents many of the issues associated with the amplification of ancient DNA such as damage and the creation of chimeras through PCR jumping.

## 09 Roman fruit growing in Switzerland: Ancient DNA from waterlogged *Prunus* species

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*Prunus* species include wild *Prunus spinosa* and many cultivated species such as *Prunus domestica*, *P. insititia*, *P. cerasus*, *P. avium* or *P. persica*. Most of the cultivated fruits were brought to the Northern Alpine region by the Romans. When and how early fruit growing occurred in the northern provinces of the Roman Imperium is not yet known.

A matter of interest is the diversity of cultivated *Prunus* species and the morphological identification which is difficult. Therefore, it is necessary to extract ancient DNA to differentiate different cultivars and obtain reliable DNA sequences data from waterlogged *Prunus* species. Fruit stones found abundantly at archaeological sites, for instance at the Roman settlement Tasgetium (Switzerland) were used. So far fragments of the *rbcL* gene (chloroplast DNA) and of the *its1* region (nuclear DNA) were successfully amplified in one *P. avium/P. cerasus* fruit stone from Tasgetium.

## 10 Origins of Cultivated Einkorn

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Understanding where agriculture arose is necessary for understanding early human culture. For each of the crops that were domesticated we can ask whether its wild progenitor was taken into cultivation only once or if there were multiple domestication events. Distinguishing between these scenarios can help to answer questions concerning the origins of agriculture in the Fertile Crescent belt.

Previous work at UMIST investigated the origins of emmer wheat. It was demonstrated that cultivated emmer is not monophyletic and was domesticated on more than one occasion and at different geographic locations in the Fertile Crescent.

The demonstration that cultivated emmer has diverse origins provides evidence in favour of the hypothesis that the transition to agriculture in SW Asia was a necessary response to a changing environment rather than the result of a chance discovery.

However, it has been suggested that einkorn, not emmer, was the first wheat to be domesticated. According to this scenario, cultivated emmer is genetically diverse because it was taken into cultivation by established and geographically dispersed farming communities only after the spread of einkorn from a unique origin. The genetic diversity of einkorn therefore becomes the critical test for distinguishing between the alternative hypotheses for the origins of agriculture.

Heuns study of the genetic diversity in einkorn suggested that cultivated einkorn is monophyletic and derived from a single domestication event that occurred in the Karacadag in SE Turkey. However, computer simulations by Allaby and Brown suggested that the analysis may be flawed. This study used microsatellite markers distributed throughout the entire einkorn genome in order to assess genetic diversity in over 400 accessions of wild and cultivated einkorn, the results were used to infer the domestication history of einkorn.

## 11 **Mitochondrial d-Loop sequence variation of cattle from the Roman period in Switzerland**

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Large amounts of cattle bones (*Bos taurus*) were excavated at the colonia Augusta Raurica (ca.15BC-300AD), one of the most important Roman archaeological site in Switzerland. At that period of time the presence of small, medium, and large individuals was shown by logarithmic size indices calculated from bone measurements. These size variations can be explained by different genetic constitution, by livestock husbandry, or by a combination of both. Livestock breeding can involve either indigenous or imported animals. In cooperation with archaeozoologists we try to support either of these explanations by analysing ancient DNA from well characterised cattle bones. DNA was extracted from the bones with a silica-based method. Three target regions of the d-Loop of the mitochondrial DNA were PCR amplified and sequenced to assess diversity in the female lineage. d-Loop sequence variation in cattle of Augusta Raurica (10AD-150AD) will be compared to rare, local Swiss breeds such as Raetian Grey cattle and Evolener cattle and to published results.

## Session: Diet

### 12 Bone chemistry and paleodiet: retrospect and prospect

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This presentation will consider the history of paleodiet research, discuss current and future trends, and present several examples representative of the techniques. The last 25 years have witnessed the discovery, development, and expansion of studies of human bone for information on diet, as these techniques have become conventional applications in archaeological research. Both trace elements and isotopes have been employed in the search for dietary signals. Elemental studies began with strontium and expanded to include zinc, barium, copper, and a number of others. Problems with the identification of sources of variation between bone and diet have not been resolved and these techniques are in decline today. Carbon isotopes in bone collagen were the focus of the initial isotopic investigations of past diet. Today stable carbon and nitrogen isotopes in collagen dominate the field and applications from all areas of archaeology have been published. Nevertheless, there is good evidence that collagen isotopes are not representative of the whole diet and that other materials, e.g. fatty acids or apatite, may provide a clearer signal of prehistoric menus. The future looks very promising for paleodiet research as more detailed information is coming into focus. Other isotopes and other questions (e.g., migration) are in the picture for bone chemistry research. Examples are given from Stone Age Denmark, Norse Greenland, and several others times and places.

### 13 Short- and long-term changes in the diet of domesticated animals from Qasr Ibrim, Egypt

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Compound-specific  $d^{13}C$  values of individual fatty acids and amino acids, obtained via gas chromatography-combustion-isotope ratio mass spectrometry, are providing new insights into palaeodietary studies of humans and animals. Traditionally, bulk  $d^{13}C$  and  $d^{15}N$  values obtained from bone collagen and apatite have been used for human and animal palaeodietary reconstruction. For the first time, compound-specific  $d^{13}C$  values of bone-derived fatty acids and



collagenous amino acids are utilised in conjunction with bulk collagen and apatite stable isotope values, to determine the ancient feeding practices of domesticated animals.

The Nubian site of Qasr Ibrim (c. 1000BC – AD1800) is situated on the Nile in modern day Egypt. Due to the extremely arid environment, organic material from the archaeological site is very well-preserved. This exceptional preservation is not only observed at the macroscopic level in finds such as bones, plant remains, leather, parchments and textiles, but is also witnessed at the molecular level.

The high degree of preservation of faunal remains at this archaeological site provides an opportunity to investigate aspects of animal husbandry, such as fodder/foraging patterns. Thirty-eight domesticated animal bones were selected for analysis; comprised of cattle and sheep/goat bones, from various periods throughout the site's occupation. Utilising models of the biochemical correlations with the dietary components and their turn-over rates, bulk stable isotope values from bone collagen and apatite combined with compound-specific stable isotope values of the collagenous amino acids and fatty acids (n-hexadecanoic and n-octadecanoic acids) were used for palaeodietary reconstruction. The collagen, apatite and amino acid  $d^{13}C$  values provided long-term indicators of the 'protein' and 'whole-diet' components of cattle and sheep/goats from the site, while the fatty acid  $d^{13}C$  values provided short-term 'whole-diet' indicators. The results indicated significant differences in the long- and short-term diets of the sheep/goat, and less pronounced differences in the cattle's diet. Indeed, cattle appear to have preferentially fed on/been fed on  $C_4$  plants, such as sorghum (*Sorghum bicolor bicolor* Moench.) and millet (*Panicum miliaceum* L.), during the later periods of the site. Furthermore, essential amino acids provided insights into the long-term protein component of the animals' diet, further illustrating changes in the animals' diet during the three thousand years of occupation at Qasr Ibrim.

## 14 A novel marine dietary indicator utilising compound-specific bone collagen amino acid $d^{13}C$ measurements of ancient humans

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The application of bone collagen stable carbon and nitrogen isotope analysis for the identification of marine food consumption in particularly arid environments may be hindered by two factors: (i) the overlap in  $C_4$  and high marine protein (HMP) consumer bulk collagen  $d^{13}C$  values, and (ii) the unreliability of bulk collagen  $d^{15}N$  values in regions of extreme aridity (<400 mm of rain per annum). Hence, the identification of HMP consumption among archaeological human populations in arid environments such as the Cape region of South Africa can be problematic. In an endeavour to identify a substitute marine palaeodietary indicator, a range of Cape region archaeological faunal and human bone collagens ( $n = 14$  and  $26$ , respectively), representing a spectrum of  $C_3$ ,  $C_4$  and HMP diets, were subjected to compound-specific stable carbon isotope analysis of their constituent amino acids as trifluoroacetyl-isopropyl (TFA-IP) esters via gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS).

