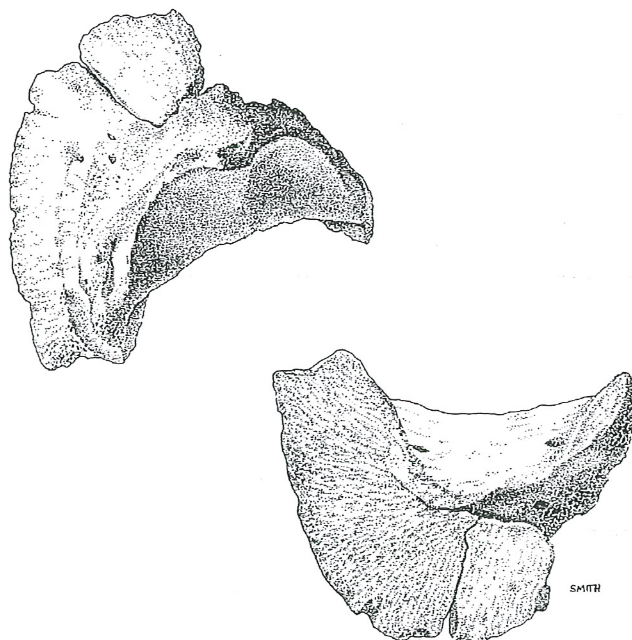
ARCHAEOZOOLOGY OF THE NEAR EAST III

Proceedings of the third international symposium on the archaeozoology of southwestern Asia and adjacent areas

edited by

H. Buitenhuis, L. Bartosiewicz and A.M. Choyke



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Cover illustration: Dorsal and palmar aspects of a
Bronze Age horse phalanx from Arslantepe, Turkey,
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Preface

This publication is the result of the third international symposium on archaeozoology of southwestern Asia and adjacent areas, held in Budapest, Hungary from 2 - 5 September 1996. The editors would like to thank all colleagues of the Working Group who helped with the translation of abstracts. Financial support for the publication was given by the Acker Stratingh Stichting, Groningen, The Netherlands.

Participants of the 3rd ASWA Conference, Budapest 1996
(Photo: Péter Komjáthy, Aquincum Museum)

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DNA ANALYSIS AND THE ORIGINS AND HISTORY OF DOMESTICATED CATTLE

David E. MacHugh[1], Ronan T. Loftus, Christopher S. Troy and Daniel G. Bradley

Resumé

Les analyses moléculaires de la variation de l' A.D.N. de trois systèmes génétiques différents ont établi que le zébu, boeuf à bosse, (*Bos indicus*) et le boeuf domestique (*Bos taurus*) ont été domestiqués indépendamment l'un de l'autre. Ces recherches ont aussi montré que les migrations historiques des populations de zébus en Afrique étaient en premier lieu conduites par des mâles et que les populations actuelles africaines présentent une mosaïque génétique de caractères taurins et de zébus. Les études qui commencent à apparaître sur l'archéologie biomoléculaire sont aussi discutées at le potentiel archéologique pour des analyses génétiques des bovinés est examiné.

Introduction

During the last forty years, the life sciences have been revolutionised by the elucidation of the structure of deoxyribonucleic acid (DNA) and the subsequent development of molecular genetics. Almost every branch of biology has been profoundly influenced by the insights and novel perspectives that molecular approaches have provided. As the 20th century draws to a close, it is increasingly apparent that a new discipline of molecular archaeology is emerging from studies of preserved DNA in ancient biological material. The purpose of this review is twofold. First, to illustrate how studies of extant molecular genetic diversity have provided new insights into the evolutionary origins and recent biological history of domesticated cattle and second, to review progress and prospects for studies of DNA molecules in cattle remains from the archaeological record.

Cattle have had an intimate and formative association with human civilisation. In historic and contemporary societies they have fulfilled key agricultural, economic, cultural, and even religious roles. The biological systematics and evolutionary history of cattle have always been highly contentious. In particular, the relationship between the two subspecies of cattle, humped zebu (*Bos indicus*) and humpless taurine (*Bos taurus*), has been an active area of research.

