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5th INTERNATIONAL ANCIENT DNA CONFERENCE

MANCHESTER CONFERENCE CENTRE
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12 - 14 JULY 2000

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PROGRAMME

Invited speakers in bold

(Abstract numbers in brackets after the title)

Wednesday 12 July

1400 – 1415 Welcome and introduction

Opening address

1415 – 1500 **Andy Merriwether** - Ancient DNA and human migrations *(1)*

1500 – 1530 *Tea*

Ancient human populations and migrations

Chair: Terry Brown

1530 – 1545 Carles Lalueza-Fox - MtDNA from the extinct Tainos (Dominican Republic) and the peopling of the Caribbean *(2)*

1545 – 1600 Marina Faerman - Transatlantic slave trade and sickle cell anemia studied by DNA analysis *(3)*

1600 – 1615 Graciela Cabana - Modeling the effects of random genetic drift and migration on the genetic diversity of ancient populations *(4)*

1615 – 1630 Jennifer Leonard - Origin of American dogs, a separate domestication? *(5)*

1630 – 1645 Ripan Malhi - Where is the location of the source population for Native Americans in the Northeast US? *(6)*

Thursday 13 July

Palaeotaxonomy and population genetics

Chair: Hendrik Poinar

0900 – 0930	**Alex Greenwood** - Nuclear DNA of the woolly mammoth (*Mammuthus primigenius*) *(7)*
0930 – 0945	Ian Barnes - Analysis of permafrost-preserved cat remains using ancient DNA *(8)*
0945 – 1000	Catherine Hänni - European bears radiation during Pleistocene: the problem of *Ursus deningeri (9)*
1000 – 1015	Uta-Dorothee Immel - Genotyping primates by microsatellite analysis from feces *(10)*
1015 – 1030	Peter Galbusera - Study on recent changes in the genetic variation of the Taita thrush (*Turdus helleri*) *(11)*
1030 – 1100	*Coffee*
1100 – 1130	**Alan Cooper** - Ancient DNA from South Parks: moas and non-mammoths *(12)*
1130 – 1145	Beth Shapiro - Pigeons, pathogens, and the Pleistocene: recovery and non-recovery of ancient DNA from archaeological and palaeontological remains *(13)*

Domestications of animals and plants

Chair: Martin Jones

1145 – 1200	Sue Haynes - Getting *Ansers* from ancient DNA *(14)*
1200 – 1215	G. Kahila Bar-Gal - Ancient DNA evidence for the transition from wild to domestic status in Neolithic goats: a case study from the site of Abu-Gosh, Israel *(15)*
1215 – 1230	Robert Blatter - Spelt-specific alleles in HMW glutenin genes and their implication on the origin of European spelt (*Triticum spelta* L.) *(16)*

1230 – 1245 Robin Allaby - Maize evolution in South America *(17)*

1245 – 1345 *Lunch*

Forensic archaeology and analysis of human remains

Chair: Susanne Hummel

1345 – 1415 **Christine Flaherty** - The archaeology of human skeletal remains: beyond ancient DNA *(18)*

1415 – 1430 Alison Graver - Mitochondrial DNA research in the Dakleh Oasis, Egypt *(19)*

1430 – 1445 N. Izagirre - RFLPs in ancient human mtDNA: greater accuracy in haplogroup typing *(20)*

1445 – 1500 Jason Eshleman - Analysis of non-ideal ancient human samples *(21)*

1500 – 1530 *Tea*

1530 – 1600 **Julia Gerstenberger** - Reconstruction of wedding patterns through genetic typing of skeletal remains of an early medieval population *(22)*

1600 – 1615 Els Jehaes - Mitochondrial DNA analysis of the putative heart of Louis XVII, son of Louis XVI and Marie-Antoinette *(23)*

1615 – 1630 Holger Zierdt - Sex hormones in ancient skeletal remains: empirical access to the fertility in historical populations *(24)*

1630 – 1645 C Savorè - Systematic evaluation of the reproducibility of ancient mtDNA sequencing *(25)*

1645 – 1700 Dongya Yang - Hypersensitive PCR, human mtDNA, and ancient DNA studies *(26)*

1715 – 1830 **Poster session**

1900 – 1930 *Drinks reception*

1930 – 2100 *Conference dinner*

Friday 14 July

Palaeodisease

Chair: Marina Faerman

0900 – 0930 **Mike Taylor** - Genetic analysis of tuberculosis in human remains *(27)*

0930 – 0945 Angela Gernaey - Molecular diagnosis of tuberculosis from the Coimbra identified skeletal collection *(28)*

0945 – 1000 A. Zink - PCR amplification of *Plasmodium* DNA in ancient human remains *(29)*

1000 – 1045 Mark Spigelman and others - The Hungarian mummy project *(30–33)*

1045 – 1115 *Coffee*

New sources and uses of ancient DNA

Chair: Alan Cooper

1115 – 1145 **Hendrik Poinar** - Molecular Coproscopy: the many uses of DNA from old poop *(34)*

1145 – 1200 Eva-Maria Geigl - DNA in fossil bones can survive longer than 100,000 years in an insoluble form *(35)*

1200 – 1215 Mim Bower - Ancient DNA from pollen preserved in sediments: a probability or a pipe-dream? *(36)*

1215 – 1230 Ruth Bollongino - Species identification: strategies and application in wildlife conservation and forensics *(37)*

1230 – 1245 Charles Greenblatt - Bacterial isolates from amber *(38)*

1245 – 1345 *Lunch*

1300 – 1400 *Roundtable discussion "A Global History of Health from Late Palaeolithic Times to the Present", led by Marina Faerman, Phillip Walker and Richard Steckel*

Ancient DNA analysis of art and artefacts

Chair: Keri Brown

1400 – 1430 **Joachim Burger** - STR profiles, mtDNA sequences, and RFLP-PCRs from art and artefacts *(39)*

1430 – 1445 Odile Loreille (talk given by Annette Müller) - Multiplex PCR in aDNA studies: application to ancient parchment analysis *(40)*

1445 – 1500 Orin Shanks - Recovery of biomolecules from stone tools *(41)*

1500 – 1530 *Tea*

Preservation, extraction and analysis of ancient nucleic acids

Chair: Terry Brown

1530 – 1600 **Franco Rollo** (talk given by Massimo Ubaldi) - DNA diagenesis: effect of environment and time on human bone and mummified soft tissue *(42)*

1600 – 1615 C. Savorè - Quantification of human DNA isolated from ancient remains *(43)*

1615 – 1630 Annette Müller - Comparison of common aDNA extraction techniques for bone material *(44)*

1630 – 1645 Karin Haack - Reduced post-PCR handling and increased specificity with a pre-PCR gel-loading buffer *(45)*

1645 – 1700 Rocio Vargas-Sanders - Ancient prehispanic tRNA and initiation protein synthesis *(46)*

1700 Close

POSTERS

Nancy Banko et al - Identification of dietary components in ancient and modern processed food *(47)*

Barbara Bramanti and Susanne Hummel - Professional tradition or import of expert knowledge in an early modern society? – a population genetics investigation by means of autosomal STRs *(48)*

Barbara Bramanti et al - Detection of the ΔF508 deletion causing cystic fibrosis in early modern skeletons *(49)*

Katharina Dittmar - Studies on parts of the 28S rDNA on 1000 year old fleas (*Pulex* sp.), recovered from animal mummies from the preincaic Chiribaya Culture, Southern Peru *(50)*

Ceiridwen Edwards et al - The analysis of microsatellite markers in ancient cattle remains *(51)*

Alison Graver et al - Ancient DNA analysis of human remains from a Late Shang Dynasty site at Anyang, China *(52)*

Jennifer Hiller et al - A systematic approach to the recovery of DNA from Pleistocene skeletal remains in cave environments *(53)*

Susanne Hummel et al - Detection of chromosomal aberrations in early human individuals *(54)*

Amy Junnila et al - Speciation of zooarchaeological remains with mitochondrial DNA: a feasibility study *(55)*

C. Keyser et al - Megaplex analysis of two skeletal remains from a frozen burial (Kazaksthan, 4th century BC) *(56)*

Oliver Krebs et al - Purifying aDNA extracts by high-performance liquid chromatography (HPLC) *(57)*

Arlene Lahti et al - DNA has gone to the dogs! *(58)*

Galit Lev et al - Spoligotyping of ancient tubercle bacilli *(59)*

Jennifer Maki et al - Spina bifida occulta in the Dakhleh Oasis, Egypt: a mitochondrial DNA analysis *(60)*

Ludovic Orlando et al - European bears radiation during Pleistocene: the problem of *Ursus deningeri (61)*

Ryan Parr et al - Ancient DNA and prehistoric archaeology in Ontario: a study of human skeletal remains from the Armstrong Mound, Rainy River, Ontario *(62)*

Ryan Parr et al - Intracemetery biological variation at Kellis 2, Dakhleh Oasis, Egypt: a report on molecular and morphological data *(63)*

Per Persson - Experiments on hybridization and biotin-streptavidin capture of ancient DNA molecules *(64)*

Zayil Salazar et al - Genomic DNA analysis of prehispanic bones and teeth samples from San Francisco Caxonos, Oaxaca, Mexico *(65)*

Robert Sallares et al - Evidence from ancient DNA for malaria in antiquity *(66)*

Tobias Schultes et al - Reconstruction of biological kinship in a skeletal collective from a Bronze Age cave *(67)*

Sergio Tofanelli et al - Target-hooked mtDNA from dental bovine remains and cattle domestication in Southern Italy *(68)*

Roco Vargas-Sanders et al - Genetic relations between different Teotihuacan populations *(69)*

Philip Walker et al - An experimental study of the effects of environmental conditions on the color and organic content of bone *(70)*

Registration

The Registration desk is situated in the Lower Foyer of the Weston Building and is open at the following times:

Wednesday 12 July	1100 – 1700
Thursday 13 July	0800 – 1700
Friday 14 July	0800 – 1700

Could delegates please wear their name badges at all times.

For those delegates wishing to see something of Manchester, information on the City and the local area is available at Weston Reception.

Messages

As it will not be possible to interrupt Conference sessions with individual messages, it is essential that delegates check for messages on a regular basis. Telephone and fax messages will be pinned to the message board, which will be adjacent to the registration desk. Messages can be left by telephoning +44 (0) 161 955 8000.

Mobile phones

Mobile phones must be switched off in the Lecture Theatre and Conference Rooms.

Posters

These will be displayed in Weston Rooms I, II and III. The poster session is timetabled for 1715 – 1830 on Thursday 13 July. Posters should be attached to the display boards using velcro or pins. A prize for the best poster will be awarded at the Conference Dinner.

Catering

Coffee and tea will be served in Weston Room I. Where lunches and evening meals have been pre-booked, these will also be served in Weston Room I. The Conference Dinner will be in Weston Room I at 1930 on Thursday 13 July, preceded by a drinks reception at 1900 in the Exhibition Lounge.

Information for speakers

The slide preparation room is Syndicate Room E/F, where slide carousel trays are available.

Facilities are available in the lecture theatre for single and dual projection and for VHS video presentation. For computer presentations, the software available is Microsoft Office 97. Alternatively, there is a Barco 3200 data projector available for speakers who have brought their own laptop computer.

Speakers are asked to hand in their slides before the commencement of the session in which they are to speak. Oral presenters should remember to collect their slides from the slide preparation room after their presentation. No responsibility can be taken for slides not collected by the end of the meeting.