While human  $C_4$  and HMP consumers were indistinguishable with respect to bulk collagen  $d^{13}C$  values ( $-10.9 \pm 3.7\text{‰}$  and  $-11.7 \pm 1.5\text{‰}$ , respectively) they were shown to be readily

distinguished based on  $d^{13}C_{\text{Glycine-Phenylalanine}}$  values ( $+4.0 \pm 1.6\text{‰}$  and  $+12.0 \pm 1.9\text{‰}$ , respectively). The relationship between HMP consumption and elevated  $d^{13}C_{\text{Glycine-Phenylalanine}}$  values was verified by: (i) the similarly elevated values exhibited by marine species when compared to terrestrial faunal species ( $+12.5 \pm 0.9\text{‰}$  and  $+3.2 \pm 4.2\text{‰}$ , respectively), and (ii) the strong correlation observed between human  $d^{13}C_{\text{Glycine-Phenylalanine}}$  and bulk collagen  $d^{15}N$  values ( $R^2 = 0.83$ ,  $p < 0.001$ ;  $n = 26$ ), the latter being a well-documented marine dietary indicator. The validity of utilising  $d^{13}C_{\text{Glycine-Phenylalanine}}$  values for identifying HMP consumption was further demonstrated in the similarly elevated values associated with both a known HMP consuming iceman (Kwaday Dän Sinchi;  $+15.6 \pm 1.2\text{‰}$ ) and a marine faunal assemblage (Dionisio Point,  $n = 7$ ;  $+14.4 \pm 2.2\text{‰}$ , respectively) from British Columbia. It was concluded that the basis for elevated  $d^{13}C_{\text{Glycine-Phenylalanine}}$  values observed in HMP consumers resulted from the direct incorporation into bone collagen of dietary glycine, which is both extremely  $^{13}C$ -enriched and present in high abundance in marine foods. Hence,  $d^{13}C_{\text{Glycine-Phenylalanine}}$  values offer considerable potential as indicators of HMP consumption and a valuable substitute for bone collagen  $d^{15}N$  values in arid regions where bulk  $d^{15}N$  values are unreliable, and also, in bones where collagen preservation is insufficient for bulk collagen  $d^{15}N$  determinations.

## 15 Determining diet using oxygen isotope ratios of bone and tooth apatite

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Here we explore the possibilities of using bone and tooth phosphate oxygen isotope ratios as a palaeodietary indicator. Currently, the primary method of determining palaeodiets is through the measurements of carbon and nitrogen isotope ratios in bone collagen. Bone collagen, however, rarely survives beyond 100,000 years in temperate environments, and for much shorter periods in arid environments. Therefore, in bone where collagen is not present or poorly preserved, it would be a great advantage to derive palaeodietary information from other tissues and/or isotopes. The phosphate ( $PO_4$ ) component of tooth enamel, and to a lesser extent bone mineral, is considered diagenetically robust and the in vivo oxygen isotope signal ( $d^{18}O_p$ ) has been reported to survive unaltered for millennia. Until recently the measurement of  $d^{18}O_p$  in bone and teeth is used primarily as a proxy for reconstructing palaeoclimates. The  $d^{18}O_p$  of mammalian bone and enamel apatite is precipitated from body water at a constant temperature and reflects the oxygen isotope signal in environmental water, which in turn is linked with climate-related processes. Theoretical and empirical models of isotopic pathways in large bodied mammals indicate that drinking water is a dominant oxygen source, but food derived oxygen can still play an integral role in the overall  $d^{18}O_p$  values. Current research using both wild animals and those on controlled diets suggest that the  $d^{18}O$  of bone and tooth apatite may offer some dietary information, but the extent and strength of this connection remains tentative. This paper will present a preliminary study investigating the potential use of oxygen isotope analysis of bone and tooth apatite ( $d^{18}O_p$ ) to discriminate between marine and terrestrial foraging strategies of animals with known diets. The outcomes of this research will help to push back the current temporal boundaries confining existing stable isotope techniques for palaeodiet reconstruction.

**16 Nitrogen and carbon stable isotope data from infant bone collagen: can we interpret more than just weaning age?**

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The use of  $\delta^{15}\text{N}$  values from archaeological infant bone collagen and other tissues for the purposes of interpreting weaning age has been established in the literature for some time. There are continuing problems with such interpretations, including our poor understanding of the turnover periods in infant collagen, the ageing of fragmentary infant skeletal material and the limitations in our knowledge of weaning practices in particular societies. We have, however, come to recognize a general pattern in the infant collagen data, with  $\delta^{15}\text{N}$  values at adult level at the point of birth, rising to a high point above adult level during the first year after birth and then dropping back to adult level as weaning occurs. This pattern is seen in most of the studies in the published literature. However, there are occasional anomalous patterns visible, such as that seen at Çatalhöyük (Neolithic Turkey) (Richards *et al.*, 2003). Here, the elevation of the values during the breastfeeding period appears to be unusually low. Such a pattern is also seen in the unpublished material from the Iron Age site at Wetwang Slack in the UK. This paper considers whether nitrogen and carbon stable isotope data can be used, particularly in the anomalous cases, to suggest factors affecting the infant cohort of such populations other than in specifically identifying weaning age. Such factors might include palaeodemographic information, the point at which other foods have been added to a breast-milk diet and the question of whether breast-milk might have been avoided in the feeding of infants at some sites.

**17 From milk to meals, variation in childhood diet from the Anglo-Saxon Blackgate Cemetery, Newcastle upon Tyne.**

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During the early medieval period Britain experienced rapid cultural change with migration and colonisation from Scandinavia and North Europe. However there is little information about contemporary dietary practice. This paper presents results of isotopic studies on Anglo-Saxon childhood diet in the 8<sup>th</sup> to 11<sup>th</sup> century cemetery at Blackgate, Newcastle upon Tyne. Oxygen, carbon and nitrogen isotopes measurements were made on 36 molars from 8 adults and 6 juveniles. The results give indications of dietary habits from deciduous teeth at 0-1 years (m1 and m2) and from permanent teeth at 0-2 years (M1) 3-6 years (M2) and 9-12 years (M3). Adult means for carbon and nitrogen were measured in rib samples, ranging in age from 15 to 39 years. Mean isotope differences were tested using paired t-test with significance at the 95% confidence interval.

Carbon and nitrogen results indicate a mixed terrestrial diet with some aquatic input. The composition of the diet changes throughout childhood, shown most strongly in the nitrogen

results. There is no significant difference between deciduous molars. However they do differ from the permanent molars indicating a transition phase towards lower nitrogen values between the age of 1 and 6 years.

Between age 6 and 12 years their nitrogen isotope values converge on adult values as represented by cortical bone. The higher  $d^{18}O$  in deciduous molars compared to permanent molars indicates a higher trophic level associated with breast feeding.

The isotopic values track alterations in diet throughout childhood. Breastfeeding ceased by 1 year, though with some continuance of high protein weaning foods until 2 years. The protein component of the diet decreases in early childhood, but rises to mirror adult levels as the child matures. We interpret that access to high quality food resources may be limited until a child is old enough to make a contribution to the household economy.

## 18 Searching for the earliest evidence for milking in Europe

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The precise nature and timing of the emergence of dairying in prehistory has been hotly debated by archaeologists over the past few decades. For example, one hypothesis suggests that following domestication, ruminant animals were at first only raised for their carcass products (i.e. meat/fat), and then approximately one millennia later their 'secondary products' (e.g. milk, wool, traction) were then also utilised by the early farmers (Sherratt, 1983). However, it was only recently that a 'direct' method of detection of dairying has been established.