Various hypotheses have been proposed to account for the morphological and genetic differences observed between the two types of cattle. One school of thought asserts that domesticated cattle arose from a single wild ancestor, the aurochs (*Bos primigenius primigenius*) during the early Neolithic phase of agricultural societies which emerged in the Near East (circa 8,000 - 9,000 BP). Humped zebu cattle populations are then thought to have been produced through adaptation to arid conditions and selective breeding from taurine progenitors (Epstein, 1971; Epstein and Mason, 1984; Payne, 1991). An alternative viewpoint contends that domesticated zebu populations were derived independently by a separate group of early pastoralists from a different wild ancestor. The southern Asian subspecies of aurochs (*Bos primigenius namadicus*) is considered the most likely progenitor of these early zebu cattle and the Neolithic cultures of Baluchistan in present-day Pakistan may have been responsible for the domestication process (Jarrige and Meadow, 1980; Meadow, 1993). Analysis of DNA from a range of extant cattle populations supports the dual-domestication hypothesis and is discussed in more detail in a later section.

Taurine and zebu cattle are completely interfertile and hybridise readily, particularly in Africa, where the history and biogeography of cattle populations represent a complex interaction of ecological, genetic and anthropological factors. The original cattle of Africa are universally considered to

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have been exclusively taurine. These domestic stocks are thought to have emerged from early migrations of pastoralists from the Near East (Payne, 1970; Epstein, 1971). Recent archaeological evidence has, however, questioned this viewpoint, suggesting that the African aurochs (*Bos primigenius opisthonomus*) may have given rise to early domesticated cattle in Northern Africa (Wendorf and Schild, 1994). Zebu cattle are thought to have been first introduced into Africa about 3,500 years ago. However, they were only introduced in significant numbers starting about 700 AD with the Arabic migrations into North and East Africa. At present, Africa represents a mosaic of cattle morphologies with zebu cattle and intermediate forms, often referred to as "sanga," predominating over most of the continent. Molecular genetic analysis has contributed greatly to our understanding of the dynamics of zebu-taurine hybridisation in Africa and has revealed distinctive patterns of sex-mediated genetic introgression which may apply in other areas where taurine and zebu populations interact.

Molecular genetic variation in extant cattle populations

Cattle, like humans, possess approximately three billion units (bases) of DNA information arranged in linear chromosomal packages. This vast repository of genetic data is transmitted faithfully through the generations, unaltered except by rare mutations and the mixing of parental contributions through sexual reproduction. Genetic variation, which at the simplest level is differences in DNA sequence between individuals, provides the raw material for evolutionary change. Although only a small proportion of the total DNA of an organism (the genome) actually codes for functional products (proteins), the fingerprints of evolutionary change and diversity can be discerned across the whole genome. This variation can be exploited by geneticists to assess the genetic relationships between individuals, populations and whole species.

The resolution achievable through the study of molecular genetic variation has increased steadily as new technological innovations have emerged in the field of molecular biology. Perhaps the most important of these developments has been the invention of the Polymerase Chain Reaction (PCR);(Saiki *et al.*, 1988; Mullis, 1990). PCR is a laboratory technique which enables geneticists to "amplify" particular DNA segments with remarkable accuracy and fidelity. These amplified DNA segments can be assayed and compared for genetic variation among individuals within or between species or populations, either directly by determining the sequence of bases on the DNA strand, or indirectly by exploiting variation in mobility between different genetic variants when separated by electrophoretic methods. Kary Mullis, the inventor of PCR, has written an insightful and readable account of the process including the historical background (Mullis, 1990).

PCR can also be used to investigate genetic variation in different components of the genome which may have separate modes of inheritance (such as the maternally inherited mitochondrial chromosome). Three DNA-based systems have been employed to-date to assess genetic differences among domesticated cattle populations. These are microsatellite genetic markers, mitochondrial DNA (mtDNA) and Y-chromosome polymorphisms.

