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The Fourth GeneTime Workshop

‘Domestication of Plants & Animals’

Tuesday 4th and Wednesday 5th September 2007

**McDonald Institute for Archaeological Research
University of Cambridge**



Hosted by:

Molecular Population Genetics Laboratory

Smurfit Institute, Trinity College Dublin, Ireland



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Schedule – Day One

Tuesday 4th September

- 9:40 **Opening Remarks**
Matthew Collins
- 9:50 **Tutorial 1 – Anne Tresset**
Archaeozoology, archaeogenetics and animal domestication in the Old World
- 10:50 **Coffee**
- 11:20 Student presentation 1 – **Yue (Vickie) Yang**
Review: Identifying early domestic cattle from Pre-Pottery Neolithic sites on the Middle Euphrates using sexual dimorphism (Helmer *et al.* 2005)
- 11:40 Student presentation 2 – **Simon McGrory**
Dairy farmers, dentists and dead cows: A biomolecular approach to a zooarchaeological problem
- 12:00 **Tutorial 2 – OI Craig**
Can stable isotopes be used to study domestication?
- 13:00 **Lunch**
- 14:20 Student presentation 3 – **Frauke Stock**
Ancient DNA and cattle domestication
- 14:40 Student presentation 4 – **Michael Campana**
Mediaeval cattle breed improvement at Dudley Castle, West Midlands, United Kingdom: A genetic approach
- 15:00 Student presentation 5 – **Julia Elsner**
Y-chromosomal markers provide no evidence for introgression between wild and domestic cattle in the early European Neolithic
- 15:20 Student presentation 6 – **Camilla Speller**
Pre-historic turkey (*Meleagris gallopavo*) exploitation in the Southwest United States
- 15:40 **Tea**
- 16:10 **Keynote Speech – Dan Bradley**
How to get ahead with domestic DNA: cattle as a case study
- 17:10 End of Day One
- 18:00 Pub, followed by workshop dinner for all participants

Schedule – Day Two

Wednesday 5th September

- 9:30 **Tutorial 3 – Emma Finlay**
Sequences and population history
- 10:10 Student presentation 7 – **Simone Jung**
Molecular factors in the evolution of cattle
- 10:30 Student presentation 8 – **Beatrice Demarchi**
Review: Multiple origins of cultivated grapevine (*Vitis vinifera* L. ssp. *sativa*)
based on chloroplast DNA polymorphisms (Arroyo-Garcia *et al.* 2006)
- 10:50 **Coffee**
- 11:20 Student presentation 9 – **Paula Campos**
Review: Genetic evidence for a second domestication of barley (*Hordeum vulgare*) east of the Fertile Crescent (Morrell & Clegg 2007)
- 11:40 Student presentation 10 – **Hugo Oliveira**
Adopting agriculture in Iberia and North Africa: an archaeogenetic study of tetraploid wheats
- 12:00 **Tutorial 4 – Greger Larson**
DNA studies of modern, historic and ancient pigs, and scientific revolutions
- 13:00 **Lunch**
- 14:20 Student presentation 11 – **Bodo Schulenburg**
Review: Animal DNA in PCR reagents plagues ancient DNA research (Leonard *et al.* 2007)
- 14:40 Closing remarks
Ceiridwen Edwards
- 14:50 **End of workshop**
- 15:00 Tea - DIY

FOR GENETIME Ph.D. STUDENTS ONLY!!

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|-------|--------------------------------|--------------------------|
| 15:30 | Genetime Ph.D. student meeting | Paula Campos |
| 15:50 | Genetime Ph.D. student meeting | Bodo Schulenburg |
| 16:10 | Genetime Ph.D. student meeting | Frauke Stock |
| 16:30 | Genetime Ph.D. student meeting | Yue (Vickie) Yang |
| 16:50 | GeneTime PI meeting | |

Keynote Speech

How to get ahead with domestic DNA: cattle as a case study

Prof. Dan Bradley

The study of animal domestication using DNA, both modern and ancient, has been illustrated by cattle studies. A progression has been from early studies of classical polymorphisms, through modern and ancient mtDNA sequencing to current examination of autosomal variants. Near-future developments will see the search for selected variants in the domestication process using resources from the genome project, wider interrogation of ancient genomes and utilisation of the new high-throughput technologies. Through all this there is an imperative for productive interdisciplinary collaboration.