During vessel use, lipids may be absorbed into the fabric of the vessel. These lipids are readily extracted with organic solvent, and quantified and characterised through the use of a suite of techniques including gas chromatography (GC), GC/mass spectrometry and GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS). Crucially, it is the compound-specific  $d^{13}C$  values of the  $C_{16:0}$  and  $C_{18:0}$  fatty acids, obtained via GC-C-IRMS, that the extracts may be classified to predominant commodity group. This is achieved through differences in the  $d^{13}C_{16:0}$  and  $d^{13}C_{18:0}$  values of ruminant adipose fats, ruminant dairy fats and non-ruminant adipose fats which reflect their specific biosynthetic origins. For example, ruminant dairy fats have lower  $d^{13}C_{18:0}$  values and  $d^{13}C$  ( $= d^{13}C_{18:0} - d^{13}C_{16:0}$ ) values than ruminant adipose fats, thus distinguishing them from other fat-types.

Using these techniques it has previously been possible to detect dairy fats in a large number of pottery vessels from British prehistory (c. 4500 – 500BC), therefore suggesting that by the time farming arrived in Britain, dairying was already an important component of farming practices (Copley et al., 2003). We are now extending our analyses geographically and temporally to cover the Neolithic in South-eastern Europe/Asia. To this end, pottery from numerous key archaeological sites from the region have been selected for organic residue analysis. Although the number of sherds yielding significant lipid concentrations is lower in these older sherds, compared to Britain, we provide direct evidence for the processing of dairy products from as early as the 6<sup>th</sup> Millennium BC in the region

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**19 Multiple isotope system determinations (O, Sr, C, N, & S) on single teeth using a new method of sequential extraction.**

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Combined isotopic analysis of oxygen, strontium, carbon, nitrogen, & sulphur on teeth and bone can provide a wealth of information on diet and place of origin. There is increasing demand for analysis of bio-apatite, collagen or other bio-molecules for all the potential isotopic systems, and essential trace elements. This presents a dilemma when only very small amounts of materials are available such as with deciduous teeth. To meet this challenge experiments have been carried out on archaeological material to determine if all five isotopic systems can be measured in a single tooth through a sequence of isotope extractions.

Single teeth were halved; for the first half, oxygen and strontium isotopes were determined on the bio-apatite fractions of separated dentine and enamel, whilst carbon, nitrogen, & sulphur isotopes were determined in collagen from the dentine. The other half of the tooth was used for sequential extraction of combined enamel/dentine bio-apatite from collagen, prior to isotopic determinations.

This new method will be described and the results discussed. In particular, the effects of the difference in age formation/maturation between enamel and dentine and post-burial diagenetic changes. The success of this technique, offers the potential for gaining the maximum information from the smallest mass of sample material.

**20 A case study of stable isotopes ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$ ,  $\delta^{18}\text{O}$ ) on human and animal bones from the passage tomb at Rössberga central Sweden.**

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The role of social complexity and migration are two of the major issues discussed in connection to the Neolithisation process. Social complexity and migration are hence also important issues

when discussing the large megalithic monuments erected during the Neolithic in Northern and Western Europe. In this study we analyse the diet and geographic origin of a sub-sample from a population buried in a Neolithic megalithic tomb in Central Sweden by means of stable isotope analysis in order to discuss the above mentioned issues. The Rössberga passage tomb, excavated in 1962, contained 128 individuals whereof some have been radiocarbon dated to between 4590 ±120 and 2440 ±120 BP. Previous analyses of the bones have shown that they are excellently preserved for both stable isotope- and DNA-analysis. In this pilot study we have analysed 30 human individuals and six animals (wild boar, hare, fox, dog and cow) from the passage tomb. The human mean value for  $\delta^{13}\text{C}$ , -21.0‰ (s.d.=0.27), is very close to the values for the terrestrial carnivores, fox -21.0‰, and dog, -21.4‰, not surprisingly indicating a complete terrestrial diet. Also the human  $\delta^{15}\text{N}$  values are terrestrial, 9.3‰ (s.d.=0.42), comparable to the dog, 9.3‰, and three permil higher than the presumably still lactating cow, 6.2‰, indicating that the protein originates from a high trophic level. All  $\delta^{34}\text{S}$  values are extremely close with a mean of 10.6‰ (s.d.=0.7) for the humans and 11.1‰ (s.d.=0.56) for the animals, which indicate that they all have the same geological origin. We conclude that this sub-sample have had a very homogenous terrestrial diet from a fairly high trophic level. We also conclude that, until we have sampled more skeletal elements from the same individual, there has been little or no migration to other geographic areas. We are still awaiting the results from the  $\delta^{18}\text{O}$  when this abstract is written.

## 21 **Different kettles of fish: identifying the consumption of aquatic foods by stable isotope analysis – examples from Medieval Britain**

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The stable carbon and nitrogen isotopic signals of freshwater organisms are known to be extremely variable and can display considerable overlap with the ranges of both terrestrial and marine animals. The consumption of freshwater foods in the past is therefore difficult to determine from routine dual isotope analysis of bone collagen, although their contribution to diet is regularly offered as an explanation for unusual, high  $\delta^{15}\text{N}$ , human isotope values. Sulphur stable isotopes ratios ( $\delta^{34}\text{S}$ ) have recently been employed to investigate modern freshwater ecosystems. As they often have very depleted  $\delta^{34}\text{S}$  values in relation to terrestrial and marine signatures, they could potentially be useful for detecting freshwater fish consumption in palaeodietary studies.

In our presentation, we aim to evaluate this suggestion for the case of medieval England, where historical and archaeological evidence strongly indicate a combination of terrestrial, marine and freshwater foods as the most likely explanation for the isotopic values observed in many human populations. We will present carbon, nitrogen and sulphur stable isotope data for humans and fauna from various sites, and discuss our findings in view of the potential of multi-isotopic approaches for dietary reconstruction.

## 22 **Stable isotope analysis of human and faunal remains from the two Bronze Age sites Aghia Triada and Perachora, Korinth, Greece**

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Here we present stable isotope data (carbon, nitrogen and sulphur) of bone collagen from two Greek Bronze Age sites: Perachora, Korinth ( Early Helladic) and Aghia Triada, Iliada (Late Helladic). Through isotope analyses we sought to characterise the general diets in these two sites and time periods, especially the amounts of marine protein, as well as animal vs. plant proteins in diets. As these are complex societies we also wanted to look for differences in diets between different social classes, as represented in different burial contexts but as well as differences between males and females and age classes.

The novel application of sulphur isotopes was undertaken to provide information on the movements of people and animals into these sites.

## 23 **Fish in a medieval fishing village along the North Sea, what do isotopes have to say?**

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In medieval archaeology, diet is often inferred from excavated faunal and plant remains. Where these give an indication of what was consumed, isotopes are better placed for assessing the relative importance of different categories of food consumed.

For the research presented here, human skeletal remains were not available, so  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$  were analysed in animal bones and soil samples from Walraversijde, a 14<sup>th</sup>-15<sup>th</sup> century fishing village along the Flemish North Sea coast. Both the amount and nature of the fish remains excavated, as well as the abundance of recovered elements of fishing equipment indicate the importance of fishing in the economy of this village.

Foraging animals (chicken, cat) showed a terrestrial  $\text{C}_3$  signal similar to that of cattle and horses. This means that they had no easy access to fish remains assumed to have been abundant in the village as a result of fish processing and fish consumption. Two dogs yielded very different  $\text{d}^{15}\text{N}$  values indicating different diets. Pigs showed the most positive  $\text{d}^{13}\text{C}$  and  $\text{d}^{15}\text{N}$  values of the terrestrial animals; the residues after fish processing were probably collected and fed to them, indicating that some kind of waste management system existed.

$\text{d}^{13}\text{C}$  and  $\text{d}^{15}\text{N}$  were also measured on bulk soil samples from excavated cesspits, and compared with soils from elsewhere on the site. Analysis of sediments from cesspits offers some advantages as an approach to palaeodietary analysis, as there is no routing of dietary components as in human bone collagen. Marine foods were apparent only in small quantities in the cesspits of Walraversijde. This was very surprising, since it apparently contradicts the abundance of fish remains found at the site. Fish seems to have been more important for its commercial value than as an item of diet.