Microsatellite variation in global cattle populations

Microsatellites are small segments of the genome where a single DNA motif of 2-5 units is repeated about 10 to 30 times in a linear array. These microsatellite arrays vary in length among individuals in a population and this length variation can be used to assess genetic differences between individuals and populations (for review see Bruford and Wayne, 1993).

Microsatellite DNA variation has been assessed using 20 different microsatellite systems which were screened in a wide range of extant populations from Africa, Asia and Europe (MacHugh *et al.*, 1994; 1997). Microsatellite length variants or alleles are received from both parents by offspring and analysis of patterns of variation at these DNA segments can reveal a great deal about evolutionary relationships among the major groups of domesticated cattle. Figure 1 shows a principal components

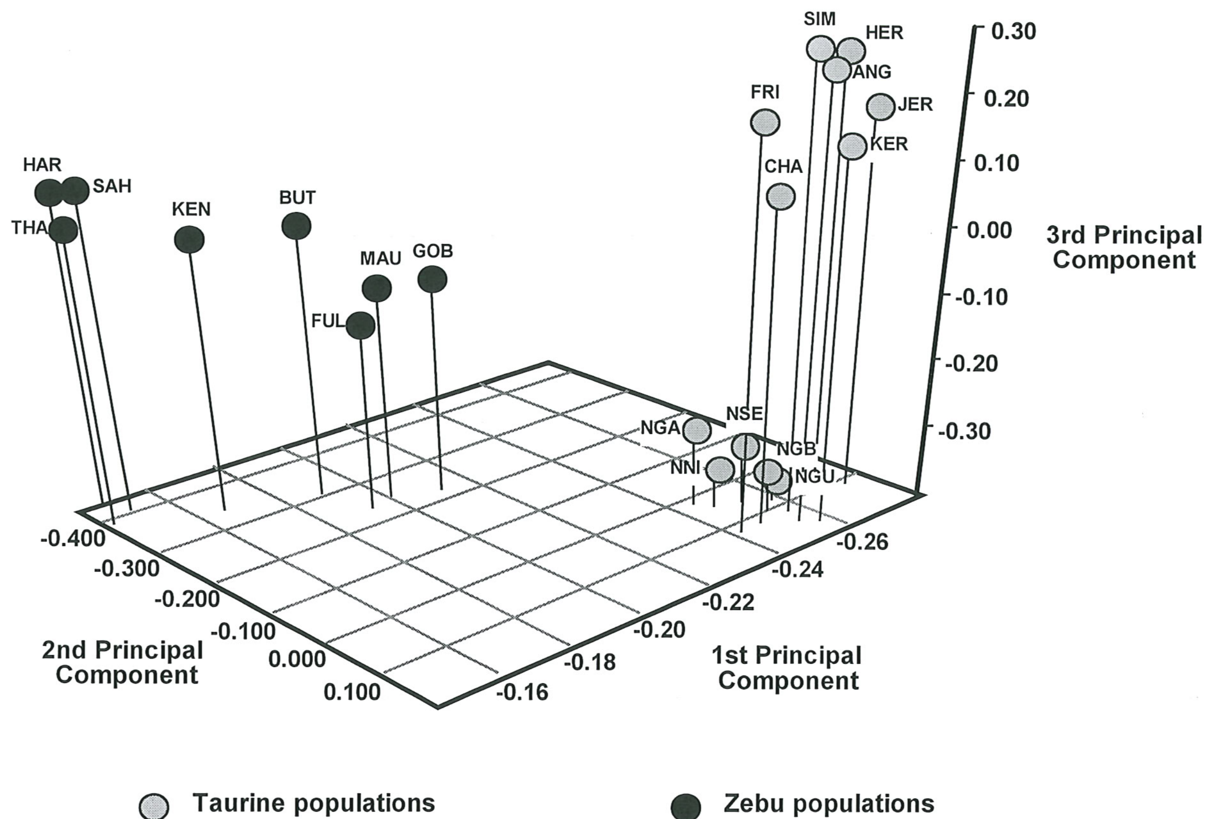

Figure 1: Principal Components Analysis of 168 microsatellite allele frequencies in 20 cattle populations from Africa, Asia and Europe.