Conference accommodation

Accommodation for delegates is one of the following, both located on Sackville Street:

- Weston Conference Centre – all rooms are en-suite with private telephone, hairdryer, trouser press, tea/coffee making facilities and satellite TV.
- Weston Hall – all rooms are en-suite with private telephone and tea/coffee making facilities.

Breakfast is served between 0730 and 1000. You can check into your accommodation after 12 noon and rooms must be vacated by 1000 on the morning of departure.

Car parking

The Manchester Conference Centre, Weston Building has its own pay car park located on Charles Street. The to two floors (L and M) are security controlled and are locked between 2000 and 0800.

Abstract 1
Ancient DNA and human migrations

D. Andrew Merriwether

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Up until the last decade, the study of human migration patterns was primarily accomplished using genetic variation from living humans and morphological variation from humans from the archaeological record. The ability to screen archaeological remains directly for genetic variation, rather than inferring supposedly genetic changes from skeletal features, has opened up an exciting new avenue of research. Ancient DNA and the archaeological approach to the study of humans are both limited by the fact that you can only test the remains that you discover. This means many questions may never be addressable by ancient DNA methods. Another drawback to using archaeological materials is that it is often difficult to obtain anything resembling a population of individuals that lived together at the same time. Sites may be occupied for thousands of years and have hundreds of individuals dispersed across that timescape. More often, sites have a narrower range of occupation and very few human remains present. Many questions require allele frequency or haplotype frequency data from an entire population, as compared to other populations, to address questions of migration and coancestry. Since finding 'true' populations in the fossil record is rare, this approach may not be available. That often leaves us with anecdotal evidence that a particular lineage was present or absent at a certain time and place in the past. For speciation level events, this may be sufficient to address the question at hand (i.e. are anatomically modern Europeans descended from Neanderthals). For recent migration analyses, we ma y see if the ancient inhabitants are the ancestors of any of the living inhabitants of an area. Thus far, much of the ancient DNA research attempted on humans has been on Native American archaeological sites to address the peopling of the New World. The vast majority of the sequences obtained from ancient specimens are only a single point mutation (or zero differences) from existing Native American sequences, and often cluster with other sequences from that region. Another growing area of research is the African origins of African American slaves. We show some evidence for the potential countries of origin for African Americans.

Abstract 2
MtDNA from the extinct Tainos (Dominican Republic) and the peopling of the Caribbean

Carles Lalueza-Fox[1], Fernando Luna Calderón[2] and Jaume Betranpetit[3]
[1]Secció Antropologia, Facultat de Biologia, Universitat de Barcelona, Avda. Diagonal 645, 08028 Barcelona, Spain; [2]Departamento de Antropología Física, Museo del Hombre Dominicano, C. Pedro Henríquez Ureña, Santo Domingo, República Dominicana; [3]Unitat de Biologia Evolutiva, Facultat de Ciències de la Salut i de la Vida, Universitat Pompeu Fabra, C. Dr. Aiguadé, 08003 Barcelona, Spain

Samples belonging to pre-Columbian Taino Indians from La Caleta site (Dominican Republic) have been analyzed. Tainos and Caribs were the inhabitans of the Caribbean when Columbus reached the Americas. The Tainos were agriculturalists that lived in chiefdoms and inhabited Cuba, Hispaniola, Puerto Rico, and probably Jamaica and some other minor islands, while the Caribs were nomadic hunter-gatherers that raided the Tainos settlements from the South and mainly inhabited the Lesser Antilles. Both human groups became extinct soon after the Contact, decimated by the Spaniards and the diseases they brought. Therefore, it is necessary to rely on the ancient DNA analysis to know the genetic affinities of these groups in relation to present-day Amerinds and to reconstruct the genetic and demographic events that took place during the peopling of the Caribbean. 27 bone samples were extracted and analyzed. The four major Amerindian mtDNA lineages were screened through amplification of the specific marker regions and restriction enzymatic digestion, when needed. The control region I was amplified with four sets of overlapping primers and sequenced in 19 of the samples. Both restriction enzyme and sequencing results suggest that only two (C and D) of the major mtDNA lineages were present in that population; 18 individuals (75%) belonging to the C haplogroup, and 6 (25%) to the D haplogroup. Sequences display specific substitutions described to correlate with each halogroup (C in np 16325 and C in np 16362 for D lineages and C in np 16298 and T in np 16327 for C lineages), as well as some other substitutions, a fact that help discard the possibility of an European DNA contamination. A set of clones from two different amplifications were obtained and sequenced for one sample, to estimate the rate of Taq misincorporations due to template damage. High frequencies of C and D haplogroups are common in South American populations, a fact that points to that sub-continent as the homeland of the Taino ancestors, as already suggested by linguistic and archaeological evidences. The Tainos show quite reduced mtDNA diversity, which can be attributed to genetic drift associated to the peopling of the Caribbean, assumed to have been a linear migratory movement from Mainland South America, following the chain configuration of the Antilles. The analysis of more pre-Columbian samples from other Caribbean islands, like Cuba and the Lesser Antilles, will help us to have a better picture of the migration process of the peopling of the Caribbean.

Abstract 3
Trans-Atlantic slave trade and sickle cell anemia studied by DNA analysis

Marina Faerman[1]*, Almut Nebel[1,2], Dvora Filon[3], Mark G. Thomas[4], Neil Bradman[4], Bruce D. Ragsdale[5], Michael Schultz[6] and Ariella Oppenheim[2]
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The potential and reliability of DNA analysis for identification of human remains are demonstrated by the study of a recent bone sample, representing a documented case of sickle cell anemia. β-globin gene sequences obtained from the specimen revealed homozygosity for the sickle cell mutation, proving authenticity of the retrieved DNA. Further investigation of the entire hypervariable segment I of the human mitochondrial control region and 12 Y chromosome DNA polymorphic markers indicated a male of maternal West African (possibly Yoruban) and paternal Bantu lineages. Corroborating evidence obtained from microscopic examination, historical and medical records facilitated a proper interpretation of the results and certified the authenticity of the genetic findings. These findings are consistent with him being a descendent of Africans brought to Jamaica during the trans-Atlantic slave trade. The reliability of DNA analysis in tracing mutations in the β-globin gene of DNA isolated from archaeological specimens will be discussed.

Abstract 4
Modeling the effects of random genetic drift and migration on the genetic diversity of ancient populations

Graciela Cabana[1], Keith Hunley[1] and Frederika Kaestle[2]
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Recently, ancient DNA work has been brought to bear on issues concerning the demographic history of past populations. Because ancient DNA work often involves the use of two or more samples from varied temporal contexts, a population genetic analysis of these samples must account for potential long-term demographic processes. In this vein, we present a simulation model that draws from ethnoarchaeological, paleodemographic and population genetic studies, and that considers the effects of random genetic drift and migration on single populations through time. We demonstrate the utility of this model by focusing on one demographic issue that has been the subject of recent ancient DNA studies: population continuity versus replacement in a single geographic area. The ability of this model to distinguish population continuity from other processes depends on a number of key variables, including population structure, size, and number of generations. We use this model to evaluate a hypothesis of population replacement in the prehistoric U.S. Great Basin. This application demonstrates the power of this simulation approach to address the general problem of reconstructing ancient population relationships.

Abstract 5
Origin of American dogs, a separate domestication?

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The archaeological record suggests that dogs were domesticated 12,000 to 14,000 years ago in the Near East or Europe, and that they were present in North America by 9000 years ago. Since the Bering land bridge closed around the same time as dogs are thought to have been domesticated, the presence of early dogs in America is a puzzle. Dogs could have been independently domesticated in America from American wolves. Alternatively, the domestication event in Eurasia could be older than usually assumed, as the genetic data suggests. This would allow more time for dogs to spread to America. Mitochondrial DNA sequences from pre-Columbian dogs show that these animals have the same origin as Eurasian dogs. These results allow us to reject the first hypothesis. The dog samples are all from Amerind archaeological sites in Mexico, Peru and Bolivia. Genetic analyses of human populations suggests that these populations resulted from the first wave of humans invading the New World. The presumed early arrival of these migrants supports the hypothesis of a very old domestication of dogs in the Old World.

Abstract 6
Where is the location of the source population for Native Americans in the Northeast U.S.?

Ripan S. Malhi
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Native American mitochondrial DNA contains polymorphic regions, consisting of a gain or loss in restriction sites or the presence of a nine base pair deletion. These mutations characterize at least five distinct lineages (haplogroups) widely represented in populations of the Americas. Linguists and archaeologists have hypothesized that the Algonquian-speaking inhabitants of Eastern North America migrated there from the Columbia Plateau 4000 yBP or earlier. After this eastward migration, the Columbia Plateau populations were replaced by proto-Penutian speaking inhabitants. Modern Native American populations residing on the Columbia Plateau are significantly different in their haplogroup frequency distribution from Great Lakes and Northeast populations. Ancient DNA was extracted and haplogrouped from tooth and bone samples from archaeological sites found in different geographic / linguistic regions of the Columbia Plateau ranging from 200 - 6500 years in age. The samples were sufficiently clustered in time and space in order to be considered populations for the purposes of this study. The haplogroup frequency distributions of these populations were then analyzed and compared to modern Algonquian speaking and other populations as well as

ancient populations. The results of this study provide insight into the occurrence and timing of this prehistoric migration.

Abstract 7
Nuclear DNA of the woolly mammoth (*Mammuthus primigenius*)

Alex D. Greenwood[1], Fred Lee[2], Cristian Capelli[3], Rob DeSalle[4], Alexei, Tikhonov[5], Preston A. Marx[2] and Ross D.E. MacPhee[1]
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Recent extinctions have reduced the diversity of Quaternary proboscideans to only two species (African and Asian elephants). As recently as a few thousand years ago, however, proboscidean species lived in many parts of the globe. Woolly mammoth (*Mammuthus primigenius*) fossils from high latitudes provide a particularly good window into Pleistocene mitochondrial and nuclear genomes because their remains are abundant, large, and often extremely well preserved, making them ideal for biomolecular research. A feature of modern elephants and the extinct woolly mammoth is the presence of large numbers of nuclear copies of mitochondrial genes that can complicate the study of mitochondrial DNA in this group. We have extracted DNA from mammoth samples collected in Alaska, mainland Siberia, and Wrangel Island, in contexts ranging from 26,000 bp to 4,000 bp. We have explored various components of the nuclear genome from these samples including single-copy nuclear genes, microsatellites, and endogenous retroviruses. Presence of nuclear DNA in these samples has been independently confirmed by two laboratories. Such sequences represent a molecular archive that can help to understand the evolution of each of these sequence types across time and space.

Abstract 8
Analysis of permafrost-preserved cat remains using ancient DNA

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In addition to brown bears, the late Pleistocene permafrost deposits of Beringia contain a wide variety of other taxa which can be useful in determining genetic change in response to alterations in climate and environment. One promising group are the cats (Felidae). There are several taxonomic issues which can be resolved using ancient DNA and in addition,

sufficient samples exist of the extinct North American lion (*Panthera leo atrox*) to allow an analysis of population history. Population-level genetic variation can be compared to the modern lion (*Panthera leo leo*), which can be determined using both modern and archival samples. The position of *P. atrox* as a top predator should make it particularly sensitive to environmental change. However, study of the cats has confounded by the recovery of mitochondrial-like sequences, which are likely to be nuclear-encoded. These sequences are recovered from various regions of the mitochondrial genome and can be present in addition to, or instead of, mitochondrial sequences recovered using both modern or ancient material as the source of DNA. Their consistent recovery from ancient material is surprising, given the supposedly much higher proportion of mitochondrial DNA in ancient remains. A preliminary quantification of these sequences suggests that the amount of nuclear DNA in the permafrost specimens is relatively high, almost equivalent to the amount of mitochondrial DNA. This has significant implications for the use of these taxa in ancient DNA studies.