## 24 **Investigating variation in human diet in the Nile Valley through stable isotope analysis**

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Stable isotope analysis is a technique enabling investigation of long term trends in dietary patterns and subsistence strategies in past societies in a direct way. Bone collagen from human and animal remains contain isotopic signatures which reflect the food resources consumed over the last decade or so of life. *Carbon isotope* analysis gives information about the aquatic vs. terrestrial components of the diet as well as the main plant type consumed (i.e. C<sub>3</sub> photosynthetic pathway vs. C<sub>4</sub>). *Nitrogen isotopes* allow the distinction of the trophic level from which dietary protein was obtained, i.e. the amount of plant food vs. animal products as well as providing some environmental information.

This poster presents new information on Egyptian diet through stable isotope analysis of bone collagen from archaeological samples.

The samples are from Egyptian and Nubian sites along the Nile Valley where archaeological evidence for subsistence practices is known, but techniques such as stable isotope analysis can add valuable new information. In later periods an art historical approach has mostly been used to produce a picture of dietary resources, showing what was available, but not necessarily what was being eaten. In some cases however such general information cannot give the fullest picture. This poster aims to compare the dietary information from isotopic data obtained between sites, as well as looking at the different subsistence strategies potentially employed along the Nile Valley.

## 25 **Deceased or Diseased? Can paleodietary studies explore aspects of death in a prehistoric mass burial in Thebes, Greece?**

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The 1997 excavation behind the archaeological museum of Thebes brought to light a long building with an arched room typical of the greek Bronze Age (3200-1100 BC). The building, approximately 12m long and 7m wide, had been very well preserved, retaining not only the stone foundations but also part of the clay walls. The construction dates to the EH II according to the pottery recovered from the site.

Further investigation of the building revealed an interesting osteological find: 15 people had been carelessly disposed of in one of the rooms. The mass burial dates to the end of EH II (from the pottery). The dead were lying in various positions, with different orientations and partly covering each other, all signs that they were not given proper burials. They were deposited on top of the destruction level of the occupation, covered by a thin layer of soil. The majority of them was under twenty years of age. What makes this site unique is the fact that after the destruction of the house and the burial, the entire site was covered by a clay tumulus. The cause of death is not evident paleopathologically. This study analyzes human and animal bones from the mass burial for carbon and nitrogen isotopes to investigate dietary patterns but also to try and see whether there was anything particular about these people that might have dictated the nature of their burial.



Their isotopic values will be compared to values from four MH tombs that were built on top of the tumulus and four undated tombs that were opened into the tumulus, to explore continuity or change in dietary habits.

We consider this site very important, since the character of the hasty mass burial of young individuals and the deliberate covering of the site attract our attention. The fact that people returned to the site to bury their dead at a later point in time provides a basis to explore other signs of (cultural?) continuity, like dietary habits.

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## Session: New Techniques and Approaches

### 26 Retrieving past human ecosystems: changing questions and emerging answers

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Biomolecular archaeology has greatly expanded our view of early human ecosystems. That view was once restricted to a study of vertebrate bones belonging to past human predators and to a particular portion of their prey, against a generalised backdrop derived from pollen analysis. With novel and emerging techniques, it now seems possible to track those ecosystems through several trophic levels and to many different types of organism and tissue. The significance of doing so arises from the central importance of the human food web to the major changes in human society. Not only do we consume an unusually wide range of species, globally numbering several thousand, but also particular diets consumed are more diverse and changeable than that recorded for any other predator. We in turn are consumed by a wide range of micropredators, a small number of which, including the organisms responsible for malaria, tuberculosis and plague, have had as profound effect on the fate of human societies as changes in our own species' feeding practices.

The application of novel methods entails not simply the provision of new answers, but also the articulation of new questions. Established questions are invariably framed around established observable data. Archaeologists have thus described and delineated past food webs in relation to archaeologically visible foods, in particular vertebrate flesh and hard grain crops. Not only are hunting and farming described in relation to those visible elements, the genetic boundary of "domestication" that separates them is defined in terms of visible morphological changes.

### 27 Documenting seaweed foddering in the Neolithic of North-western Europe using tooth enamel carbon and oxygen isotope ratios

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The use of seaweed as fodder for sheep, and to a lesser extent cattle, horses and pigs, is, or was until recently, a widespread tradition in insular and coastal environments of North-western Europe. Marine resources complement animal diet during the bad season when the access to terrestrial pastures is considerably reduced by constraining climatic conditions.

Historical accounts relate the practice over the past fourteen centuries. Historical and ethnographical sources mention the use of red (Rhodophyceae) and brown (Phaeophyceae) seaweed, among which most commonly used species will be listed.

Antiquity of seaweed foddering in prehistoric times was investigated through analysis of tooth enamel carbon and oxygen isotope ratios. The method was first tested on modern seaweed eating sheep from the North-Ronaldsay island. Seaweed consumption is reflected in high stable carbon isotope ratios, and intra-tooth sequential sampling permits to highlight seasonal changes in the contribution of seaweed to diet. The procedure was then applied to sheep and cattle teeth from two Neolithic sites from the Orkney islands. At the Knap of Howar (~ 3500 BC), neither sheep nor cattle were fed seaweed, as shown by carbon isotope ratios reflecting a pure C3 terrestrial diet. At the Holm of Papa Westray (~ 3000 BC), the results from isotope analysis of sheep tooth enamel clearly suggest grazing on terrestrial pastures during the warm season and a significant contribution of marine resources to the sheep diet during the coldest months. These results constitute the earliest evidence for this practice in the area under consideration.

## 28 **Determining template copy number from ancient DNA extracts**

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Ancient DNA (aDNA) typically deals with low numbers of DNA templates that are often heavily damaged. Many of the authenticity problems associated with field originate from low levels of contaminate DNA coupled with a lack of information about protein and/or DNA preservation in samples. The accurate quantification of starting template copy numbers in a PCR reaction can provide valuable insights into the preservation of DNA and is a useful tool in establishing authenticity of novel sequence data. We present a series of case studies in which quantitative real-time PCR was used to estimate starting template numbers in a variety of different aDNA extracts. DNA extracted from a variety of species, tissues and storage conditions is compared. Some of the problems associated with PCR inhibition and use of templates standards are discussed together with a how these techniques might be beneficial in optimising both the extraction and amplification of aDNA.

## 29 **Preservation of biomolecules in carbonized organic residues on ceramics from Indigenous Roman settlements – a combined DTMS and solid-state <sup>13</sup>C CP/MAS NMR spectroscopic study**

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The chemical characterization of organic residues found on ceramic vessels can provide direct information on the original vessel use and on the diet and cooking practices of people in the past. A combined solid-state CP/MAS <sup>13</sup>C NMR and Direct Temperature-resolved Mass Spectrometric (DTMS) approach was chosen to chemically identify and quantify a collection of

charred and non-charred solid surface residues preserved on vessels recovered from indigenous Roman settlements in the Netherlands. DTMS gave information about a broad range of organic compounds and their mode of occurrence. The volatile lower molecular weight part of the residue includes lower temperature desorption products, such as fatty acids, sterols and fatty acylglycerols.

The compounds formed at higher temperatures are derived from relatively intact proteins and polysaccharides, and from more condensed cross-linked aromatic materials evolved during (partial) carbonization. Residues situated on the exterior of vessels show a series of condensed aromatic compounds in the higher temperature region, indicative of wood smoke and soot. Solid-state CP/MAS  $^{13}\text{C}$  NMR spectroscopy was applied to a small number of charred (5) and non-charred (1) residues in order to verify the DTMS results and obtain qualitative information about the relative amounts of extractable and non-extractable compounds present. Charred residues show resonances of aromatic and aliphatic structures (with a aromatic fraction  $f_a$  varying from 0.25 to 0.73), and in some cases resonances for carboxylic acid groups and carbon-nitrogen bonds found in proteins. The non-charred residue only shows aliphatic and carboxylic resonances while the resonances of aromatic structures are absent. The CP/MAS  $^{13}\text{C}$  NMR results confirm earlier DTMS classifications and quantifies for the first time the functional group distribution in complex solid organic residues. It also demonstrates that the micro-scale molecular characterization of carbonized residues on ancient pottery can be done directly on the powdered sample.