KEY: ANG - Aberdeen Angus (Scotland); HER - Hereford (England); JER - Jersey (Channel Islands); KER - Kerry (Ireland); CHA - Charolais (France); FRI - Friesian (Holland); SIM - Simmental (Switzerland); NGA - N'Dama (Gambia); NGU - N'Dama (Guinea); NGB - N'Dama (Guinea Bissau); NNI - N'Dama (Nigeria); NSE - N'Dama (Senegal); BUT - Butana (Sudan); KEN - Kenana (Sudan); GOB - Gobra (Senegal); MAU - Maure (Mauritania); FUL - White Fulani (Nigeria); HAR - Hariana (India); SAH - Sahiwal (Pakistan); THA - Tharparker (India).

analysis (PCA) of microsatellite variation in 20 cattle populations from Africa, Asia and Europe. A clear dichotomy between zebu and taurine populations is evident in the first and second PCs on the diagram which together account for almost 60% of the total variation.

In the three-dimensional space encompassed by the first three PCs, the African zebu populations form an almost linear array stretching towards the Asian zebu cluster at one corner and to the five African taurine N'Dama populations at the other corner. On closer inspection, the position of the various African populations corresponds to the relative proportions of zebu and taurine alleles in their gene pools (see MacHugh *et al.*, 1997). When this microsatellite data is used to produce phylogenetic dendrograms or trees, the most salient feature is the large divergence between the *Bos taurus* and *Bos indicus* clades. Microsatellite data from bison can be used in conjunction with the palaeolontological literature to derive a rough estimate of the time of divergence between the ancestors of taurine and zebu cattle. These analyses suggest that the taurine and zebu clades diverged at least 600,000 years ago (MacHugh *et al.*, 1997), effectively ruling out the possibility that zebu cattle were derived from taurine populations during the Holocene.

The distribution of microsatellite variants in cattle populations from Africa has shown a cline of zebu influence running across Africa, with the highest concentration of zebu alleles being found clos-

est to the original points of entry for zebu cattle into Africa from Asia. The distribution of the total zebu genome across Africa is complicated however, by sex-related differences in the dispersal of other genetic elements which are detailed below.

Mitochondrial DNA variation in cattle

Mitochondrial DNA (mtDNA) is present in mammalian cells in the form of a closed circular chromosomal element of approximately 16,000 units (bases) of DNA. Each cell may possess thousands of copies of the mtDNA chromosome. MtDNA is maternally inherited (males do not pass on their mtDNA), and is subject to a high rate of sequence change (mutation). Variation between individuals can be readily assayed using PCR and direct sequence determination. Normally, a particularly hypervariable region of mtDNA termed the displacement-loop (D-loop) is studied by comparing sequences from a range of individuals from different populations. This approach has contributed a great deal to our understanding of human evolution, migration and genetic anthropology (for review see von Haeseler *et al.,* 1995).

Mitochondrial genetic diversity has been assayed using over 100 DNA sequences originating from the three Old World continents (Loftus *et al.*, 1994a, 1994b; Bradley *et al.*, 1996). Figure 2 shows a schematic tree of relationships among the major cattle groups. As observed with microsatellite data, the main feature is the deep split between Indian *Bos indicus* and *Bos taurus*. However, an additional somewhat puzzling aspect of this phylogenetic tree is the clustering of African zebu mtDNA with African taurine samples. As discussed above, analysis of nuclear DNA polymorphisms (microsatellites) indicates that African zebu breeds are actually hybrid combinations of Asian zebu and African taurine populations. It may therefore be expected, that some Asian zebu mtDNA should be present in African cattle populations. This paradoxical situation can be understood in the light of genetic data from analysis of DNA variation on the bovine Y-chromosome.