Abstract 9
European bears radiation during Pleistocene : the problem of *Ursus deningeri*

Ludovic Orlando[1], Marylène Patou-Mathis[2], Michel Philippe[3], Pierre Taberlet[4] and Catherine Hänni[1]
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The evolution of European Pleistocene bears is poorly resolved. Palaeontological data allow the definition of several species and lineages, but the relationships between them are unclear. In particular, the relationships between the cave bear (*Ursus spelaeus*), its putative ancestor *Ursus deningeri* and the brown bear (*Ursus arctos*) remain blurred. Here, the DNA analysis of 30 samples of bears coming from several European deposits, ranging from 20 000 to 130 000 years BP, allowed us to resolve this issue. Two mitochondrial DNA regions, the control region and the cytochrome b gene, converge toward the notion that cave bear split largely before the lineages of brown bears. In addition this study led us to conclude that *Ursus deningeri* is the sister group of *Ursus spelaeus* and not its ancestor as proposed by classical palaeontological studies. The study of genetic distances led us to propose that climatic fluctuations explain the splits that took place between the 3 species *U. arctos*, *U. spelaeus* and *U.deningeri*. Given their abundance, their wide distribution in space and time, and their large morphological and molecular diversity, bears are a powerful model to study the setting up of different lineages inside species, shaped by climatic changes during the Pleistocene, as well as extinction periods.

Abstract 10
Genotyping primates by microsatellite analysis from feces

Uta-Dorothee Immel, Susanne Hummel and Bernd Herrmann
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Molecular genetic techniques provide immense opportunities for population genetics, assigning biological relationships and genotyping man and animals by the analysis of modern DNA and ancient DNA. In this study, DNA was extracted from fecal samples of orangutans (*Pongo pygmaeus* ssp.) obtained from individuals kept at different Zoological Gardens in Germany. Their kinship is known and documented in the 'International Studbook of the Orangutan'. Fecal samples were prepared for an automated CTAB-Phenol-Chloroform extraction. Different human short tandem repeat loci and a system for sex determination utilized in forensic case work were amplified by means of a multiplex PCR with a commercial human STR kit (AmpFlSTR Profiler Plus). Five of the nine primer pairs designed for human autosomal STRs in question amplified successfully on orangutan DNA. For these STR systems orangutans reveal polymorphisms comparable to humans. Fragment length determination carried out on a DNA-sequencer enabled us to reconstruct kinship. The technique of typing individuals by analyzing their feces will have important implications for investigations on the genetic diversity of regional populations and the genetic management of wild and breeding populations of orangutans.

GERLOFF U, HARTUNG B, FRUTH B, HOHMANN G, TAUTZ D (1999) Intracommunity relationships, dispersal pattern and paternity success in a wild living community of bonobos (*Pan paniscus*) determined from DNA analysis of faecal samples. Proc R Soc Lond [Biol] *266* (1424): 1189-1195.

IMMEL U-D, HUMMEL S, HERRMANN B (1999) DNA Profiling of orangutan (*Pongo pygmaeus*) feces to prove descent and identity in wildlife animals. Electrophoresis *20* (8):1768-1770.

LAUNHARDT K, EPPLEN C, EPPLEN J T, WINKLER P (1998) Amplification of microsatellites adapted from human systems in faecal DNA of wild Hanuman langurs (*Presbytis entellus*). Electrophoresis *19* (8-9): 1356-1361.

Abstract 11
Study on recent changes in the genetic variation of the Taita thrush (*Turdus helleri*)

Peter Galbusera, Luc Lens and Erik Matthysen
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This research project aims to study the changes in genetic structure of bird populations caused by changes in their habitat. A loss in genetic variability in small and/or fragmented populations and a reduced gene flow between them can have severe effects like inbreeding depression and a reduced capacity for adaptation to a rapidly changing environment. Until recently only contemporary populations (in altered versus unaltered habitats) were compared to study the effect of habitat changes. To unambiguously demonstrate any changes in popoulation genetic structure one needs to know the original structure, in the time span before the changes took place. Our study object, the Taita thrush, is hence situated in the recently fragmented regions of the Taita Hills in Kenya. Recent populations have already been sampled and studied (Galbusera et al. 2000). A pilot study on museum samples started in early 2000. The DNA present in the dried tissues is partially degraded but still suitable for sequence analysis or for amplification in a PCR (with polymorphic microsatellite markers). Hypotheses towards the causes of genetic changes will be tested by correlating the genetic data to historical and recent information of the habitat.

Abstract 12
Ancient DNA from South Parks: moas and non-mammoths

Alan Cooper
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Mammalian bones preserved in permafrost deposits are a unique opportunity for ancient DNA research, because the large numbers of specimens, dating from 10,000 to more than 50,000 years, allow evolutionary change to actually be recorded. As an example, studies of the omnivorous brown bear, hyper-carnivorous short-faced bear and climatic records from Eastern Beringia demonstrate apparently dramatic effects of climate change during the Last Glacial Maximum. The potential of other permafrost studies will also be discussed. In other studies, the complete mitochondrial genomes of two genera of moa, the extinct New Zealand ratite bird, have been sequenced at Oxford - and sections replicated in two additional laboratories. Extensive cloning and quantitation experiments were used to authenticate the sequences, which showed limited amounts of damage-related artefacts. The data was compared to that of the living ratites, and the genetic distances calibrated with fossils to evaluate hypotheses of flighted or vicariant origins for the ratites. The information gained during this project provides some interesting views about the practicality of other large ancient DNA sequencing projects.

Abstract 13
Pigeons, pathogens, and the Pleistocene: recovery and non-recovery of ancient DNA from archaeological and paleontological specimens

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We extracted DNA from tissue from the skull of the Oxford dodo, *Raphus cucullatus*, using both silica and phenol/chloroform techniques. We amplified the 12S and cytochrome *b* genes from this specimen and from other pigeons, including the closely related Rodrigues solitaire, *Pezophaps solitaria*. the Tooth-billed pigeon, *Didunculus strigirostris* and the Passenger pigeon, *Ectopistes migratorius,* to construct a phylogeny containing 38 of the 43 genera in the family Columbidae. Both the 12S and cytochrome *b* phylogenies show the dodo and solitaire to be most closely related to the nicobar pigeon, *Caleonas nicobarica.* In addition, we detail a number of negative results, including failure to amplify several pathogens, such as *Treponema pallidum* and *Coccidiodes immitus* from archaeological and paleontological collections. Attempts to replicate a previous amplification of *Mycobacterium tuberculosis* from a Late Pleistocene bison specimen found in Natural Trap Cave, Montana were also unsuccessful. Studies of La Brea tarpit material have also been negative, although new extraction techniques have been determined. There negative results have important implications for ancient DNA studies, particularly those involving pathogens.

Abstract 14
Getting *Ansers* from ancient DNA

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Species identification is a common problem in zooarchaeological assemblages. Traditionally, interpretations were based on techniques using morphology and biometry. However, for many species, including geese, these criteria do not exist, or are wholly inadequate for specific identification to be realistic. Previous studies at the University of York have shown that DNA retrieved from goose bones at the Saxon site of Flixborough (North Lincolnshire) provided a means of accurately identifying material to species level. In the present study, 323 goose humeri from deposits spanning nearly 4 centuries has provided information on DNA survival in bones of different degrees of preservation and contexts, and has demonstrated the occurrence of both wild and domestic goose species at the site. The results presented here provide for the first time, data on the real (not assumed) significance of domestic geese to the Saxon economy. In conjunction with data from modern domestic breeds, the data from

Flixborough tentatively suggests that DNA may be used to determine the presence of different domestic varieties of geese at this site. Results from modern domestic geese have also provided an insight into the origins of goose domestication.

Abstract 15
Ancient DNA evidence for the transition from wild to domestic status in Neolithic goats: a case study from the site of Abu-Gosh, Israel

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Abu Gosh is a Neolithic site located 12 km west of Jerusalem, Israel. The archaeological remains revealed the presence of two occupations; the Pre-Pottery Neolithic B (PPNB, 9500–8000 BP) period, and the Pottery Neolithic (PN, 7500-5500 BP) period. The fauna from the PPNB levels is characterized by wild fauna including goat remains. The quantity of the goats remains in the assemblage and their morphology suggests that they represent an early phase of domestication. In contrast, the faunal remains from the PN levels are predominantly those of domestic animals, primarily goat (*Capra hircus*). A genetic study was carried out on a small sample of caprine bones from both periods, to determine whether the morphometric criteria used for separating wild and domestic caprines are expressed at the genetic level. In general, aDNA findings agreed with those obtained from the morphometric studies in distinguishing between PPNB and PN goats. However, in the PPNB levels, two species of wild goat were identified on the basis of their mtDNA sequences, but could not be differentiated on the basis of the morphometric analysis. This suggests that post-cranial morphometric criteria for distinguishing between these two species still need to be defined. The findings show that at Abu Gosh, *Capra hircus* was established by the PN period and that the genetic changes which accompanied the shift from wild to domestic goats, occurred within a relatively short period of time (>1000 years).

Abstract 16
Spelt-specific alleles in HMW glutenin genes and their implication on the origin of European spelt (*Triticum spelta* L.)

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Free-threshing bread wheat (*Triticum aestivum* L.) and hulled spelt (*T. spelta* L.) share the same genomes (AABBDD) but the relation of the two wheats remains controversial. Hexaploid spelt originates either by crossing a tetraploid wheat (AABB) with wild *Aegilops tauschii* (DD) growing in the southern areas of the Caspian Sea or by introgression of a hulled tetraploid wheat into bread wheat. In the first case spelt spread into Europe with agriculture, in the second spelt also emerged independently in Europe. To distinguish between those possibilities, molecular markers have to differentiate any introgressing tetraploid wheat from allelic variation in the original hexaploid wheat. We investigated the high molecular weight (HMW) glutenin promoter region of the Glu-A1-2 and Glu-B1-1 gene from 65 recent wheat accessions. Species-specific differences and geographical patterns were detected. To examine genetic differences of spelt and bread wheat in the past and to assess possible correlations due to recent translocations of wheat breeding stocks, we analysed desiccated chaff of spelt and bread wheat, retrieved from the ceilings of two historical buildings (300 and 250 years old, respectively) in the region of Basel, Switzerland. The results from the historical samples are in agreement with the data obtained with modern wheat.

Abstract 17
Maize evolution in South America

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The high genetic diversity of maize (*Zea mays mays*) coupled with its morphological distinctiveness from the wild progenitor(s) teosinte (*Z. mays parviglumis* and *Z. mays mexicana*) has lead to the suggestion that maize has undergone an accelerated rate of evolution following the domestication founder event. In this study we examined the *Adh*2 locus in primitive races of maize grown by the indigenous peoples of South America and archaeological samples from a lowland cave site in Brazil. The biogeography of the *Adh*2 alleles and complex microsatellite structures suggest that there were at least two genetically distinct expansions of maize into South America. An absolute readiocarbon date for the founding of one allelic expansion allows the comparison of ancient and modern diversity and calculation of evolutionary rates. The data supports the hypothesis that maize has undergone an accelerated rate of evolution contrary to the findings of previous aDNA analysis of maize evolution.