## 30 Ancient treponemal DNA: - high expectations

Abigail Bouwman and Terry Brown

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At the end of the 15<sup>th</sup> century AD an epidemic occurred in Europe. This epidemic is generally believed to be of syphilis. There are three theories for the introduction of syphilis into Europe.

- 1) The Columbian theory is that Columbus introduced syphilis from the Americas in 1494.
- 2) The Pre-Columbian theory is that syphilis was present in Europe before 1494 and an epidemic occurred due to lower immunity in the population or mutation of the organism.
- 3) The Unitarian Theory is that another treponemal disease was present in Europe and adapted to different climate/cultural practices become venereal syphilis around the end of the 15<sup>th</sup> century.

Three human treponemal diseases cause bone pathology and can therefore be identified in the archaeological record. The venereal (syphilis) and non-venereal (yaws and bejal) forms have different pathology and therefore the bone changes can also be different. However, not all bone pathology occurs in most skeletal samples and therefore most individuals can only be identified as treponemal and so it is difficult to identify which theory is correct with conventional osteology. Therefore a biomolecular approach was suggested.

A number of genetic differences between the venereal and non-venereal treponemal genomes and these could theoretically be used to identify the presence of syphilis in Europe before 1494, additionally, the entire genome of the syphilis treponeme has been sequenced and a number of potential virulence factors identified.

A project was undertaken to amplify these genetic markers from archaeological bone samples. Forty-seven samples were obtained and analysed using nine different treponemal specific PCR primers. Although 24 bones yielded human DNA and seven bones yielded TB DNA no treponemal DNA was recovered. This indicates that either treponemal DNA does not survive in the archaeological record or that the present biomolecular techniques are not yet sensitive enough to recover it.

## 31 Fishing for evidence of the processing of marine animals in archaeological pottery vessels

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Organic residue analysis is now a well-established technique for determining vessel use in Antiquity, thus providing useful insights into the exploitation of animal and plant resources in the past. Through a variety of techniques including gas chromatography (GC), GC/mass spectrometry (GC/MS) and GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS), it has been possible to detect the processing of a diverse range of commodities in pottery vessels, e.g. ruminant adipose fats, ruminant dairy fats, various non-ruminant fats, leafy vegetables, palm fruit, other plant and bee-products. One of the obvious omissions from this list are marine animal products. Fish bones are often recovered at various archaeological sites (especially coastal sites), yet their presence as absorbed lipid residues has so far eluded us.

We present new evidence for the processing of marine animals. Potsherds excavated from coastal pre-contact sites situated on Santa Catarina Island, Brazil (c. 1000AD and yielded large quantities of marine shells and bones) were submitted for organic residue analyses. The residues yielded saturated fatty acid distributions (dominated by C<sub>16:0</sub>, with lower abundances of C<sub>14:0</sub> and C<sub>18:0</sub>). Interestingly, the isoprenoid fatty acids 4,8,12-trimethyltridecanoic acid and phytanic acid (both common constituents of marine organisms) were detected in many of the extracts. Further compelling evidence for a marine source was obtained through the detection of unusual series of cyclic compounds, namely ω-(o-alkylphenyl)alkanoic acids, with 16, 18, 20 and 22 carbon atoms. Such compounds are believed to be formed during the protracted heating of triunsaturated fatty acids (C<sub>16:3</sub>, C<sub>18:3</sub>, C<sub>20:3</sub> and C<sub>22:3</sub>), which are commonly found in the tissues of marine animals. This product-precursor relationship was indeed demonstrated through laboratory thermal degradation studies.

Thus, the detection of these unusual cyclic compounds together with the isoprenoid fatty acids and faunal evidence provide the first secure detection of the processing of marine animal products in archaeological pottery vessels (Hansel *et al.*, in press).

### Reference

Hansel, F. A., Copley, M. S., Madureira, L. A. S. & Evershed, R. P. (in press). Thermally produced α-(o-alkylphenyl)alkanoic acids provide evidence for the processing of marine products in archaeological pottery vessels. *Tetrahedron Letters*.

### 32 **Authenticity of long curated historical hair samples - the case of Newton's hair**

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Six samples of hair attributed to Sir Isaac Newton (1642-1727) were obtained for hair analysis from five different sources in both public & private collections. The samples varied in length from 2 to 17cm. None of these samples had a fully documented curation history. A primary research concern was to determine whether the samples were authentic through biomolecular (genetic and isotopic) analysis.

Serial sub-sections of the hairs were submitted for carbon and nitrogen stable isotope analysis. Each hair showed discrete variation in stable isotope values in the sub-sections, which reflected normal seasonal availability of foodstuffs. However, there was a 6‰ range in  $\delta^{15}\text{N}$  and a 2‰ range in  $\delta^{13}\text{C}$  values between individual hairs which indicates significantly different dietary inputs for at least four of the six hairs.

A single fibre from each sample was subdivided and submitted for mitochondrial (mt)DNA analysis and independent replication. MtDNA was amplified from four of the six samples and each contained a different DNA sequence. It is unlikely that these differences were due to contamination, since it was assumed that all hairs would have been handled extensively in the past and thus underwent rigorous decontamination prior to extraction. It would be extremely unlikely that only a single contaminant sequence would survive per hair based on the results of independent replication.

The mtDNA results support the evidence from stable isotope analysis and indicate that these six samples represent a minimum of four separate individuals. Importantly, whilst Sir Isaac Newton's position as a major figure in history meant that multiple samples attributed to this individual were available from different sources for analysis, the authenticity of all such material can be brought into question. Hair samples attributed to high profile individuals with a long undocumented curation history must be viewed with caution.

### 33 **Is ancient DNA present in siliceous phytoliths?**

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Plant organic tissue is rarely preserved in a state where ancient DNA (aDNA) can be extracted. To broaden the sources of aDNA we checked the possibility of extracting aDNA from the siliceous parts of plants, namely phytoliths. Phytoliths are silica bodies deposited by plant cells that have morphologies specific to the plant family and sometimes species. Because the phytoliths are relatively stable, they accumulate in archaeological sites.

If plant DNA was trapped inside these silica bodies, it would be protected from the environment. Furthermore, the aDNA could be extracted in the lab under clean conditions after all the exposed DNA was removed prior to demineralization. This would enable us to study the genetics of many species without the fear of contamination.

We therefore addressed the question of whether or not DNA is present within phytoliths.

We chose to study an archaeological layer from the Iron Age of Tel Dor, Israel, which consists mainly of phytoliths. Phytoliths were cleaned and washed with acid. Then they were dissolved at neutral pH in ammonium fluoride under conditions that do not degrade DNA. After dissolving the silica, bio-molecules were collected by dialysis. Our analysis showed that no DNA could be amplified from these samples by PCR, under conditions where controls containing picogram amounts of template gave positive results and negative controls showed no contamination. Mass spectroscopy gave no evidence for short oligo-nucleotides.

We conclude that in this particular sample, either there is no DNA or it is in minute amounts and in a preservational state that does not enable amplification by PCR. We suggest ways to further investigate the potentially exciting prospect of detecting DNA in phytoliths. We did note that modern bread wheat phytoliths contain macromolecules with some characteristics that suggest glyco-proteins, and the material is easily accessible for  $d^{13}C$  analyses.

### 34 New analytical methods for sex determination of human skeletal remains

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The anthropological research uses two different techniques for the estimation sex, in one hand the morphological methods, in the other hand the DNA analyses, mainly determination of the sex-specific amelogenin sequences. However, the classical metric measurements we can use only for good conditioned complete adult skeletons, moreover extraction and purification of human DNA are common problems for anthropological laboratories.

This paper presents two non-morphological analytical methods for sex determination of anthropological findings. These simply developed chemical techniques help us in sex determination of very fragmented skeletal remains, and bring new survey data for paleoanthropological and forensic research.