Tutorial 1

Archaeozoology, archaeogenetics and animal domestication in the Old World

Dr. Anne Tresset

For many years, archaeozoology has mostly relied on bone metrics and bone shape modifications to highlight domestication processes and trace the diffusion of domesticates outside of the distribution area of their progenitors. Though useful in many cases, these traditional tools have limits. First, they do not allow alone to spot domestication processes at very early stages. For these reasons, these criteria have often been used in conjunction with others, such as demographic indicators of human control over animal populations but, even then ambiguities subsist. Size and shape modifications are also difficult to use alone to trace domesticate diffusion in areas where the wild forms exist, as early domesticates are very similar to wild individuals. A good illustration of this is given by the difficulties to trace the diffusion of domestic bovines and suids out of the Near East into Europe, together with the Neolithic flows, a story stretching from mid-9th millennium cal. BC (introduction of domesticates to Cyprus) to the 4th millennium cal. BC (introduction of domesticates to northern and north-western Europe). Here, archaeogenetic studies have been a real breakthrough, as ancient DNA brings less ambiguous information than bone size and shape in a number of early Neolithic contexts in Europe, even if mitochondrial DNA only documents the maternal aspects of the story. The comparison between genotypes and phenotypes (documented by bone characteristics, as size, shape, discrete characters ...) also brings interesting elements. In a number of cases, it allowed confirmation or information of the identification of bone remains to a given form or species. The study of ancient genetic diversity is also a very promising tool to document the dynamics of domesticate dispersion and thus the herding management underlying it. Bottleneck effects or, on the opposite side, a good preservation of genetic diversity, have distinct signatures in terms of herd sizes involved in diffusion, as well as in terms of isolation between domestic herds – and thus pastoral communities – over the Neolithic territory.

Thus, tight cooperation between archaeozoology and archaeogenetics has recently provided very convincing and stimulating scenarios of animal domestication in the Near East and domesticate diffusion outside of it, describing processes to a degree of detail never achieved so far. Mutual enrichment between the two scientific communities, and a better understanding of each other's limits and prospects, has allowed us to broaden the scope of research on animal domestication history by extremely substantial degrees. But, fortunately, much remains to do in the field for joint teams of archaeogeneticists and archaeozoologists!

Tutorial 2

Can stable isotopes be used to study domestication?

Dr. Ol Craig

Stable isotope analysis has been widely applied to human and animal remains from a range of periods and geographical locations to study palaeodiet, migration/mobility and palaeoclimate. However, there are relatively few stable isotope studies that have directly addressed issues of plant and animal domestication. After providing some general background information on stable isotope analysis, we will discuss how isotope data may be used, either directly or indirectly, to infer domestication in the archaeological record. After evaluating a few case studies, we will then attempt to sum up the potential of this approach and especially how it may be integrated with other lines of enquiry more commonly used to study domestication.

Tutorial 3

Sequences and population history

Dr. Emma Finlay

What can we deduce about how population size has changed over time by looking at modern DNA sequences? Participants will be given sequences simulated under different demographic histories and asked to identify the networks, phylogenies and skyline diagrams that were generated under different scenarios.

Tutorial 4

DNA studies of modern, historic and ancient pigs,
and scientific revolutions

Dr. Greger Larson

Domestication is nothing else if not a story. This tutorial will focus on the relationship between empirical data, hypothesis formulating, and hypothesis testing in developing and relaying that story. The focus will especially be on the main message of Kuhn's "*The Structure of Scientific Revolutions*" as a way of thinking about the derivation of narratives from data, and what to do when new evidence comes to light that seems to contradict that data. Lastly, we will discuss the applicability of Occam's Razor and the importance of biological and archaeological contexts when discussing competing interpretations of the same dataset.