Y-chromosomal variation and the sex-mediated dispersal of the zebu genome in Africa

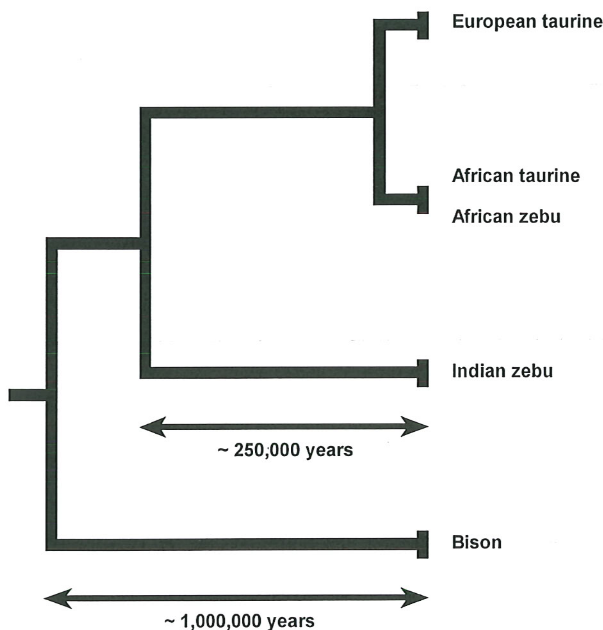

Figure 2. Simplified mtDNA phylogeny for the major cattle groups with Bison (*Bison* sp.) as an outgroup.

The sex-determining or Y-chromosome is exclusively transmitted through the paternal lineage and for the purposes of evolutionary comparisons among populations, it can be considered analogous to the mtDNA in female lineages. Both molecular and cytogenetic techniques can readily distinguish between the Y-chromosome types of *Bos taurus* and *Bos indicus.* Bradley *et al.* (1994) have carried out a molecular survey of cattle Y-chromosomes in European and African cattle and have found that Y-chromosomal distributions in Africa are a symmetrical mirror-image of the mtDNA distribution.

As outlined above, the zebu mtDNA seems to be completely absent from African cattle populations. However, when the same populations were assayed for Y-chromosome type, the zebu Y-chromosome was present at high frequency across the whole of Africa and even present in populations which are morphologically taurine. These asymmetrical distributions

of the uniparental inherited genetic elements, highlight the male-mediated nature of zebu gene flow on the African continent. The paternally inherited zebu Y-chromosome has spread like wild fire throughout African cattle populations while the maternally inherited zebu mtDNA has been unable to perturb the genetic inertia of the taurine mtDNA gene pool. The biparentally inherited microsatellite markers, on the other hand, present an intermediate picture between these two extremes and reflect the overall penetrance of the zebu genome more accurately. Taken together, these molecular surveys strongly suggest that zebu bulls played an extremely important role in the genetic introgression of zebu genes into African cattle populations (for a more detailed discussion see MacHugh *et al.,* 1997).

Future prospects for the study of cattle molecular biogeography

The broad evolutionary history of cattle has been fairly well established using molecular genetic techniques. The status of the zebu as an independent domesticate has been clarified and the sex-mediated dispersal of zebu populations in Africa has been demonstrated. However, there is still one important piece of the jigsaw missing - the status of cattle populations originating from the rest of Asia. Perhaps the most pressing issue is the evolutionary history of populations which trace their origins to Southwest Asia, particularly those breeds located in the Fertile Crescent region where it is likely that taurine cattle were first domesticated.