### 35 Fishing for ancient SNPs, and pyrosequences

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There are several factors that need to be accounted for when working with ancient DNA. The material must be well preserved, protected from contaminating DNA, and sufficient to last for

independent replications. All this has limited the scope of ancient DNA studies. However, with our 'fishing' approach, we believe that we have found the solution to at least a few of the problems, without compromising the checklist for authentication. We hybridise DNA in the extract to biotinylated oligos, and thereafter use magnetic separation to 'fish' out the DNA. This technique allows for several re-fishings, and provides the selectivity needed to give an extremely pure template to work with in the amplification step. The use of pyrosequencing technology for post-PCR typing can cut out amplicon cloning in some cases. This protocol has allowed us to get sequence data as well as SNP data from a series of prehistoric materials.



## Session: Degradation

### 36 **Tracking degradation: multiple approaches to the study of the deterioration of archaeomaterials**

Matthew Collins

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Living in York one is all too aware of the importance of molecular degradation, its foundations and economic success are built upon slowly decaying Medieval rubbish. Arguably, the foundation of biomolecular archaeology is an understanding of molecular diagenesis, but all too often the importance of molecular diagenesis has been overlooked. Perhaps this is because it is all too obvious; the same factors that preserved ancient foods - (semi)sterile, dry or cold conditions - are those which intuitively will enhance the preservation of ancient biomolecules. Concentrating on bone, and beginning with burial practice, the two 'extremes' identified are burial of an intact corpse and surface scatter of butchered bones. These two common alternative practices should yield markedly different preservations, controlled by the physical environment and the detritivore community. Following the (predominately biological) processes occurring post-mortem, physicochemical processes operating on the organic (hydrolysis, oxidation, condensation) and associated inorganic (dissolution, recrystallization) phases become more apparent. The key role is played by water, Shakespeare's 'sore decayer' (Hamlet Act V, Scene I) which both forms the medium for biological and chemical processes and is a key reactant in organic matter diagenesis; the other main player is oxygen, important both directly and by promoting further biological decay. The final factor to be considered, as archaeomaterials transform over time towards theoretical equilibrium with their burial environment, is temperature; temperature also has an important role 'thresholding' biological activity. Hopefully the presentation will help to identify the major cultural, biological and chemical processes which control preservation, so that effort can be target at the most promising samples.

### 37 **Recovering ancient pathogen DNA from skeletal material**

Ian Barnes

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Bacterial pathogen DNA sequences recovered from archaeological specimens would seem to have significant applications in confirming diagnoses, determining evolutionary history, and establishing past population dynamics.

However, the application of ancient DNA techniques in this area has been disappointing, as the data produced are generally based on inconclusive methodologies and are not sufficiently informative to address real archaeological questions.

In order to address these problems, samples have been taken of individuals from two osteological archives: the Hammond-Todd collection dating to the early 20<sup>th</sup> century, and the Hunterian collection dating to the late 18<sup>th</sup> century. The specimens sampled from these collections have well-recorded causes of death from either tuberculosis or syphilis, are of relatively young age, and have not been buried. They therefore provide a useful baseline to determine the potential for recovery of pathogen DNA from other ancient materials.

This presentation discusses the results of extensive PCR, cloning and sequencing experiments designed to establish the survival of both host and pathogen DNA.

These data are placed in a context of the published work on ancient pathogens, with a particular consideration of the survival and decay of DNA and the role of soil bacteria.

### 38 DNA damage in 100 – 100,000 year-old ancient DNA

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20 years ago, the field of ancient DNA started with the analyses of a small piece of mitochondrial DNA sequence from a 140-year old museum specimen of the quagga, an extinct form of zebra. As it was later shown, already this very first ancient DNA sequence showed two incorrectly determined positions, most likely caused by DNA damage to the template molecule. Surprisingly, for a long time, little effort was made to determine the chemical nature and frequency of DNA damage in ancient DNA, although it has been pointed out once and again, that miscoding lesion may threaten the authenticity of ancient DNA sequences and thus the field of ancient DNA as a whole. Recently, we have shown that the predominant miscoding lesion in a large set of cave bear sequences was uracil, derived by deamination of cytosine, a common type of hydrolytic damage in DNA. Further studies have shown that this type of damage is common for a wide range of samples used for ancient DNA studies and already occurs frequently in samples less than 100 years old. Moreover, there is a negative correlation between the frequency of cytosine deamination and the amount of both amplifiable DNA and amino acids preserved in ancient samples. The high prevalence of cytosine deamination damage requires that either the amount of template DNA obtained from ancient DNA samples is quantified or all positions are amplified at least twice. Both calculations of error probability and analyses of substitution patterns of sequences obtained under the latter criterion show that such ancient DNA sequences are highly reliable.

### 39 Estimation of the diversity of bacterial communities in wooden foundation poles across Europe using molecular biology techniques

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The identification of bacteria associated with decay of wood pilings up to 800 years old is discussed. The identification techniques used were based on nucleic acid extraction from wood pilings across Europe, Polymerase Chain Reaction (PCR) amplification of 16S rRNA genes, separation of 16S rRNA genes based on sequence differences using Denaturing Gradient Gel Electrophoresis (DGGE), and sequence analysis using phylogenetic approaches. Bacterial isolates capable of cellulose degradation were cultured from archaeological timbers and characterised molecularly. Environmental sequences retrieved from the same wood samples were identified after homology searches and phylogenetic analysis. Many of these sequences fell within the same bacterial groups as those from the isolates, particularly those characterised as *Pseudomonas* and *Cellvibrio*. Some sequences, however, remained unidentified. Molecular biology techniques have revealed identities of a broad range of species within wood samples: *Pseudomonas*, *Cellvibrio*, *Psychrobacter*, *Acinetobacter*, *Telluria*, *Duganella*, *Massilia*, *Nitrosomonas*, *Sphingomonas*, *Brevundimonas*, *Rhodobacter*, *Rhizobium*, *Rosebacter*, *Sinorhizobium*, *Paenibacillus*, *bacillus*, *Sulfobacillus*, *cellulomonas*, *Flavobacterium*, *Cytophaga*, *Bergeyella*, *Chroyseobacterium*, *Desulfovibrio* and *Desulfohalobium*

### 40 Could DNA survive under extreme pH conditions?

Virginia Bessa-Correia<sup>1</sup>, Pablo Martín<sup>2</sup>, Colin Smith<sup>1,4</sup>, M<sup>a</sup>Dolores Pesquero-Fernández<sup>1</sup>, Antonio Alonso<sup>2</sup>, Begoña Sánchez-Chillón<sup>1</sup>, Eva-Maria Geigl<sup>3</sup> and Yolanda Fernández-Jalvo<sup>1</sup>

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Modern, archaeological and palaeontological bones in highly acidic (pH<4) and alkaline (pH>10) sediments have often been found to be macro and microscopically degraded on the surface. In the case of teeth, dentin and root seem to be preferentially altered in alkaline environments and enamel in acidic environments. Thus, a pattern for pH-dependent degradation of buried tooth tissue emerges pointing to a correlation between organic content of tooth tissue and sensitivity to the pH of the surrounding sediment: the higher the mineral part of the tooth tissue the more it is sensitive to lower pHs. In contrast, a higher organic part renders them more sensitive to higher pHs. Subsequently surface, isotopic, DNA and protein preservation were analysed. Here we show the results of an experiment of artificial diagenesis of teeth that we exposed for different times to aqueous solutions of extreme pH values. In particular, the level of DNA loss, DNA fragmentation and nucleotide base modification, as analysed by PCR, quantitative Southern hybridisation and STR analysis, equalled the one of control DNA isolated from untreated tissue.

Diagenetic parameter values (% collagen, IRSF, mineral carbonate phosphate ratio, and porosity) and isotopic signals (oxygen and carbon from mineral carbonates) have also been studied. Our results indicate that although the surface of the samples showed already signs of degradation, DNA, protein content and isotopic signals were not significantly altered.

#### 41 **Molecular biological methods used in the analysis of waterlogged wooden artefacts from Nydam Mose**

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Waterlogged archaeological wood is mainly degraded by microorganisms. In order to investigate what kind are involved in degradation of waterlogged archaeological wood from an Iron Age offering site, molecular biological methods have been used.