Student Presentation 1

Title:	Review: Identifying early domestic cattle from Pre-Pottery Neolithic sites on the Middle Euphrates using sexual dimorphism
Paper details:	Helmer <i>et al.</i> (2005) In: <i>The First Steps of Animal Domestication: New Archaeozoological Approaches</i> (ed. Vigne, Helmer & Peters), pp. 86-95. Oxford: Oxbow Books
Presenting author:	Yue (Vickie) Yang

The identification of domestication in ruminants is usually carried out using biometrical analysis and comparing the results with data from wild specimens. For cattle as well as other taxa, the major difficulty when one observes a reduction in size is to establish if this reduction is due to climatic factors, a cynegetic stress (over-hunting), a preferential hunting of females, or as a result of domestication. While in the first two cases, one must rely on other studies or even complementary disciplines, biometrical analysis is essential for sex differentiation which is fundamental for distinguishing between selective hunting of females and domestication. The methods regularly employed in archaeozoology have a major drawback because it is not possible to determine the number, the mean or the standard deviation for each sex. Thus, we had used two new methods: the Kernel analysis and the mixture analysis.

The recent excavations (Göbekli, Dja'de, Halula) and the study of material from Mureybet provide a significant sample from a relatively small region with comparable climatic conditions, where sites are separated by less than 150 km and range from PPNA to Middle PPNB.

On condition that the distinction between male and female specimens is carried out, it is possible to provide evidence for cattle domestication in the region from the Early PPNB onwards.

Student Presentation 2

Title:	Dairy farmers, dentists and dead cows: A biomolecular approach to a zooarchaeological problem
Author(s):	Simon McGrory
Author(s) affiliations:	BioArch, University of York, U.K.
Presenting author:	Simon McGrory

Domestication of animals led to the development of many different husbandry strategies to exploit different species and products. These differing husbandry regimes can theoretically be distinguished, from each other and from hunting behaviour, by differences in their culling or “kill-off” patterns which are reflected in the different mortality profiles of faunal assemblages recovered from archaeological sites. The nature of archaeological faunal remains means that it is impossible to generate a true mortality profile unless both sex and age at time of death can be determined from a single bony element something that is not currently possible. This project seeks to rectify this problem in cattle. A proteomics approach is used to discriminate the sexually dimorphic forms of the enamel protein amelogenin. This approach allows the sexing of cattle from their mandibles, an element already used for age at time of death estimation.

Student Presentation 3

Title:	Ancient DNA and cattle domestication
Author(s):	Frauke Stock, Ceiridwen J. Edwards & Daniel G. Bradley
Author(s) affiliations:	Molecular Population Genetics, Trinity College, Dublin, Ireland
Presenting author:	Frauke Stock

The aim of the project is a phylogeographical study of ancient European cattle remains. Mitochondrial DNA is the traditional DNA marker used to approach those questions, but is of little informative value here as all European cattle divide into only two haplogroups (T and T3). The genotyping of ancient cattle remains might allow a more detailed study of the geographical distribution of cattle genotypes and its subdivision within the European cattle population.

Therefore, out of a variety of microsatellite markers that are well known and documented in cattle, a subset will be selected that have a high informative value (regarding heterozygosity, allele frequency and mean number of alleles).

The redesign of the primer pairs will be carried out considering the characteristics of ancient DNA. Due to strong degradation, the possible PCR product length is limited and the probability of null alleles is increased. Additionally, deamination of the DNA strand might lead to incorrect genotyping. Several techniques will be employed to avoid these issues.