Our laboratory in Trinity College, Dublin is currently extending the survey of genetic variation in cattle into the Middle East and other regions of Asia. A large number of DNA samples have been collected from cattle breeds in Turkey, Syria, Iran and Iraq. This material is currently being assayed for microsatellite and mtDNA variation. When these data have been analysed it should become apparent whether these populations share a common origin with European cattle as would be expected from the known history of cattle husbandry. It has also been suggested, with some tentative molecular support, that the indigenous African taurine cattle may have been domesticated independently from a local race of aurochs which was present in North Africa during the early Holocene (Bradley *et al.,* 1996; MacHugh *et al.,* 1997). A thorough survey of molecular variation in Middle Eastern cattle populations would provide a robust comparative dataset to assess the validity of this hypothesis.

THE EMERGING SCIENCE OF MOLECULAR ZOOARCHAEOLOGY

Ancient DNA: a dream come true?

In 1985, a remarkable report appeared in the scientific journal Nature. Svante Pääbo, a Swedish molecular biologist had successfully cloned DNA from a 2,400 year-old Egyptian mummy (Pääbo, 1985). This discovery was greeted with amazement in the scientific community and created a major stir in the world's media. During the previous year, Russell Higuchi and his colleagues had identified DNA from a 140-year old quagga skin (an extinct relative of the zebra); (Higuchi *et al.,* 1984), however scientists had never imagined that DNA could survive for thousands of years as Pääbo's results suggested. These twin landmark papers were responsible for a new branch of science - the study of ancient DNA.

The next major breakthrough in ancient DNA research was the application of PCR to the retrieval of DNA molecules from ancient biological material (Pääbo and Wilson, 1988). PCR was seen as the ideal tool for analysis of minute quantities of DNA preserved in ancient biological material. It is exquisitely sensitive and allows geneticists to characterise DNA sequence information from a tiny amount of starting material. Coupled with the demonstration the following year, that archaeological bone could serve as a suitable source of DNA (Hagelberg *et al.,* 1989), it became apparent that molecular genetic archaeology was fast becoming a reality.

As an increasing number of research groups became involved with ancient DNA research, competition to extract and characterise increasingly older DNA became intense and attention switched to

truly ancient "fossil" DNA - preserved for millions of years. During the period 1990 to 1994, a number of research groups published reports detailing the characterisation of DNA from a diverse range of fossil material (see Table 1 for a full chronology of both ancient and fossil DNA research since the 1980's). These reports included analyses of fossil leaves entombed in clay sediments, insects trapped in amber and putative dinosaur bones (Golenberg *et al.,* 1990; DeSalle *et al.*, 1992; Cano *et al.*, 1993; Woodward *et al.*, 1994). Unfortunately, the dream of accessing genetic information from organisms which existed millions of years ago has been thwarted. Many scientists always doubted the detection of truly fossil DNA and considered it either wishful thinking or due to contamination from extraneous sources. In light of these objections, rigorous reanalysis of many specimens and a better understanding of the processes of DNA diagenesis has led to the consensus that DNA sequences are unlikely to be retrievable from material older than about 100,000 years (Sidow *et al.*, 1991; Poinar *et al.*, 1996; Austin *et al.*, 1997).

Not surprisingly, the first applications of DNA technology to archaeological remains have been in the areas of anthropology and human history. Analysis of mtDNA from ancient Native American remains has contributed substantially to the debate concerning the origins of the aboriginal peoples of North and South America (Stone and Stoneking, 1993; Merriwether *et al.*, 1994; Lalueza *et al.,* 1997; Monsalve *et al.,* 1996). Perhaps the most striking demonstration of the potential that ancient DNA studies have for anthropological studies is the work carried out by Erika Hagelberg and her colleagues on early human remains from the central Pacific islands of Polynesia (Hagelberg and Clegg, 1993; Hagelberg *et al.,* 1994). Using a diagnostic mtDNA polymorphism, they were able to demonstrate that the earliest inhabitants of Polynesia came from Melanesia and not Southeast Asia as previously thought.