Identification of the microorganisms causing deterioration is important, because it becomes more and more common not to excavate archaeological sites, but preserve archaeological artefacts in situ. To understand the deterioration process and to make a risk assessment of a site, it is necessary to gain knowledge of the degrader's and their nutrients demands.

Here, we will present our methods in analysing the microorganisms in wood; i.e.

DNA extraction from wood, amplification of DNA with three different primer sets (bacteria, fungi, archaea), creation of clone libraries, analysis of PCR products using Restriction Fragment Length Polymorphism, sequencing of clones and phylogenetic affiliation.

In addition preliminary results from an anaerobic growth experiment with material from the interior of an archaeological sample and the analyses of the obtained cultures will be presented

#### 42 **Fingerprinting the Early Deterioration of Collagen Fibrils And its application for the identification of cooking in archaeological bone**

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We are interested in identifying thermal alteration in archaeological bone. Previous studies have used Transmission Electron Microscopy (TEM) to look at collagen fibrils from fish bone and mineralised sheep tendon after low temperature heating. The results indicated that heat induced morphological changes occur at temperatures as low as 60°C. These findings have now been replicated on sheep humeri however similar changes were observed in unheated sheep bone which had been buried in a low pH (3.5-4.5) soil. Within a given burial however, cooked and uncooked bone was easily distinguishable.

Research on the thermal alteration of collagen fibrils has now been expanded to encompass archaeological and modern mammal bone from different species (cow, dog, horse, human) and with different ages-at-death; non-mineralised collagen has also been analysed.

The results so far have suggested that collagen degradation is more complicated than first thought and may involve different mechanisms dependant on the age-at-death and tissue type. The research also highlights the lack of available information from studies on modern collagenous tissues relating the chemical properties of the collagen triple helix and fibril with their physical properties.

An understanding of these mechanisms is important not only for the identification of cooked bone but also for predicting the state of preservation of archaeological bones and for obtaining optimum yields of intact collagen for isotopic analysis. The most recent results will be discussed.

## 43 Residue analysis of sealed containers from Qumran, Israel

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A valuable source of palaeodietary and cultural information may be found in organic residues preserved in archaeological vessels. Fired and unglazed clay often functions as a trap for organic biomolecules which may be preserved over many millennia. Our aim is to determine whether preservation of organic residues also occurs in archaeological ceramics from the Levant. We have therefore analyzed what appears to be a very well preserved sample from Qumran, Judean Desert, Israel. The site is dated from the ca. late 9<sup>th</sup> – 8<sup>th</sup> century BC until its abandonment during the roman period at 70 AD. During the last season of excavations eighteen sealed jars were found in the Roman Period context. Organic residues extracted from the walls of one of the jars contained over 60 different peaks identified by GC-MS, which may represent degraded fatty acids and monoglycerides, while the vessel's rim produced no significant peaks. A large piece of black material was present in this jar. Infrared spectroscopy showed that it contains a large amount of organic material rich in carboxylate groups, indicated by the 1620cm<sup>-1</sup> absorption, and calcium oxalate monohydrate (1316 and 784 cm<sup>-1</sup> peaks), suggesting a plant origin. Raman spectroscopy showed that the organic material was not carbonized. The black material is soluble in water, slightly soluble in dimethylsulfoxide and dissolves sparingly in chloroform, methanol and chlorobenzene. Low values of amino acids (0.1%) were detected, indicating that little protein is present. The material stains blue with colloidal iron, supporting the notion that it is composed mainly of compounds of polysaccharides. After basic hydrolysis, the GC-MS chromatogram indicated the presence of C<sub>16:0</sub> and C<sub>18:1</sub> as free fatty acids and monoglycerides, known to be the major components of palm oil. As the archaeology of the site indicating that palm trees were grown in abundance, it is possible that the jar contained palm honey.

## 44 The histology of bone degradation

Miranda M.E. Jans and Henk Kars

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In the framework of a European funded project more than 250 bone samples from over 40 different archaeological sites in The Netherlands, the United Kingdom, Sweden, Italy and Turkey were analyzed. These samples have been examined using several techniques, among which thin-section histology. Subsequent monitoring and quality-assessment projects have provided 140 samples from 13 sites in The Netherlands, which have been histologically analyzed as well. The large amount of samples and their varied preservation circumstances have allowed us to study the large variation in bone preservation.

In this poster we will attempt to not only show histology examples of the different mechanisms of degradation such as microbial alteration or cracking, but also examples of several atypical forms of bone preservation.

A more elaborate collection of photomicrographs will be published as a concise and practical histological atlas of bone preservation, primarily aimed at researchers or students in archaeological heritage management.

## Session: Migration

### 45 Using mitochondrial DNA to reconstruct the dynamics of ancient populations

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For the past two decades, ancient DNA studies have promised to actually record evolution, but have been severely limited by the small number of suitably preserved specimens and limited time frame accessible. Recently, sophisticated phylogenetic techniques have been developed to allow temporal, geographic, and genetic data to be included in population-level analyses. These techniques have revealed the full potential of ancient DNA by making it possible to reconstruct the genetic history of large mammal populations through time, including dispersals, localized extinctions, and long-range migrations. To illustrate these techniques, we present a detailed analysis of the last 200,000 years of bison evolution in Beringia and North America, including the contribution of climate change and humans to the North American megafaunal mass-extinctions around 12,000 years ago (12 ka BP). Mitochondrial DNA sequences were obtained from >350 bison ranging in age from >65 ka BP to modern. Phylogenetic analyses depict a large, morphologically and genetically diverse population living throughout Beringia until the Last Glacial Maximum (LGM), around 22-18 ka BP. Around this time, extant populations were separated by advancing continental ice sheets, and shortly afterwards began to decline dramatically in genetic diversity. The mitochondrial data are used to reconstruct the post-glacial dispersal of these populations, and to correlate the observed patterns with the predicted and described movements of other species, including humans. Interestingly, The initial decline in bison genetic diversity correlates closely with environmental changes associated with the onset of the LGM, whereas archaeological evidence does not support the presence of large populations of humans in Eastern Beringia until more than 10,000 years later.

### 46 The Phylogeography of the Extinct Cave and American Lions (*Panthera spelaea* and *Panthera atrox*)

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Humankind has long been fascinated by the “King of Beasts” *Panthera leo*. Indeed, some of the earliest figurative art seems to be an attempt at fusing the form of the lion with that of man.

Numerous factors make the study of lion taxonomy, phylogeography and migrational history interesting.

The spread of anatomically modern *Homo sapiens* out of Africa into Europe, Asia, Siberia and finally North America is almost perfectly mirrored by the lion. Therefore the study of the distribution of *P.leo* forms should provide insights into the barriers encountered by *H.sapiens*.

The study has concentrated on amplifying the control region HVR in subfossil and museum remains of lions from a wide geographic range (Africa, Middle East, India, Europe, Russia, Canada, Alaska and the lower 48 states of the US). Due to numerous peculiarities in the DNA of Felids this region should give the highest resolution picture of genetic variation in lions.

The poster will represent our main findings to date and address such questions as;

Is *Panthera atrox* (American Lion) a valid taxonomic distinction and what is its range?

Is *Panthera spelaea vereschagini* (Beringian lion) a distinct genetic form?

Are Canadian and Alaskan lions the easternmost range of *P.spelaea* or the northernmost range of *P.atrox*?

What geographical/ecological barriers may account for the variation found in lions?