Abstract 18
The archaeology of human skeletal remains: beyond ancient DNA

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An ancient DNA analysis of human skeletons from the early Anglian cemetery at West Heslerton, North Yorkshire, was part of a multidimensional research approach to answer questions about gender, kinship, and social identity in the past. The potential of ancient DNA analysis in archaeology is enormous, but is still limited by cost and the state of the art. This research used many lines of evidence, including analyses of DNA, osteological materials, spatial relations, and material culture, to better understand the complexities of burial rites and the subsequent reflection of early Anglo-Saxon England culture and social behaviors. Seventy-seven skeletons were analyzed by extracting DNA from teeth and bones. The amelogenin PCR method was used to differentiate between males and females, and a variety of short tandem repeat analyses were used to determine familial relationships between individuals. Absolute sexing of juvenile skeletal material had not been feasible before the use of PCR; this study has provided not only sex, but an analysis of the relationship of potentially gendered material culture to biological sex, giving us an insight into the development of gender construction.

Abstract 19
Mitochondrial DNA Research in the Dakhleh Oasis, Egypt

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Molecular genetic research is being conducted as part of the Dakhleh Oasis Project (DOP), an international and multi-disciplinary research initiative in the western desert of Egypt. Mitochondrial DNA (mtDNA) is being analyzed from both ancient human skeletal remains associated with the Roman period town of Kellis (100 to 450 AD) and contemporary inhabitants of the Dakhleh Oasis. The primary objectives of this research are to derive paleogenetic information about the inhabitants of ancient Kellis, and to develop a picture of change over time within this desert oasis. Preliminary mtDNA restriction site data and control region sequence variability suggest significant genetic differences exist between the ancient and modern oasis populations.

Abstract 20
RFLPs in ancient human mtDNA: greater accuracy in haplogroup typing

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In this work we have analyzed mtDNA restriction polymorphisms in the necropolis of Aldaieta (Alava) (VI-VII c.). The samples were typed for a restricted set of RFLPs that were diagnostic of European haplogroups, following the hierarchical scheme from Macaulay et al. (1999). We have analyzed 90 samples corresponding to 64 individuals, 26 of them by duplicate. We have examined thoroughly the definition of some of the haplogroups through the analysis of a greater number of polymorphic sites (11 enzymes vs. the 7 commonly used in our previous works). This has allowed us to ascertain some of the problematic haplogroups, like T, J and X. On the other hand, 11% of the haplotypes do not correspond exactly with the haplogroups defined by Macaulay et al. (1999).

Abstract 21
Analysis of non-ideal ancient human samples

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Successful analyses of ancient DNA require stringent controls on contamination from modern sources. Ideal samples for aDNA analysis will have had minimal exposure to potential contamination sources. For human skeletal material, material curated exclusively for aDNA analysis handled only by those individuals performing the aDNA analysis is ideal. However, this is a small subset of potential informative material. Ignoring non-ideal samples greatly reduces potential sample-size as well as the hypotheses that aDNA can address. Studies of human population expansions and migrations rely heavily on adequate sample size. We have been able to obtain verifiable aDNA RFLP and sequence data from human bone previously shown to be contaminated. We present methods for preventing and eliminating contamination from samples that may have been exposed to a modern contamination source prior to analysis. Under certain circumstances, these methods make it possible to obtain reliable data from specimens that would otherwise be inappropriate for study.

Abstract 22

Reconstruction of wedding patterns through genetic typing of skeletal remains of an early medieval population

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The study is carried out on a randomly drawn sample of 200 individuals from the early mediaeval graveyard at Weingarten, Germany, dating from the 5th – 7th century. This skeletal population comprises 800 individuals, who show variation of their social standing through characteristic sets of grave goods that display wealth and rank differentials. Several DNA-typing approaches are applied to reconstruct wedding patterns in this socially stratified population. For one, the simultaneous amplification of nine autosomal short tandem repeats generates the genetic fingerprint that is unique to an individual. Furthermore, through the analysis of Y-chromosomal STRs individuals of the same paternal lineage can be identified, whereas sequencing analysis of the hypervariable region of the mitochondrion can determine which members of a population belong to the same matrilineage. Comparisons between the social groups will show if differing variabilities can be detected for the analysed DNA sequences. In the case of patrilocal residence a high variability of mitochondrial sequences will be detectable, whereas a population practising matrilocal residence should be discernible by a high number of deviating Y-haplotypes. With the results of the aDNA typing it should be determinable if different wedding patterns were applied in the various social groups of this historical population.

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Abstract 23

Mitochondrial DNA analysis of the putative heart of Louis XVII, son of Louis XVI and Marie-Antoinette

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According to official historiography Louis XVII died of tuberculosis in the Temple of Paris during the French Revolution on June 8 1795. Since then, the official version of his death has been repeatedly questioned. One of the most persistent theories claims that it was a substitute who died, while Louis-Charles escaped out of France. At the beginning of the 19th century a

number of men came forward claiming to be the son of Louis XVI. In 1845, one such man, Karl Wilhelm Naundorff, was buried in the Netherlands as 'Louis XVII'. DNA analysis showed that the remains of Naundorff could not be identified as those of Louis XVII (1).This conclusion was based on a comparative mitochondrial DNA (mtDNA) analysis of Naundorff's bone and DNA samples of maternal relatives of Louis XVII. The crucial question of the mystery remained whether Louis XVII died in the Temple or not. In order to put an end to the theory of a substitute, we performed a mtDNA analysis of the heart of the young boy who died on June 8 1795. The heart has been removed from the body during autopsy. In order to obtain unquestionable results, analysis of the heart was independently performed by both laboratories. The results show that the consensus mtDNA sequence obtained in both laboratories is identical to that of the living maternal relatives and to the Habsburg sequences obtained previously.

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Abstract 24
Sex hormones in ancient skeletal remains - empirical access to the fertility in historical populations

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Classic sources used in historical demography enable a hypothetical insight on the population level, which can be a considerable indicator for collective behaviors. For example, the possible causes of the population explosion in Central Europe in the second half of the 18. Century are widely discussed by historians. It is indisputable that it can be observed at the same time of the introduction of the potato as a major nutritional component (e.g. Matossian 1984, Netting 1981, von Gundlach 1989). However, no information about individual or collective biographic events is supplied. Therefore, the sex hormones (steroids) are a suitable endogenous group of materials for the systematic, empirical investigation of the fertility in historical populations. Steroids have been detected early in archaeological material (Lin et al. 1978; Sobolik et al. 1996), and finally the extractability from archaeological bone has been demonstrated with the introduction of GC/MS analysis (Wischmann et al. 2000). This investigation presents the analysis of steroids of the female endocrinium extracted from modern and archaeological bone with methods adapted from food chemistry by means of gas chromatography/ mass spectrometry. The quantification of the hormones permits the reconstruction of the endocrine state of the individuals. Apart from validating biographic events, e.g. the proof of pregnancy in presumed mother/child burials on the basis of specific hormone samples (e.g. Pregenolon), the investigation of skeletal collectives with individuals of different age enables the reconstruction of the fertility course over life span (determination

of menarche and menopause). Exemplarily shown by the analysis of steroids, the preservation of the endogenous biomolecules in the archaeological tissues opens a new perspective for the reconstruction of both individual and collective biographic events.

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Wischmann H et al. (2000) Trace analysis of endogenous and exogenous biomolecules from archaeological skeleton materials. Anthrop Anz *58*:77-83

Abstract 25
Systematic evaluation of the reproducibility of ancient mtDNA sequencing

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It is difficult, if not impossible to establish the authenticity of DNA sequences obtained from ancient remains. Strict adherence to aDNA laboratory protocols can minimize the potential for contamination, but can never eliminate the problem. Increased confidence in the authenticity of aDNA results can be achieved through repeated analysis by an independent laboratory. We have conducted a multi-center study of 2000-year-old human skeletons from the Isola Sacra, Italy to assess the reproducibility of aDNA mtDNA sequence data. Bones from 35 individuals were analyzed by two independent laboratories using a variety of DNA extraction protocols and PCR amplification strategies. Both laboratories obtained mtDNA sequences for 17 of the individuals examined, providing a measure of inter-laboratory reproducibility. Multiple sequences were generated for more than half the individuals examined in each laboratory, allowing an assessment of intra-laboratory reproducibility. Overall, the reproducibility of the aDNA mtDNA sequence data was inversely proportional to the number of variables in the analysis. Reproducibility was greatest when the 'independent' analysis was conducted in the same laboratory using the same protocols. However, this type of analysis may provide a false sense of security since the concordant results may simply indicate the presence of a common contaminant.

Abstract 26
Hypersensitive PCR, human mtDNA, and ancient DNA studies

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PCR amplifications often fail because of degradation and impurities in ancient DNA samples. One approach to overcome amplification failure is to use highly efficient polymerases to try to increase PCR sensitivity. In a study of human mtDNA from 2,000 year old Italian skeletal samples we observed a strong boost of PCR amplifications using AmpliTaq Gold™. But we also found an increase in false signals indicating systematic contamination or non-repeatable sequencing results. A series of experiments using different amplification conditions were carried out to tackle this problem. Results indicate the higher sensitivity of AmpliTaq Gold™, the higher vulnerability of human mtDNA to contamination with modern sources, and the lower number of ancient mtDNA molecules, are the probable causes of false signals. We propose an optimization of the number of cycles during PCR amplification and the consistent use of a series of diluted and quantified positive DNA controls to monitor the levels of contamination in ancient DNA samples.

Abstract 27
Genetic analysis of tuberculosis in human remains

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Information from genome sequencing projects has started to furnish insights into the evolution of *Mycobacterium tuberculosis* complex members as well as to provide likely strategies for differentiating causative species and strains. Whole-genome comparisons, between *M. bovis* and attenuated BCG vaccine strains with *M. tuberculosis* strain $H_{37}Rv$, has highlighted the importance of deletion events (RDs) rather than point mutations, as a source of genetic variation. Completion of an *M. bovis* sequencing project, expected in late summer 2000 will permit the reverse comparison to be made and for additional deletion events in *M. tuberculosis* to be described. Knowledge of unidirectional loss of DNA, gleaned from contemporary studies, will be necessary for development of molecular methods for assessing the balance of disease caused by *M. tuberculosis* and *M. bovis* in past populations and for studying possible virulence factors. To assess the future potential for microbial

palaeogenetics, a series of genotyping techniques have been applied to nine skeletons with osteological evidence of tuberculosis from a medieval skeleton collection from the deserted village site of Wharram Percy, Yorkshire, UK. PCR amplification methods included assays for IS6110, mtp40, *oxyR*285, *rpoB*, *plcD*, spoligotyping and deletion region 7 (RD7), which has occurred in *M. bovis* isolates with diverse genotypic characteristics recovered from a wide host range. In all nine cases, several markers for *M. tuberculosis* complex DNA were found and in all instances it appeared that disease was due to *M. tuberculosis* rather than *M. bovis*. These observations have implications for understanding sources of tuberculous infection in rural agrarian communities.

Abstract 28
Molecular diagnosis of tuberculosis from the Coimbra identified skeletal collection

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The Identified Human Skeletal Collection curated at the Museum of Anthropology, Coimbra University, Portugal, has provided an excellent opportunity for a pilot study of tuberculosis (TB) diagnosis by modern molecular techniques. The collection comprises 505 males and females who died between 1904 and 1936; records detailing age at death, occupation and probable cause of death are available and the osteological examination of the collection, with particular reference to TB, is documented[1]. Since a significant number of the population are recorded to have suffered from TB, this pilot study has been designed to assess the sensitivity and reliability of two biomarkers for *Mycobacterium tuberculosis* – IS*6110*[2] and mycolic acids[3] for the diagnosis of ancient disease; the morphology of the lesions in skeletal remains can often be non-specific[4]. We investigated whether the documented medical historical diagnosis of tuberculosis in eight juvenile individuals correlated with the molecular detection of IS*6110* and mycolic acids. Preliminary results indicated that these biomarkers were detectable in the majority of samples; mycolic acid detection was more in agreement (87.5% match from 8 skeletons) with the documented clinical history than was *IS6110* detection (50% match from 6 skeletons). An interpretation of these analyses will be discussed.