Student Presentation 4

Title:	Mediaeval cattle breed improvement at Dudley Castle, West Midlands, United Kingdom: A genetic approach
Author(s):	Michael G. Campana ¹ , Mim A. Bower ¹ & Richard M. Thomas ²
Author(s) affiliations:	¹ Archaeogenetics Laboratory, McDonald Institute for Archaeological Research, University of Cambridge, U.K. ² School of Archaeology and Ancient History, University of Leicester, U.K.
Presenting author:	Michael G. Campana

Recent analysis of a substantial faunal assemblage from Dudley Castle, West Midlands, has revealed marked changes in the nature of animal husbandry in the later 14th century, including a dramatic increase in the size of many domestic species (Thomas 2005a, 2005b). This new evidence provides the earliest indication of animal improvement in mediaeval England. One of the key unresolved questions from this study, however, is the cause of the size change. Two theories have been proposed to explain this phenomenon: (1) the size increase reflects improved upkeep of the animals, possibly facilitated by the post-Black-Death expansion of pasture; (2) the size increase reflects selective breeding or the introduction of new genetic stock—a process that may have been advanced by the increased numbers of wage-earning peasants following the Black Death, who would have had intimate knowledge of animal husbandry and much greater incentive to improve the profitability of their stock.

Since traditional zooarchaeological methods lack the sophistication to answer this question, we have attempted to analyse ancient microsatellites, genetic markers which have been well-characterised in modern cattle (e.g. López Herráez *et al.* 2005). We present here the initial findings of this research.

López Herráez, D. *et al.* 2005. Comparison of microsatellite and single nucleotide polymorphism markers for the genetic analysis of a Galloway cattle population. *Zeitschrift für Naturforschung* **60**, 637–643.
Thomas, R. 2005a. *Animals, Economy and Status: The Integration of Zooarchaeological and Historical Evidence in the Study of Dudley Castle, West Midlands (c.1100–1750)*. BAR British Series 392. Archaeopress, Oxford.
Thomas, R. 2005b. Zooarchaeology, improvement and the British Agricultural Revolution. *International Journal of Historical Archaeology* **9** (2), 71–88.

Student Presentation 5

Title:	Y-chromosomal markers provide no evidence for introgression between wild and domestic cattle in the early European Neolithic
Author(s):	Julia Elsner ¹ , Amelie Scheu ^{1,2} , Joachim Burger ¹ & Ruth Bollongino ^{1,3}
Author(s) affiliations:	¹ Arbeitsgruppe Paläogenetik, Institut für Anthropologie, Johannes Gutenberg-Universität, Saarstrasse 21, D-55099 Mainz, Germany ² Deutsches Archäologisches Institut, Eurasien-Abteilung, Im Dol 2-6, Haus II, D-14195, Berlin, Germany ³ UMR 5197, Archéozoologie, Histoire des Sociétés Humaines et des Peuplements Animaux CNRS - Muséum National d'Histoire Naturelle, Case Postale N° 56 (Bâtiments d'Anatomie Comparée), 55 Rue Buffon, F-75231 Paris Cedex 05, France
Presenting author:	Julia Elsner

Morphological and genetic research on prehistoric animal bones have traced back the origin of today's domesticates to the Fertile Crescent from where they were imported into Europe during the early Neolithic. However, some authors suggest an autochthonous domestication or subsequent introgression of European wild aurochs.

To clarify the possibility of prehistoric hybridization, we analysed Neolithic samples from wild (*B. primigenius*) and domesticated cattle (*B. taurus*) originating from various sites all over Europe and western Asia using molecular genetic methods. Based on our previous studies on the mitochondrial genome, a significant introgression of aurochs cows can be ruled out since the European aurochs lineage is not found among the haplotypes of modern cattle (Bollongino 2006).

Since these data were limited to matrilineages, we performed molecular sexing of the samples and subsequently investigated the paternal lineages via two markers on the Y chromosome. These markers were suggested to discriminate between *B. primigenius* and *B. taurus* by Götherström *et al.* (2005, *Proc. R. Soc. B.* **272**). In contrast to this, we detected no correlation between mitochondrial and Y chromosomal haplotypes and the distinction of aurochs and imported cattle by means of Y-loci can not be supported. Thus, it is not possible to make any inferences on possible male introgression.