## 47 **Tracing the origin of ancient wheat in Egypt: new prospects by looking at ancient DNA**

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Wheat is one of the principal 'founder crops' that started food production in the Near Eastern 'arc' c. 10,000 years ago and has been the traditional staple of Europe and West Asia. It is one of the first domesticates that appears in Egypt, some 3000 years after its first domestication. During the Graeco-Roman period, Egypt and the Fayum became a breadbasket for the Roman Empire. A research project in the Fayum (Egypt) has been started up, which is focused on the origins of agriculture in the Neolithic and the organisation of agriculture during the Graeco-Roman period. This project will provide new desiccated wheat samples from both periods in addition to the collection that is already available from this area and other sites in Egypt. The analysis of the ancient DNA of wheat occupies an important position. The aim of DNA research is to present a typing of ancient wheat samples in relation to their spatial and temporal distribution. In a wider context, the aim is to reconstruct the early spread and trade patterns of this staple food. As a first step in this reconstruction, DNA fingerprints will also be made from ancient wheat samples from other Egyptian sites, in order to compare them with fingerprints obtained from the Fayum. In this way, botanical remains can be used as a unique type of proxy data for the reconstruction of the distribution and trade of food.

The multidisciplinary research project in the Fayum is a partnership between the University of Groningen and the University of California in Los Angeles (UCLA; Department of Near Eastern Languages and Cultures). The study of ancient DNA is carried out in co-operation with the Research group Molecular Biology of Plants (MBP/RUG), which participates in the Research school Groningen Biomolecular Sciences and Biotechnology Institute (GBB/RUG).



## 48 Population stability, migration and ecological differentiation in cave bears

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Until recently, migrations of species have been deduced either from the palaeontological record or from the pattern of genetic diversity in extant populations. The application of ancient DNA techniques on large numbers of fossil samples allows combining the two approaches. The European cave bear, *Ursus spelaeus*, has one of the best fossil records of all large Pleistocene mammals. At the same time, cave bears have long been known to display large morphological differences between geographically close populations, a finding interpreted as evidence for low migration capabilities of cave bears. By analyzing mitochondrial DNA sequences from diachronical series of cave bear fossils from four different caves in combination with fossils from more than 20 additional caves we find that at least some cave bear populations were indeed genetically stable for tens of thousands of years and in some cases in fact from the end of the penultimate glacial maximum close to the last one. However, we also find evidence for long distance migration. The large morphological distance between geographically close, contemporaneous populations which is accompanied by a lack of mitochondrial DNA exchange can thus not be explained by low migration capabilities but is rather due to reproductive isolation between cave bear populations. Finally, we find evidence that human – cave bear interactions may have destabilized cave bear populations leading to genetic replacement.

## 49 Genetic variation in Southern German cave lions

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Phylogenetic affiliation between the pleistocene cave lion (*Panthera leo spelaea*) and modern *Pantherinae* has been widely discussed. Whereas some authors consider the cave lion as a subspecies of *Panthera leo*, *Panthera leo spelaea* accordingly, others regard him as an own species, *Panthera spelaea*. In some cases he has even been assigned to the tiger clade and identified as *Panthera tigris spelaea*.

Through phylogenetic analysis of the complete cytochrom b sequence of four different cave lions, we can show a definite affiliation of *Panthera leo spelaea* to modern *Panthera leo* as a sister taxon. The genetic variation lies within the range of modern lion populations.

Nevertheless, whether cave lion is a subspecies of *Panthera leo* or an independent species, is only a matter of the convention. The example of *Panthera leo spelaea* shows that genetic distance alone is an insufficient measure to define ancient and modern taxonomic units.

Another notable result of the molecular analysis is that *Panthera leo spelaea* was isolated from populations of its African and Asian relatives for 600 ky until it went extinct, probably during Bronze-Age.

## 50 **Immigrants on the isle of Lewis: radiogenic isotope evidence for status and mobility in the Iron Age and Viking periods**

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This study reports an investigation of diet and migration in the Outer Hebrides using radiogenic isotope analysis of tooth enamel. We analysed burials from two sites on Lewis: the Iron Age cemetery at Galson and the only known Norse family cemetery in the Hebrides, at Cnip. These sites date from two crucial periods in the history of the island: the coming of Christianity and the Vikings. The aim was to characterise the isotope signature of Hebrideans in both periods, and to establish whether immigrants were present amongst the burial populations.

We found the dietary strontium signature of Lewis to be dominated by Quaternary drift in the form of marine-derived shell-sand (machair), rather than the ancient Lewisian gneiss bedrock, whereas human lead signatures were dominated by terrestrial gneiss. In later periods, lead signatures are dominated by lead of anthropogenic origin, but in the earlier Iron Age, both natural and anthropogenic human lead signatures exist, indicating different status, and in conjunction with the strontium results, possibly mainland origins for two adults.

At Cnip, three adults were immigrants to Lewis. Two were buried in adjacent, identical graves, the context and form of which were enigmatic, being neither clearly pagan nor Christian. However, their strontium signatures indicate that they did not originate in Norway, nor from the same place as each other. The third was a female buried with a classic Viking jewellery assemblage. Whilst the results can be explained within the archaeological period, site and context, it is clear from this study that *geological* interpretations of human isotope signatures are not always in accord with the archaeological evidence and the data raise questions about how we may interpret geologically derived elements in biological tissues.

## 51 A molecular genetic approach to the study of ancient wheat

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Since the onset of agriculture in the Near East, wheat (*Triticum*) has been a staple crop. In Roman times, Egypt was an important production area of this staple food. Written sources and archaeological research show that wheat was transported from its production areas along the Nile to other regions, both within and outside Egypt. Berenike was a Graeco-Roman harbour on the Red Sea coast of Egypt to which wheat was transported, both for local consumption and for export overseas. The Groningen Institute of Archaeology has conducted several seasons of excavation in Berenike, which was found to have been inhabited from the 1<sup>st</sup> century B.C. to the early 6<sup>th</sup> century A.D. During the excavations, substantial amounts of desiccated wheat remains were recovered. Neither written sources nor morphological analysis of the excavated wheat remains have been conclusive in establishing the origins of wheat found in Berenike. Here, we propose the analysis of DNA from ancient wheat remains to further elucidate these origins. The initial goal of this project is to establish the technology to extract, amplify, sequence and analyse DNA from desiccated ancient wheat remains. In this context, we recently analysed a 300 year-old sample from a mill near Basel, Switzerland. This sample consists largely of spelt (*Triticum aestivum* ssp. *spelta*, with an admixture of weeds. On the DNA extracted, we performed PCR directed at chloroplast and nuclear loci. PCR products were cloned and sequenced. The resulting sequences from the spelt sample were compared with the results of morphological botanical analysis of the same sample carried out earlier, and to modern wheat sequences. Currently, we are analysing the almost 2000 year-old wheat remains from Graeco-Roman Berenike.

The long-term aim of this work is to combine molecular genetics with traditional archaeological approaches to resolve wheat transport routes in Graeco-Roman times, including those to Berenike.

## 52 Lerna, 2000 – 1500 BC: the biography of a community

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This poster presents part of a new 5-year research project financed by the NWO, and focuses on the changes affecting the community at Lerna, Greece during the Middle Bronze Age. Angel's 1972 publication of the human bones from the intramural cemetery at Lerna remains one of the few large-scale studies of human skeletal material from the Aegean. However, this material needs to be re-examined in the light of new analytical techniques.

The project will combine traditional archaeological analyses with innovative techniques. First, a contextual statistical analysis of the funerary data will be undertaken in order to establish variation among burial groupings. This information will be combined with stratigraphic observations (associations between tombs and houses) in order to establish possible kin groups on purely archaeological grounds.

Second, the human skeletal material will be systematically re-examined: an osteological analysis will enable us to reconstruct the biological history of Lerna's skeletal population (demography, health and oral status, activity patterns) and to examine variation between different sub-groups. The examination of dental lesions, the analysis of stable isotopes (carbon and nitrogen) as well as dental microwear analysis will help us establish dietary patterns of specific age and/or sex groups.

Third, DNA analysis will target genomic and mitochondrial DNA in order to identify gender and reconstruct kinship relations.

We will use teeth as our sample -or long bones, if teeth are not available. A pilot analysis of 10 samples will give us preliminary indications about the preservation of the ancient DNA and the success rate of DNA isolation and amplification. A much larger number of samples will be analyzed, if the first results are encouraging. The selection of skeletons to be sampled will be informed by the archaeological analysis of burial (kin?) groupings; an effort will be made to include different age, sex and wealth categories and modes of disposal from each phase.