Methodological challenges

The work that has been carried out using human material has helped to clarify the particular difficulties faced by scientists working with ancient DNA. The little DNA that is present in ancient material is heavily degraded and chemically modified. This situation, coupled with the sensitivity of the PCR means that contamination from modern sources is the perennial bugbear of ancient DNA studies. Rigorous precautions must be taken to ensure that modern DNA does not produce a false signal in experiments designed to detect the presence of genuine preserved DNA molecules. This is particularly pertinent for studies of ancient human DNA, but in principle the same procedures must be adopted for studies of other species. All reagents and laboratory consumables must be devoid of contaminating DNA and a dedicated laboratory must be set up with a strict physical separation of pre- and post-PCR work. In addition, the only sure way to monitor for the presence of contamination is through the use of a range of control extractions and amplifications. Another major problem is the presence of soil-derived molecules such as fulvic and humic acids which inhibit PCR chemistry and must be removed before DNA can be amplified. These extra purification steps add to the cost of each extraction and also increase the likelihood of contamination. Once DNA has been extracted, amplified and analysed from an archaeological specimen it is absolutely vital that the result is verified by performing at least one independent extraction and analysis. Given the uncertainties of working with ancient DNA, this is undoubtedly the best way to allay concerns about contamination.

The vast majority of ancient DNA work to-date has concentrated on sequences amplified from the mtDNA molecules present in archaeological samples. Unfortunately the single-copy nature of many of the most informative regions of the nuclear genome (such as microsatellites), has with a few exceptions, precluded their use as tools in ancient DNA research. Perhaps in the future, as our knowledge of PCR technology and DNA degradation improves, it may be possible to routinely assay many different types of DNA markers in archaeological samples. However for the time being, most ancient DNA research will continue to be pursued using variation in the mtDNA genome.

1984	Characterisation of DNA from extinct quagga (*Equus quagga*)	(Higuchi *et al.*, 1984)
1985	DNA cloned from an ancient Egyptian human mummy	(Pääbo, 1985)
1988	First use of PCR to amplify DNA from ancient biological material	(Pääbo and Wilson, 1988)
1989	Demonstration that amplifiable DNA could be extracted from archaeological bone	(Hagelberg *et al.*, 1989)
1989	The taxonomic status of the extinct marsupial wolf (*Thylacinus cynocephalus*) is resolved using ancient DNA	(Thomas *et al.*, 1989)
1990	Chloroplast DNA sequences obtained from 17-20 Myr old Miocene *Magnolia* species (since discredited)	(Golenberg *et al.*, 1990)
1992	DNA characterised from a fossil termite preserved in 25-30 Myr old Oligo-Miocene amber (lack of reproducibility has led many scientists to doubt these findings)	(DeSalle *et al.*, 1992)
1993	Amplification and sequencing of DNA from a 120-135 Myr old weevil found in Lebanese amber from the Early Cretaceous (since discredited)	(Cano *et al.*, 1993)
1994	Isolation and characterisation of DNA from an 80 Myr old dinosaur bone is claimed (since discredited)	(Woodward *et al.*, 1994)
1996	Amino acid racemization is found to be an accurate indicator of DNA survival and many fossil DNA claims are discredited	(Poinar *et al.*, 1996)
1997	Extensive re-evaluation of previous work on amber-preserved DNA indicates that all results to-date are spurious	(Austin *et al.*, 1997)
1997	First demonstration that DNA analysis can reliably distinguish sheep and goat bones from the archaeological record	(Loreille *et al.*, 1997)

Table 1. Milestones in the study of ancient DNA molecules.

Analysis of DNA from cattle archaeological remains

In 1996 our laboratory was awarded funding from the Wellcome Trust for a project entitled "Biomolecular Archaeology and the Domestic Origins of Cattle." This project was envisaged as a complement to our existing work on the genetic origins and molecular diversity of extant cattle populations (Bradley *et al.*, 1994, 1996; Loftus *et al.*, 1994a, 1994b; MacHugh *et al.*, 1994, 1997).