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Abstract 29
PCR amplification of *Plasmodium* DNA in ancient human remains

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The successful detection of ancient bacterial DNA in human remains depends significantly on the availability of adequate primer pairs to obtain specific PCR results. In a previous study on mycobacterial DNA we were able to demonstrate that the application of primers derived from current medical practice may lead to cross-reactions with soil bacteria usually not present in clinical samples. In the present study in a series of bone samples partly presenting paleopathological evidence for chronic anemia from a Southern German ossuary (Rain/Lech 1400-1800 AD) and on mummy material from Egypt (appr. 1500-500 BC) we applied different sets of primers for the detection of plasmodial DNA. Subsequent sequencing of the positive PCR results was performed to confirm the PCR results. Interestingly, the application of a primer pair, which has been developed to identify plasmodial DNA in ancient human remains, revealed a high number of false positive results mostly due to the amplification of fungal DNA. With other primer pairs a number of non-specific DNA fragments was amplified, as evidenced by additional bands on agarose gels. Ongoing studies will use more stringent reaction conditions in order to avoid the additional amplification of non-specific bacterial or fungal DNA, thus allowing the proper identification of plasmodial DNA.

Abstract 30
The Hungarian mummy project

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During reconstruction of the Dominican Church of Vác, Hungary in 1994-1995, remains from 265 individuals were discovered, many naturally mummified. The crypts were continuously utilized for burial by middle class families from 1731-1838. TB was studied because initial X-ray and histological examination suggested the presence of the organism. In addition, the thick lipid rich walls of the tubercle bacillus and its ability to survive after death of the host suggests good preservation of its DNA. Over 400 samples including lung, abdomen, ribs, hair, teeth and clothing from 174 of these individuals are being screened for *Mycobacterium tuberculosis* DNA at the Medical Microbiology Department, UCL. Contemporary written records are available for many individuals and include date of death, age, sex, family name, relatives and a brief description of cause of death. The presence of significant TB infestation in a middle class population of a small township, well before the Hungarian industrial revolution is of interest. These specimens will be examined for signs of other infectious diseases, and host factors indicating host resistance or susceptibility to specific microbial diseases e.g. NRAMP, will be investigated. The presentation will commence with a short video showing the material and mode of sampling. (Funded by a grant from the Wellcome Trust UK.)

Abstract 31
Mycobacterium tuberculosis complex DNA in middle class individuals from 18th-19th century Hungary

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Initial X-ray and histopathological examination of several bodies from the late 18th and early 19th Century found in Vác, Hungary, revealed evidence suggestive of TB infection. Therefore a comprehensive study was undertaken. Samples were collected from 174 individuals. Many were naturally mummified so soft tissues were available. Ribs were sampled from skeletalised material. At present 166 bodies have been examined by specific PCR for *Mycobacterium tuberculosis* complex (MTB) DNA. Results have been analysed by age of the individual, sex, sampling site, and type of PCR used. Overall, 38% of the individuals were positive. Nested PCR based on a 123bp MTB-specific fragment of IS*6110* with a product of 92bp was the most sensitive method. Single-stage IS*6110* PCR detected only 43% of positive samples. Other target sequences detected by PCR include the 19kD antigen gene (131bp); *dnaA-dnaN* spacer region (151bp); *gyrA* (194bp); *katG* (220bp) and the MPB70 antigen gene (372bp). Only 20% of samples positive by nested IS*6110* PCR were also positive for PCRs with a target sequence >300bp, which suggests that amplicon size is a crucial factor and that some specimens contained well-preserved MTB DNA. This contrasts with the extremely poor recovery of human DNA from the same mummified material.
(Funded by a grant from the Wellcome Trust UK.)

Abstract 32
***Mycobacterium tuberculosis* complex DNA from 18th-19th century Hungarians: molecular aspects**

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Mycobacterium tuberculosis complex (MTB) DNA was successfully amplified from 63/166 (38%) of naturally mummified Hungarians from the 18th-19th century. Using written records, several bodies can be placed into family groups. This information combined with analysis of MTB DNA sequencing data and molecular fingerprinting techniques such as spoligotyping can be used to determine the epidemiology of TB infection in this community. Better-preserved specimens were examined for the presence of an IS*6110* insertion in the *dnaA-dnaN* region specific for the multi-drug-resistant Beijing family of TB , although none of the TB isolates screened to date have this specific insertion. TB strains can be placed into genotypic groups 1, 2 and 3 on the basis of silent point mutations in codon 463 of *katG* and codon 95 of *gyrA*. Sequencing of 12 samples has placed these strains into genotypic groups 2 or 3. However, as it has been proposed that group 1 is older in evolutionary terms than groups 2 and 3 (Sreevatsan *et al*., 1997) we may have expected to see more representatives of group 1. We are also screening for the presence of point mutations associated with drug resistance in the *rpoB*, *katG*, *inhA* and 23S rRNA genes. (Funded by a trust from The Wellcome Trust, UK.)

Abstract 33
Tuberculosis in ancient populations: a linkage study

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Tuberculosis is the primary focus of this research in the analysis of disease linkage in past populations. The linkage analysis will focus primarily on the natural resistance associated macrophage protein 1 (NRAMP1) gene, where it has been shown that a number of mutations cause susceptibility to tuberculosis, leprosy, leishmania and other pathogenic infections. This work requires the recovery of both pathogenic DNA and host nuclear DNA from the remains.

The Hungarian mummified remains from Vác, Hungary, are suitable for linkage analysis due to the static nature of the collection. A static population has no gene flow, no influx or efflux of members and no reproduction. This is ideal for studying diseases in a population. There is no resistance or immunity developing to the disease, no ageing, no transmission of the disease, no dietary change to affect the disease state, no contraction of other diseases to complicate the analysis. The samples pre-date the industrial revolution, which has increased the rate of infection from tuberculosis in other locations, however the collection represents both sufferers and non-sufferers from one contained population group. The group have no dietary stress or malnutrition and have adequate hygiene. The significance of this study will be to provide useful information on the history of tuberculosis and in particular the history of host-pathogen interactions.

Abstract 34
Molecular Coproscopy: the many uses of DNA from old poop

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Modern dung has become the item of choice for conservation genetic studies, as it allows the noninvasive typing of individuals and their phylogeographic study. Coprolites, are the fossilized remains of extinct animals and recently we have shown that the DNA from these remains is accessible once Maillard derived crosslinks are resolved by the addition of PTB, a chemical known to cleave these products. Mitochondrial DNA allows the identification and phylogenetic study of the defecating animal and chloroplast DNA can reveals novel aspects of their diet and nutrition. The identified plants can also then be used to reconstruct the paleoenvironment and climate for the surrounding area at that time point. Analysis on ground sloth coprolites from three different time periods from Gypsum Cave Nevada will be discussed as well as preliminary analysis of archaic human coprolites.

Abstract 35
DNA in fossil bones can survive longer than 100,000 years in an insoluble form

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Depurination of DNA in solution occurs at a rate which has led to the prediction that DNA older than 5000 years could not be preserved (1). However, mitochondrial DNA in fossils estimated to be around 40,000 years old has been amplified (2) showing the limits of this extrapolation. Indeed, particular fossilisation processes could protect DNA molecules in

some fossils from microbial, oxidative and hydrolytic attack and preserve them for a much longer time. I have shown (3) that this is indeed the case and that DNA can be found preserved in 465,000 years-old fossil bones (originating from a layer dated by ESR of the Lower Palaeolithic site of Menez-Dregan, Brittany, France [4]). However, this DNA is present in an insoluble form associated to the mineral matrix, and resists up to now standard extraction procedures and thus PCR analyses. I could demonstrate the presence of this DNA using a molecular hybridisation technique with modern genomic DNA as probes. This technique, which is insensitive to enzyme inhibitors present in all fossil extracts and less sensitive to contaminations with modern DNA, allowed me to identify the taxa of the bones (*Perissodactyla* or *Artiodactyla* in the various bones analysed) by comparison with control experiments using DNA of extant species (3). Furthermore, the technique allows the analysis of the original fossil DNA molecules without modifying them which is impossible during PCR. The exceptional stability of this genetic material could be due to its adsorption to hydroxyapatite and other bone molecules occurring immediately after the death of the individual and later on during the fossilisation process. The price to pay for such preservation is that the association is so tight that it prevents up to now conventional extraction and PCR amplification. To validate this approach, more recent, morphologically well preserved and palaeontologically determined bones from various periods (from Neolithic to modern times) are now being investigated. Current results show that molecular hybridisation is still the most adequate approach to analyse DNA in fossil bones regardless of its solubility. So far, soluble DNA can be found and analysed by PCR only in the more recent bones. However, some of them also contain preserved DNA in an insoluble form. In conclusion, DNA can be preserved over much longer periods of time than previously expected in a form stably associated to other fossil components. This presumably shields DNA from chemical and microbial degradation allowing this amazing preservation. However, this association presently hampers sequence analysis of the genetic material as current extraction procedures, developed for present day, biological samples are inefficient.

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Abstract 36
Ancient DNA from pollen preserved in sediments: a probability or a pipe-dream?

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Ancient DNA has been proven to be extant in a number of archaeologically preserved materials so why not pollen? Pollen is preserved stratified in waterlogged sediment, an environment which is stable, cold, in many cases anaerobic and where DNA/mineral interactions can occur. All factors which promote the preservation of DNA over long periods. Additionally the gross morphology of pollen also promotes the preservation of genetic material. It has a UV filtering, hydrophobic pollenkitt coating overlying one of the most resistant biopolymers known in the biological world - sporopollenin; adding up to a biological system designed to preserve DNA. Taking these and other factors into account the probability of DNA being preserved in sediment stratified pollen is high. Pollen from different types of plants are morphologically distinct, skilled palynologists can identify pollen to genera and in many cases to species level. This information is used to reconstruct past environments, data useful, not only to ecologists but also to archaeologists who seek to understand the factors which influenced human behaviour in the past. However, there is a large and significant genus for which morphological identification of pollen is not possible to species level: the grasses. Grassland covers vast tracts of the Eurasian continent encompassing many archaeological civilisations and time periods. Within the grasses is counted not only cereals, an important food source, but also the wild wheats, progenitor species of the cereals used today. Because of the problems with pollen identification, the ecology and past spread of these species is poorly understood, leaving a large part of the past human environment unquantifiable. Thus a genetic test for the identification of the presence of grass, cereal or wild wheat species in a pollen assemblage would have great significance to the archaeological community.

Abstract 37
Species identification – strategies and application in wildlife conservation and forensics

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Illegal trade is a serious threat to many wildlife species. In order to convict anyone suspected of illegal trade evidence has to be identified. This is particularly difficult when only traces

are at hand or when animal products (e.g. 'medicines') are available that are not morphologically identifiable. Such cases may be solved by genetic analysis. Elephants for example are still poached on account of their flesh and tusks, whereas rhinoceroses are extremely endangered since their horns are regarded an aphrodisiac in many asian countries. Therefore primer pairs which are located at the mitochondrial cytochrome b gene were developed for elephants and rhinoceroses. These primers amplify a 151 bp / 192 bp sequence and make it possible to identify animals at the species level. The analysis is carried out through direct sequencing. To avoid contamination the primers reveal 3´mismatches compared to the respective human sequence. Studies on ivory and rhinoceros horn confiscated by the customs have already been performed. Furthermore, a semi-universal primer pair matching most Mammalia was designed for those samples without any indication of the species of origin. In order to demonstrate the wide applicability to different materials (hair, fur, skin, antlers), museum specimens are about to be analysed additionally.