Student Presentation 6

Title:	Pre-historic turkey (<i>Meleagris gallopavo</i>) exploitation in the Southwest United States
Author(s):	Camilla Speller & Dongya Yang
Author(s) affiliations:	Department of Archaeology, Simon Fraser University, Burnaby, British Columbia, Canada
Presenting author:	Camilla Speller

As one of the few New World animal domesticates, the turkey (*Meleagris gallopavo*) served as an important resource for the Ancestral Pueblo of the Southwest United States. Despite the rich history of Southwest archaeology, several questions concerning the domestication and use of turkey remain unanswered, including the geographic origin of turkey domestication, the subspecies exploited, and the process by which turkey stocks were bred and traded within the region. This study applied ancient DNA analysis to over 60 archaeological turkey remains to investigate the distribution of turkey subspecies and domestic stocks within the Southwest. Although several different turkey mitochondrial haplotypes were identified, the bulk of the sample was represented by a single turkey haplotype, most closely related to a wild subspecies currently found outside the Southwest US. The results of this study indicate that several subspecies of turkey were exploited by the Ancestral Pueblo, including both local and non-local subspecies.

Student Presentation 7

Title:	Molecular factors in the evolution of cattle
Author(s):	Simone Jung & Daniel G. Bradley
Author(s) affiliations:	Molecular Population Genetics, Trinity College, Dublin, Ireland
Presenting author:	Simone Jung

Rinderpest, also known as cattle plague, is a disease of large ruminants, with descriptions of its effects dating back to Roman times. It is caused by a *morbillivirus* closely related to human measles virus. This Rinderpest virus (RPV) enters the cells via a special kind of cellular receptor named *signalling lymphocyte activation molecule* (SLAM, also known as CDw150).

SLAM is a member of the immunoglobulin superfamily and mediates different functions, depending on the availability of downstream molecules, within the signal transduction pathways. The receptor is expressed on immature thymocytes, memory T cells, a fraction of B cells, activated lymphocytes, macrophages and mature dendritic cells (DC) and regulates, amongst others, the production of interleukin 4 (IL-4), as well as the production of IL-12 and the tumour necrosis factor α (TNF- α).

The bovine SLAM gene, located on chromosome 3, consists of seven exons and has a total length of 39,047 bp. The aim of this investigation is to figure out if there is any genetic difference or, more precisely, any evidence for positive or negative selection, between the coding sequences of SLAM in different cattle breeds. Additionally, it might be interesting whether these differences could give their carrier a benefit, for instance, concerning the susceptibility to the cattle plague virus. For that reason, the seven exons of the SLAM gene of diverse European, African and Indian cattle breeds were amplified, aligned and will be investigated with standardized population-genetic methods.

Student Presentation 8

Title:	Review: Multiple origins of cultivated grapevine (<i>Vitis vinifera</i> L. ssp. <i>sativa</i>) based on chloroplast DNA polymorphisms
Paper details:	Arroyo-Garcia <i>et al.</i> (2006) <i>Molecular Ecology</i> 15 , 3707–3714
Presenting author:	Beatrice Demarchi

The domestication of the Eurasian grape (*Vitis vinifera* ssp. *sativa*) from its wild ancestor (*Vitis vinifera* ssp. *sylvestris*) has long been claimed to have occurred in Transcaucasia where its greatest genetic diversity is found and where very early archaeological evidence, including grape pips and artefacts of a 'wine culture', have been excavated. Whether from Transcaucasia or the nearby Taurus or Zagros Mountains, it is hypothesized that this wine culture spread southwards and eventually westwards around the Mediterranean basin, together with the transplantation of cultivated grape cuttings. However, the existence of morphological differentiation between cultivars from eastern and western ends of the modern distribution of the Eurasian grape suggests the existence of different genetic contribution from local *sylvestris* populations or multilocal selection and domestication of *sylvestris* genotypes. To tackle this issue, we analysed chlorotype variation and distribution in 1201 samples of *sylvestris* and *sativa* genotypes from the whole area of the species' distribution and studied their genetic relationships. The results suggest the existence of at least two important origins for the cultivated germplasm, one in the Near East and another in the western Mediterranean region, the latter of which gave rise to many of the current Western European cultivars. Indeed, over 70% of the Iberian Peninsula cultivars display chlorotypes that are only compatible with their having derived from western *sylvestris* populations.