The origins and history of domesticated cattle represents in many respects, an ideal subject for the application of ancient DNA techniques. In evolutionary terms, artiodactyls are sufficiently distant from *Homo sapiens* that contamination from human sources should not be an issue. In addition, cattle are large-bodied animals and have correspondingly large bones which may aid in the preservation of DNA embedded in the central osteological matrix. The time period of interest (10,000 BP to the present) is comfortably within the accepted limits for the survival of DNA in ancient biological material (Poinar *et al.*, 1996; Austin *et al.*, 1997). Another favourable aspect is that the evolutionary relationships of the major groups of extant domesticated cattle have been determined and a large body of molecular data currently exists which can be used as a framework to compare information from an-

cient cattle sequences. With this in mind, some of the archaeological issues which we wish to address include the following:

- Are the cattle remains found at Norse settlements in Dublin of Scandinavian or Irish origin?
- What is the genetic status of cattle remains found at Çatal Hüyük in Anatolia, a Neolithic site with the earliest known evidence for cattle domestication?
- Were the early domesticated cattle of Baluchistan in Pakistan zebu or taurine?
- Can genetic analysis of early bovid remains in North Africa determine whether cattle were domesticated independently in Africa?
- Will molecular gender identification prove a more reliable method than traditional morphological approaches?

The initial focus of the work has been on the analysis of a relatively large panel of bones from the Norse Wood Quay site in central Dublin dating to about 1000 AD. MtDNA was extracted and analysed from 11 individual bones from a total of 20. Independent extractions were carried out on all the bones and appropriate controls were used throughout. Five different independently authenticated mtDNA variants were detected in this group of 11 ancient cattle and these will be compared to mtDNA sequences from three extant Norwegian breeds and the one remaining indigenous Irish breed, the Kerry cattle from south-west Ireland.

Preliminary analysis of Çatal Hüyük material donated by Louise Martin of University College London, has shown some promise. It has proved possible to amplify small mtDNA fragments from some of the bones and we will be able to compare these to the large database of mtDNA sequence data from Middle Eastern cattle populations currently being assembled in our laboratory. This should provide some insights into the origins of these early domesticated cattle and their relationship to present-day populations.

A gender-identification system for cattle archaeological remains using a Y-chromosome-specific DNA segment has also been developed. This method is extremely reliable in differentiating modern cattle material. However, it still needs to be fine-tuned to make it suitable for analysis of ancient DNA. A reliable method for sexing cattle bones as an independent verification system for morphometric studies, would be a very useful addition to the methods currently available to archaeologists.

The zooarchaeological community and ancient DNA

Inevitably a review and outline of research into the origins of domesticated cattle written for archaeologists is going to end with an appeal for samples! With this in mind, included below are guidelines for the collection and storage of zooarchaeological specimens intended for DNA analysis. These guidelines are modified from those discussed in Brown and Brown (1992). Our research group would be very interested to explore collaborations with zooarchaeologists working on Neolithic sites in Southwest Asia or North Africa. Cattle osteological material collected on previous field trips would also be very welcome. Further details can be obtained via electronic mail using the following address: *dmachugh@mail.tcd.ie*.

Guidelines for collection and storage of archaeological samples intended for DNA analysis

1. Wear clean gloves when excavating and handling material for DNA analysis (this is not so crucial if the material is of non-human origin).
2. Remove excess soil and other deposits with a brush or tweezers. Do not wash the sample.
3. Keep the material dry at all costs. If the sample is suspected to be damp, it should be placed on a clean surface and left to dry thoroughly.
4. Store the dried samples in a clean, dry, airtight container. If possible, avoid plastic bags as they may encourage moisture and the growth of microbes.
5. Store the samples in a cool dark place. Keep out of direct sunlight.
6. If possible obtain more than one bone from an animal for DNA analysis

Remember, because of the high failure rate, you can never have too many samples for DNA analysis!

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