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Abstract 38
Bacterial isolates from amber

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A number of reports on the isolation of organisms from amber have been published dating back to Galippe's publication in 1920. We have recently isolated an assemblage of characterized organisms from Dominican and Lebanese amber. These seem to be related to deep soil, an anaerobic environment, plant associated species, and insect gut symbionts - all fitting the expectations of an amber habitat. The characterized bacilli and cocci are found to be phylogentically distant from extant relatives. Characterization by fatty-acid profiles (FAME) and rDNA are often disparate in their species designations. A 'gene scan' of the original amber before prolonged culture by random priming of the rDNA and restriction enzyme fragment analysis showed that major forms of the isolated organisms were present before the possibility of contamination during the culture. On the other hand, microscopic mapping of the amber revealed delineated areas of stress around micro-inclusions and even an intruding fiber, so it is impossible to deny that bacteria could not have entered at a later date. Morita has recently suggested that organisms in amber as well as in other extreme environments may take their energy for DNA repair but not growth from hydrogen utilization. Survival mechanisms of paleo-organisms will be considered.

Abstract 39
STR profiles, mtDNA sequences, and RFLP-PCRs from art and artefacts

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The only factors involved in the preservation of DNA in primary biological materials, like bones or teeth, are the milieu, duration, and conditions of deposit. But with secondary biological materials, manufacturing processes also exert an influence. The term secondary biological materials refers to those that were submitted to intentional modification by human beings, usually for the purpose of preserving or protecting against aerobic activity. This includes e.g. the tanning of animal skins to manufacture leather, the pickling, marinating, or cooking of foodstuffs, and the boiling down of fish bones or parchment scraps to manufacture glues and binding agents. Most historical art and handcraft objects contain raw materials consisting of organic components, which in turn may contain the DNA of their animal or plant of origin. Restorers, archivists, and archaeologists are usually interested in identifying the species of origin of a biological component. Along with mtDNA sequencing, an RFLP-PCR method was also applied to this end; it permits relatively easy identification even of a mixture of various species. Beyond that, STR-profiling enables the identification of individuals of origin, as has been shown for historical parchments. The DNA profiles not only enabled the re-assembly of individual finds or fragments, but are also an excellent means of revealing contaminations and of authenticating less variable markers, such as mtDNA sequences. And finally, it will be shown how targeted primer design permits the effective exclusion of potential contaminators, like humans and cattle.

Abstract 40
Multiplex in aDNA studies: application to ancient parchment analysis

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Experiments on human aDNA revealed that it's possible to amplify simultaneously several autosomal loci (STR). Since multiplex save sample material and ensure the authenticity of the results, they are especially suitable for aDNA studies. Here, we report the design of two multiplex systems for animal aDNA amplification. The goal is to enable precise genetic characterizations of the species that have been used in the Middle Ages to prepare parchments. Although animal skins of e.g cattle, sheep or goat reveal morphological traits that are characteristic for each species, time induced alterations often prevent a successful morphological determination as well as the assignment of isolated pieces to the original scroll. The first multiplex will amplify mtDNA for species identification and two nuclear fragments for sexing. The second multiplex will include different microsatellites markers, all

polymorphic in cattle, sheep and goat. Those markers should also give valuable information about the genetic diversity of past Bovidae populations.

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Abstract 41
Recovery of biomolecules from stone tools

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Traces of protein and DNA preserve on stone tools used to process animals. Microcracks produced during the manufacture of stone tools may harbor ancient biological residues. Our studies tested this hypothesis using light, scanning electron, fluorescence, and confocal microscopy to characterize microcracks produced in obsidian microblades by pressure flaking. Cell-sized fluorescent latex beads entered microcracks at depths exceeding 50 um below the microblade surface. Using fluorescently labeled protein and DNA, we documented subsurface penetration of blood into microcracks. Biological residues trapped in these microcracks were not removed by surface washing. However, we found that sonication in 5% ammonium hydroxide or an 18-hour incubation in 4M guanidinium hydrochloride released 60-80% of the trapped DNA and protein. We sequenced DNA from residues and hair recovered from chert flakes used in an experimental butchery 14 years ago. These experiments suggest that residues trapped in microcracks represent an important and often overlooked source of ancient DNA and protein.

Abstract 42
DNA diagenesis: effect of environment and time on human bone and mummified soft tissue

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We are investigating the phenomenon of DNA decay in human bone and mummified soft tissue with the aim of understanding the precise role played by environmental factors such as the action of soil bacteria and fungi. In this view we collected a group of 30 human femurs dating 1800 A.D. and submitted them to a series of analyses to check the following parameters: state of preservation of collagen; racemization of aspartic acid; presence and state of preservation of total DNA; state of preservation of mitochondrial DNA; presence of bacterial DNA; presence of the DNA of fungi and other eukaryotic microorganisms. The results are consistent with the hypothesis that most of the endogenous human DNA vanishes long before the bone structure undergoes a significant diagenesis and before soil microorganisms can penetrate in a massive way. This hypothesis seems to hold also for mummified soft tissue. In this case, however, we must take into account the development and possible action of the cadaveric microflora before (natural or artificial) mummification takes place.

Abstract 43
Quantification of human DNA isolated from ancient remains

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Ancient DNA studies are hampered by a number of intrinsic factors. Different strategies have been proposed to overcome problems such as the degradation, modification and low level of DNA templates, the presence of inhibitors, the presence of contaminating DNA from non-human sources, the presence of contaminating modern DNA templates and the efficiency and reliability of the extraction and purification protocols. Commonly, the results of PCR reactions are used to assess the efficiency of the extraction and the presence of DNA at the source, since a major problem is assessing the presence of endogenous DNA in early stages of the analysis. We discuss the use of a simple protocol for detecting and quantifying the amount of human genomic DNA in ancient extracts. This hybridization-based method is capable of quantifying amounts of human genomic DNA as low as 10-20 picograms and is not affected by the presence of other biological material. We have used this method to assess the efficiency of different extraction protocols, to track the presence of DNA along different stages of the extraction and to optimize the amount of input DNA necessary to obtain reliable PCR amplification of aDNA.

Abstract 44
Comparison of common aDNA extraction techniques for bone material

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A comparative study was accomplished to test the effects of different DNA extraction methods on quality and quantity of DNA obtained from a skeletal bone sample. The ability of six DNA extraction methods to recover amplifiable DNA from skeletal bones was tested on a three thousand year old Tibia, which was taken from the Lichtenstein cave (Harz Mountains, Osterode, Germany). A phenol/chloroform/silica based DNA extraction method (Baron et al. 1996), a Guanidiniumthiocyanate/silica based extraction method (Höss 1995), a CTAB based extraction method (Yang et al. 1997), a simple boiling method (Meijer et al. 1992), a Chelex based method (AmpFlSTR Profiler Plus™ Users Manual) and a commercially available kit (InViSorb Forensic Kit I, InViTek) were tested. Amplification as a measure of DNA yield and absence of inhibitors were performed using a multiplex PCR (AmpF*l*STR Profiler Plus™, PE, Applied Biosystems). The study showed that only few DNA extraction methods could isolate chromosomal aDNA from the sample, whereas almost all methods recovered mitochondrial DNA. The most effective way to isolate amplifiable chromo somal DNA from bone was the phenol/chloroform based DNA extraction method.

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Abstract 45
Reduced post-PCR handling and increased specificity with a pre-PCR gel-loading buffer

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Minimal handling of samples as well as specificity of PCR is vital in ancient DNA and forensic applications. Here, we present an evaluation of the influence of the 'Yellow Sub' polymerase chain reaction (PCR) additive on the specificity and yield of the PCR. The additive is a viscous yellow non-ionic polysaccharide gel-loading buffer which we however apply *before* rather than after the PCR. This procedure lowers the risk of contaminating the laboratory with amplicons during post-PCR handling procedures. To evaluate the effect of 'Yellow Sub' on the PCR, we amplified part of the mitochondrial DNA control region in a gradient thermocycler at various series of annealing temperature ranges with and without Yellow Sub. Subsequently, we tested five additional polymerases with and without Yellow Sub. The experiments, derived from the same mastermixes, show that Yellow Sub consistently reduces non-specific products while retaining the same yield, and that Yellow Sub does not significantly change the optimal annealing temperature, pH value or Mg^{2+} concentration optimum.

Abstract 46
Ancient prehispanic tRNA and initiation protein synthesis

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The cytoplasm of eukaryotic cells contains two species of methionine specific tRNA: $tRNA^{imet}$ and $tRNA^{mmet}$. The in vitro initiation reaction includes at least four basic steps: 1) Formation of an eIF-2- GTP-met-$tRNA^{i}$ ternary complex; 2) transference of this complex to the 40S ribosomal unit; 3) binding of mRNA to the 40S complex and ; 4) joining of the 40S initiation complex to the 60S subunit Protein factors required for initiation of polypeptide synthesis in cell-free systems are commonly prepared from the crude mixture of ribosomal salt-washed proteins. Recently, it has been technically possible to recover DNA and RNA from ancient tissues of prehispanic Mexican bones. Moreover, Venazzi and Rollo (1990) presented evidence that nucleic acids in ancient samples are composed mainly of RNA, and suggest that the longevity of ribosomal RNA could be due to its substantial abundance

compared with other cellular nucleic acids. In this work it was utilised tRNA belonging to prehispanic Mexican bones in an in vitro initiation protein synthesis system, the results show that tRNA was functional for the two first initiation steps.

Abstract 47

Identification of dietary components in ancient and modern processed food

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The investigations on the composition of processed food are often limited to either plant or animal ingredients. In this study we combine different experimental strategies for both the identification of animal *and* plant components. Historical samples were taken from ceramics in the Ethnological Museum in Berlin. Modern processed food was used for the development of extraction protocols adapted to different methods of processing. The analysis of plant materials is carried out by the separation of plant components by means of High Performance Liquid Chromatography (HPLC). This technology enabled to prove e.g. specific carotenoids which are characteristic for maize. Components of animal origin are identified by the amplification of a part of the cytochrome b using an universal primer for several species usually processed in Middle European food. The site on the cytochrome b was chosen so that the treatment with a restriction enzyme leads to different fragment lengths (PCR-RFLP) that are characteristic for various animal species (*Bos taurus*, *Ovis aries*, *Sus scrofa*, *Capra hircus*, *Gallus gallus* and *Meleagris gallopavo*).

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Abstract 48

Professional tradition or import of expert knowledge in an early modern society? – A population genetics investigation by means of autosomal STRs

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In this study, the human skeletal collective of Goslar (Germany, 18th century) was genetically characterised in comparison with a present-day German sample. The historical individuals studied have the peculiarity to have shared the same kind of work as metallurgists. The matter of interest was whether specialised workers continued to come from different regions to work in Goslar – as was the case in the Middle Ages – or if the metallurgy had meanwhile become a family tradition in a close society. The genetic analysis was performed with highly polymorphic markers (STRs) commonly employed for population genetics. STRs are ideal markers also for degraded DNA because of their short fragment size and because they are easily and unambiguously detectable. A commercial kit (AmpFlSTR Profiler Plus™, Applied Biosystems) that permits the simultaneous amplification of nine microsatellites together with the sex marker amelogenin was adopted. After a first study that amplified HUMVWA from 60 medieval individuals (Zierdt et al. 1996), this was the first time that the results of aDNA investigation by means of STRs were submitted to statistical analysis for population genetic aims. Different statistical tests excluded the hypothesis of a continuos migration flow – or a know-how import - to Goslar in the considered period.