Student Presentation 9

Title:	Review: Genetic evidence for a second domestication of barley (<i>Hordeum vulgare</i>) east of the Fertile Crescent
Paper details:	Morrell & Clegg (2007) <i>PNAS</i> 104 , 3289–3294
Presenting author:	Paula Campos

Cereal agriculture originated with the domestication of barley and early forms of wheat in the Fertile Crescent. There has long been speculation that barley was domesticated more than once. We use differences in haplotype frequency among geographic regions at multiple loci to infer at least two domestications of barley; one within the Fertile Crescent and a second 1,500–3,000 km farther east. The Fertile Crescent domestication contributed the majority of diversity in European and American cultivars, whereas the second domestication contributed most of the diversity in barley from Central Asia to the Far East.

Student Presentation 10

Title:	Adopting agriculture in Iberia and North Africa: an archaeogenetic study of tetraploid wheats
Author(s):	Hugo Oliveira & Diane Lister
Author(s) affiliations:	Department of Archaeology & McDonald Institute for Archaeological Research, University of Cambridge, U.K.
Presenting author:	Hugo Oliveira

The routes of agricultural spread can be studied by looking at phylogeographical patterns of certain genetic markers. Wheat cultivation was introduced in the Iberian Peninsula (5th millennium BC) and North Africa (approximately 4th millennium BC) during the Neolithic. Possible contacts between both shores of the Mediterranean have been poorly studied and archaeological evidence is scarce, consisting of little more than cardial pottery sherds.

Tetraploid wheats were among the first domesticated plants to be introduced into both regions. A study of the genetic relationships between tetraploid wheat landraces (traditional cultivars that have not been improved by modern genetic techniques) from Portugal, Spain, Morocco and Algeria could provide information about past movements of crops, during the Neolithic or later periods.

To assess the potential for the genetic analysis of wheat landraces to be informative about past events, we studied genetic markers in 5 historical accessions present in the Percival Collection of Wheats (dating from the 1920's) with their corresponding modern landrace accessions from germplasm banks. Five chloroplast DNA microsatellites were genotyped and sequenced to search for variation in the number of repetitions of a nucleotide motif.

Preliminary results indicate that DNA can be successfully amplified from historical seeds from these regions. The use of mononucleotide repetition microsatellites, however, poses experimental pitfalls. A reasonable degree of genetic stability appears to exist from the early 20th century to the present. Modern wheat landraces can provide us with information about past contacts between both shores of the Mediterranean.

Student Presentation 11

Title:	Review: Animal DNA in PCR reagents plagues ancient DNA research
Paper details:	Leonard <i>et al.</i> (2007) <i>Journal of Archaeological Science</i> 34 1361–1366
Presenting author:	Bodo Schulenburg

Molecular archaeology brings the tools of molecular biology to bear on fundamental questions in archaeology, anthropology, evolution, and ecology. Ancient DNA research is becoming widespread as evolutionary biologists and archaeologists discover the power of the polymerase chain reaction (PCR) to amplify DNA from ancient plant and animal remains. However, the extraordinary susceptibility of PCR to contamination by extraneous DNA is not widely appreciated. We report the independent observation of DNA from domestic animals in PCR reagents and ancient samples in four separate laboratories. Since PCR conditions used in ancient DNA analyses are extremely sensitive, very low concentrations of contaminating DNA can cause false positives. Previously unidentified animal DNA in reagents can confound ancient DNA research on certain domestic animals, especially cows, pigs, and chickens.