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Abstract 49
Detection of the ΔF508 deletion causing cystic fibrosis in early modern skeletons

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Human population history is at least to a reasonable extent dedicated to selective processes among which genetic diseases play a major rule. There is evidence that during the process of peopling Europe the adaptation to environmental constrains affected the number of pathological genes. Certainly this lead to balanced and unbalanced polymorphism in the genetical record of Europeans. Recently, for the cystic fibrosis a heterozygote advantage was suggested to explain its high incidence (1:25 carrier individuals in Europeans). This selective advantage was speculated to be due to a high resistance to chloride-secreting diarrhea, including cholera. Up to now the major efforts to test directly this hypothesis have been limited to animal models. We propose to verify the hypothesis directly on a sample of human individuals that died during a cholera epidemic spread in the Mediterranean basin at the beginning of nineteen century. In this preliminary investigation we checked off the presence of amplifiable DNA in the human remains simultaneously in terms of genetic fingerprints and for the cystic fibrosis mutation ΔF508.

Abstract 50
Studies on parts of the 28S rDNA of 1000 year old fleas (*Pulex* sp.), recovered from animal mummies from the preincaic Chiribaya Culture, Southern Peru

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Generally, well preserved arthropod remains are rarely found among archaeological material. The outstanding preservation of some mummified animals (mainly dogs and guinea pigs) from several archaeological sites in Southern Peru gave rise to the idea of recovering ectoparasitic arthropod remains, that once infested the animals during life time. The excavation sites belong to a complex of settlements and grave sites, that are dated to the Chiribaya Culture, a coastal affiliation of the Tiahuanako Culture, some 1000 years ago. From the animal mummies, various species of ectoparasites could be obtained, among them specimen of the flea-genus *Pulex*. The experimental studies were carried out at the Laboratory of Insect Genetics of the Brigham Young University, Provo, Utah, USA and the Institute of Biomedical Research in Borstel, Germany. Independently, 211 base pairs of the same region (EF) of the 28S rDNA could be sequenced. Contrarily to the statement of low cloning efficiency of ancient DNA, the method was successful. A largely unresolved problem is the taxonomic status of *Pulex simulans/irritans*. Interestingly, comparison to recent species revealed a difference of several base pairs in an otherwise highly conserved region. Studies about a correlation of morphological variability towards genetic variability have not yet been conducted. Due to the unclear taxonomic position of *Pulex simulans* and *Pulex irritans* I refer to them as *Pulex simulans/irritans.*

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Abstract 51
The analysis of microsatellite markers in ancient cattle remains

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Ancient DNA research in animals has largely concentrated on the analysis of mitochondrial DNA (mtDNA). Such studies have been informative in tracing the underlying patterns of European cattle domestication, but the resolution of cattle mtDNA haplotypes imposes limitations on their use. Microsatellite loci have been shown to give resolution to the breed level within cattle. To investigate archaeological questions within Europe, it is clear that an approach involving microsatellite analysis would be informative. It has been possible to

redesign primers that will amplify a small (50 to 200 bp) region of a number of microsatellite loci, making them amenable for use with ancient material. A panel of cattle bones excavated from a 1000-year-old Viking site in Dublin was chosen as previous analyses indicated that these remains are well preserved. Several microsatellite loci have been successfully typed for seventeen specimens. These initial results indicate that microsatellites will be useful in the study of ancient cattle, but that certain criteria need to be satisfied, including well preserved samples, informative loci, short amplification products, and reproducible results. This development will allow the comparative analysis of ancient samples with extant cattle breeds, making it possible to address specific questions about the origins of European cattle.

Abstract 52
Ancient DNA analysis of human remains from a late Shang Dynasty site at Anyang, China

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A pilot ancient DNA study was conducted on skeletal remains from the late Shang Dynasty Heiheru Site at Anyang, China (c. 1300 B.C.). Mitochondrial DNA (mtDNA) extraction and PCR amplification were performed on a test sample of human skeletal elements representing 12 individuals. Recovered DNA was assessed for the presence or absence of RFLP-site markers defining Asian haplogroups and mitochondrial HVI sequence polymorphisms. Nine ancient samples yielded PCR amplifiable DNA. Technical problems included poor preservation of skeletal samples, pre-laboratory contamination of samples with human DNA, and low ancient DNA yield. Preliminary data suggest endogenous DNA is preserved in skeletal samples from the Heiheru site and that further investigation will yield information useful for the paleogenetic study of the ancient Shang.

Abstract 53
A systematic approach to the recovery of DNA from Pleistocene skeletal remains in cave environments

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Questions of recent human genetic evolution have turned to ancient DNA studies for resolution. Sometimes this results in successes, notably the sequencing of DNA from the

Neandertal I specimen, and thereby provides a powerful insight into questions surrounding the origins of anatomically modern humans. In many cases, however, samples simply fail to yield amplifiable DNA. This is doubly unfortunate, as DNA analysis is both expensive and destructive. This project seeks to address the difficulties encountered with the recovery and analysis of ancient DNA from Pleistocene hominid samples across Europe, by devising non-destructive and microscale analyses which can be used to predict the likelihood of DNA recovery. In order to achieve this, the project will focus primarily on faunal remains associated with hominid remains in karstic caves. Cave sites have been chosen because they tend to be more thermally and geochemically predictable and less microbially active than open sites. Caves are more likely to remain undisturbed by reuse over time and represent the major sources of Pleistocene bone material. Climate data and soil conditions will be taken into account, and a suite of destructive and nondestructive techniques to experimentally determine the diagenetic condition of the faunal bones will be employed.

Abstract 54
Detection of chromosomal aberrations in early modern individuals

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In human genetics diagnosis the search for possible chromosomal aberrations is frequently achieved by comparative genome hybridisation (CGH). Unlike PCR the hybridisation technique does not involve an amplification of the investigated DNA resulting in an extreme robustness against contaminating cells. Additionally, the CGH technique does not require a certain hypothesis - like it is represented e.g. by probes and primers - since the total chromosomal set of an individual is examined simultaneously. Until today two studies using CGH have been carried out on ancient DNA. The experimental results of the first study were able to prove a monosomy of chromosome 17 in a 262 year old fetus revealing multiple severe malformations. The second study presently investigates the DNA extracted from an early modern skeleton that reveals a microcephalic cranium. The CGH results indicate a partial deletion of chromosome 8p. By Human Genetics such deletions on 8p are known to be linked to cardiac defects, craniofacial malformations and cranial dysplasia.

Tönnies H et al. (1998) Chromosome analysis of a 262 years preserved fetus with multiple congenital malformations: first application of comparative genomic hybridisation to ancient DNA. Eur. J. Hum Genet. 86:

Hummel S et al. (1999) Proving authenticity of ancient DNA by Comparative Genomic Hybridisation. Naturwissenschaften 86: 500-503

Abstract 55
Speciation of zooarchaeological remains with mitochondrial DNA: a feasibility study

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North American archaeologists often encounter ambiguities when trying to distinguish *Bison bison* from *Bison antiquus* in archaeological and paleobiological contexts. The following pilot study was designed to address the problem of bison differentiation by examining a small, conserved portion of their mitochondrial 12s ribosomal RNA gene. The first goal of this study was to extract DNA from the ancient *Bison antiquus* specimens and the 400-year-old *Bison bison* specimen. The second goal was to sequence the region of interest, looking for species specific motifs. If such distinctions can be confirmed in future studies, then archaeologists have a new means of speciation using hard tissue remains. Examination of a population of suitable specimens will be required to determine if any individual variation occurs in the 12s region within populations of bison, both extinct and extant.

Abstract 56
Megaplex analysis of two skeletal remains from a frozen burial (Kazaksthan, 4th Century BC)

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The discovery in eastern Kazakhstan of a barrow dating from 4th century BC had provided the opportunity to excavate a frozen burial. The burial chamber contained a wood sacrophagus with two human bodies, a young adult man and an old woman. They were well-preserved and some part of flesh and muscles were still present. During the excavation, it could be proven that the woman had been buried 15 to 30 years after the sacrophagus had been pillaged, which certainly took place some times after the first body had been inhumed. DNA analysis was undertaken to determine whether these human remains represented closely related individuals. Analyses were performed on muscle, brain and bone samples with the AmplF*l*STR® kit. Amplifications were successful from bone samples only. The molecular sexual determination allowed to confirm and clarify archaeological data. Haplotypes of the two subjects were determined for 8 STRs and revealed that they were closely related. The present investigation highlights the idea that molecular biology represents a very useful tool for archaeologists, nevertheless the state of skeletal preservation remains a determinant factor. Because the bodies had been frozen by exceptional climatic conditions., their DNA was preserved better than other samples from the same period but not exhumed from frozen soils.

Abstract 57
Purifying aDNA extracts by High-Performance Liquid Chromatography (HPLC)

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The analysis of ancient bone samples might be hampered by the inhibition of the PCR. Especially soil components like fulvic and humic acids, which invade archaeological skeletal material, are potential PCR inhibitors. These substances form a very diverse group, varying in their chemical structure, molecular weight and their solubility. In some aspects the chemical properties of several of these acids are very similar to those of DNA. Therefore they often cannot be removed from DNA extracts without removing considerable amounts of DNA as well. Nevertheless, purification of DNA extracts from inhibitory fulvic and humic acids with minimal loss of DNA can be achieved by using a chromatographic separation by means of HPLC. To prove the effectiveness of this method bone samples which did not reveal results with conventional techniques (phenol-chloroform extraction, DNA purification kits from different suppliers) were subjected to HPLC. The samples were ground, submitted to a decalcification step and then the extract was injected into the HPLC, using ethanol and water (aqua bidest.) as eluents. After this treatment no inhibition occurred within the PCR. It can therefore be shown that purification by HPLC is a reliable method to remove inhibitory substances from aDNA.

Abstract 58
DNA has gone to the dogs!

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The viability of DNA extraction from skeletal material was tested on six cranial fragments of *Canis lupus*. Specifically, a 250 base pair region within the D-Loop of the mitochondrial (mtDNA) genome was targeted for analysis. Adhering to standard operation procedures developed by the Lakehead University Paleo-DNA Laboratory, all bone specimens yielded the targeted DNA. Results were verified by repeated trials, and sequence data was obtained from the relevant region. Findings can be used to determine efficiency of mtDNA extraction from bone material on larger populations, as well as to assess biodiversity within the *Canis lupus* species.

Abstract 59
Spoligotyping of ancient tubercle bacilli

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Taylor et al. (1999), first applied the technique of Spoligotyping to ancient DNA of tuberculous bone. The method, developed for the rapid diagnosis of tuberculosis by Kamerbeek et al. (1997) utilyses PCR amplification of repeated short sequences in the tuberculosis genome which are separated by variable spacer elements. It combines an exceedingly sensitive detection system with hybridization of the short PCR amplicons to oligonucleotides linked to a membrane. It may help to provide an answer to an important question in the evolution of tuberculosis, that is the differentiation of *Mycobacterium bovis* from *M. tuberculosis*. Spoligotyping can also contribute to identification of the tuberculosis reservoir in ancient times and the method of transmission. Specimens suspected on pathological grounds of being positive tuberculosis were screened for the Mycobacterium IS 6110 sequence and the rpsL gene which encodes for the S12 ribosomal protein of the bacillus. The 33 samples ranged from 17,400 years of age to those of barely a hundred years. The amplification products were all sequenced to be certain of their authenticity. Positive samples were examined by Spoligotyping. The results will be discussed from the point of view of the problematics and possibilities of the method.

Abstract 60
Spina bifida occulta in the Dakhleh Oasis, Egypt: a mitochondrial DNA analysis

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The prevalence of spina bifida occulta evident in skeletal remains form the Kellis 2 (circa A.D. 100-450) site of the Dakhleh Oasis, Egypt has been determined to be approximately 22%. The high incidence of this specific congenital malformation may be indicative of a highly inbred population, a reflection of regional paleodietary deficiencies such as folic acid and zinc, or alternatively a result of a genetic predisposition to spina bifida occulta induced by environmental variables. The examination of a 274 bp segment of the HV1 region of the mitochondrial genome through automated sequencing techniques, coupled with restriction site data were utilized to determine correlations between the presence of spina bifida occulta and maternal descent.

Abstract 61

European bears radiation during Pleistocene: the problem of *Ursus deningeri*

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The evolution of European Pleistocene bears is poorly resolved. Palaeontological data allow the definition of several species and lineages, but the relationships between them are unclear. In particular, the relationships between the cave bear (*Ursus spelaeus*), its putative ancestor *Ursus deningeri* and the brown bear (*Ursus arctos*) remain blurred. Here, the DNA analysis of 30 samples of bears coming from several European deposits, ranging from 20 000 to 130 000 years BP, allowed us to resolve this issue. Two mitochondrial DNA regions, the control region and the cytochrome b gene, converge toward the notion that cave bear split largely before the lineages of brown bears. In addition this study led us to conclude that *Ursus deningeri* is the sister group of *Ursus spelaeus* and not its ancestor as proposed by classical palaeontological studies. The study of genetic distances led us to propose that climatic fluctuations explain the splits that took place between the 3 species *U. arctos*, *U. spelaeus* and *U.deningeri*. Given their abundance, their wide distribution in space and time, and their large morphological and molecular diversity, bears are a powerful model to study the setting up of different lineages inside species, shaped by climatic changes during the Pleistocene, as well as extinction periods.

Abstract 62

Ancient DNA and prehistoric archaeology in Ontario: a study of human skeletal remains from the Armstrong Mound, Rainy River, Ontario

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Ancient DNA analysis was conducted as part of the bioarchaeological investigation of human skeletal remains recovered from the Armstrong Mound, a Middle Woodland Laurel mortuary site in northwestern Ontario. Mitochondrial DNA (mtDNA) was extracted from long bone sections and screened for coding region restriction sites defining New World haplogroups A, B, C and D. Of the 11 skeletal samples analyzed, 6 contained PCR amplifiable ancient DNA and could be assigned to a specific haplogroup. Five individuals have been assigned to haplogroup A, while one individual carries the 9 bp delection characteristic of haplogroup B.

Possible familial relationships within the mound were investigated through sequence analysis of a segment of mtDNA hypervariable region 1 (HV1).

Abstract 63

Intracemetery biological variation at Kellis 2, Dakhleh Oasis, Egypt: a report on molecular and morphological data

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The emerging field of molecular archaeology can contribute significantly to the study of intracemetery biological variation. The study of human skeletal remains from Kellis 2, a Roman period cemetery in the Dakhleh Oasis, Egypt, involves the integration of molecular genetic data with traditional morphological approaches. The Kellis 2 skeletal series presently includes 378 burials, all of which have been analyzed for non-metric trait morphology. Molecular data are presented for a small subset of individuals and the relationship between the two paleogenetic data sets is examined.

Abstract 64

Experiments on hybridization and biotin: streptavidin capture of ancient DNA molecules

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Tofanelli et al 1999 (Ancient Biomolecules, Vol. 2, pp. 307-320) have proposed the use of streptavidin coated beads to capture DNA that has hybridized with a biotin labeled probe, to isolate ancient DNA from bone extracts. I have done experiments with a modified version of this method. In a series of experiments with the addition of known amounts of recent human DNA the method seem to give a 100% recovery of a mtDNA fragment. In experiments on ancient bone and teeth samples from horse, the method also prove to be very successful.

Abstract 65
Genomic DNA analysis of prehispanic bones and teeth samples from San Francisco Caxonos, Oaxaca, Mexico

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Identification of human remains by DNA analysis has proven to be a powerful tool on forensic investigations. On the archaeological context of the pre-Columbian site of San Francisco Caxonos, Oaxaca inhabited by highland zapotecs, have been found some burials that belong to at least to the Classic (circa AD 400) to the late Post-Classic period (AD 1300-1521). The conjunction of dietary studies and DNA analysis will provide a useful element to make clear distinctions between individuals on the same archaeological context. To make DNA analysis we used bones and teeth samples that were treated under different cleaning conditions to avoid PCR inhibitors. On this work we present the results obtained with genomic DNA and dietary studies which show that individuals of this population are slightly different from those of other ancient Mesoamerican groups; besides, we try to find the relationship between the different burials found at this ancient highland zapotec settlement through Amplitype PM and DQA-1 probes.

Abstract 66
Evidence from ancient DNA for malaria in antiquity

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PCR-based techniques were applied to samples from human skeletal remains from a Late Roman infant cemetery at Lugnano in Teverina, Umbria, Italy (5th century AD). Using circumstantial evidence, the excavators (D. Soren *et al.*) hypothesised that the burials were the product of an epidemic of *Plasmodium falciparum* malaria (1). We have attempted to test their hypothesis directly by studying ancient DNA. One skeleton (a 2–3 year old infant) has yielded amplifications of 18S ribosomal DNA specific to *P. falciparum* malaria (2). These data prove that *P. falciparum* malaria was present and active, although it remains possible that other diseases were also involved. Thus the archaeologists' hypothesis has been at least partially vindicated by biomolecular methods. We have also searched for human genetic mutations associated with malaria and have found two cases of glucose-6-phosphate dehydrogenase deficiency in other skeletons from this site.

1. D. Soren, T. Fenton and W. Birkby (1995) *J. Palaeopathol.* 7:13–42.
2. R. Sallares and S. Gomzi *Ancient Biomol.* forthcoming.

Abstract 67
Reconstruction of biological kinship in a skeletal collective from a Bronze Age cave

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Until now kinship analysis has focused on situations where the burial site suggested concrete biological relationships between certain individuals (e.g. Gerstenberger et al. 1999). The aim of this paper is to show that kinship analysis can be widened to skeletal collectives with no prior information about biological relationships. The applied strategy is based on the genetical assignment of individuals to paternal and maternal lines, which consequently allows the formulation of testable hypotheses concerning concrete genealogical relationships. Skeletal remains of 36 individuals of the Lichtenstein cave (Harz Mountains, Osterode, Germany), dated to the Late Bronze Age, were investigated. Identification of paternal relationship could be established through multiplex amplification of four Y-STR-systems (Schultes et al. 1999). Maternal relationship was identified by multiplex amplification and direct sequencing of three loci of the hypervariable regions 1 and 2 of the mitochondrion (Schultes et al. in prep.). Testing of direct biological relationship was achieved through analysis of nine autosomal STRs with the kit Profiler Plus™ (PE Applied Biosystems). Biological relationship of varying degree could be documented in the Lichtenstein cave. These results challenge the archaeological interpretation of the cave site, that was assumed to have been used as a place of ritual sacrifice. Identification of family structures supports the interpretation of the cave as a burial site and opens perspectives for understanding kinship systems in prehistoric societies.

Gerstenberger J, Hummel S, Schultes T, Häck B, Herrmann B (1999) Reconstruction of a historical genealogy by means of STR analysis and Y-haplotyping of ancient DNA. – Eur J Hum Genet *7*: 469-477.

Schultes T, Hummel S, Herrmann B (1999) Amplification of Y-chromosomal STRs from ancient skeletal material. – Hum Genet *104*: 164-166.

Abstract 68
Target-hooked mtDNA from dental bovine remains and cattle domestication in Southern Italy

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Target-Hooking (Tofanelli et al., 1999) is an efficient method for the isolation of rare and degraded nucleic acids from crude lysates, based on the biomagnetic immobilisation of the

target sequence by affinity capture. In detail, the protocol exploits the large binding capacity of streptavidin-coated magnetic beads (Dynabeads M-280, Dynal AS, Oslo) and 5'-biotinylated oligonucleotides as recovery probes. We *target-hooked* DNA strands embracing a 173 bp segment of the bovine mitochondrial control region (nucleotides 16166-16338 of the reference sequence, Anderson et al. 1982) from crude extracts of ancient dental remains from Southern Italy, whose estimated age ranged between 9,000 and 500 BP. Specifically-designed oligos were used as PCR primers in two-rounds standard amplification assays and as sequencing primers on gel-purified target bands (with the Dye terminator Cycle Sequencing Kit, PE Biosystems). Appropriate contamination controls were always as expected. We successfully sequenced PCR products in 7 out of 13 samples. The results of phylogenetic and diversity analyses were tested against two hypotheses: 1) a recent introduction of domesticated animals from the Near East; 2) an *in situ* domestication of local *Bos primigenius* populations.

Anderson S, de Bruijn MH, Coulson AR, Eperon IC, Sanger F, Young IG (1982). Complete sequence of bovine mitochondrial DNA. Conserved features of the mammalian mitochondrial genome. *J. Mol. Biol.* 156:683-717.
Tofanelli S, Dell'Amico MC, Nencioni L, Paoli G, Borgognini Tarli SM (1999). Recovering ancient DNA by streptavidin-coated magnetic beads and biotinylated oligonucleotides. *Ancient Biomolecules*, 2:307-320.

Abstract 69
Genetic relations between different Teotihuacan populations

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Teotihuacan is one of the most important archaeological sites from Mesoamerican cultures. Teotihuacan culture was represented by different epoch: Formative Period (800 BC to 200 AD), Classic Period (200-650 AD) and Epiclassic and Postclassic Period (650-1500 AD). Archaeological research conducted by many investigators have found bone remains from individuals that lived in Classic, Epiclassic and Postclassic periods. First period was represented by skeletons belonging to Temple of Quetzatcoatl, La Ventilla B, Oztoyahualco and Teopancazco sites. The human bone remains that belong to second period, were excavated from Tunnels and Caves at Teotihuacan. This work will deal with prehispanic nuclear DNA and archaeological data coming from five archaeological sites that have important information concerning Classic and Epiclassic Teotihuacan populations. DNA purified from 30 samples have been able to give positive results of amplification and typing using Perkin Elmer Cetus forensic probes, enabling to do comparisons with well known genetic markers between the individuals from different temporalities and archaeological sites of Teotihuacan. Thus, the possibility arises of studying genetic variability in ancient and actual Mexican populations.

Abstract 70

An experimental study of the effects of environmental conditions on the color and organic content of cremated bone

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Preliminary research indicates that DNA is sometimes preserved in burned bone. The environmental variables responsible for the survival of organic material under various cremation conditions are a matter of debate. A series of controlled burning experiments was conducted to determine the effects that temperature, time, and oxidizing environment had on bone color and collagen content. These experiments show that collagen persists in bones exposed to temperature as high as 600 degrees centigrade. They also indicate that bone color changes during burning are strongly influenced by the availability of oxygen, and that color provides a reliable indicator of collagen preservation. This research suggests that color is a useful index of the presence of biomolecules in burned bone. We are currently studying the samples used in our experiments to determine the correlation between collagen preservation and the preservation of DNA.