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THE ROYAL SOCIETY

Early medieval cattle remains from a Scandinavian settlement in Dublin: genetic analysis and comparison with extant breeds

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A panel of cattle bones excavated from the 1000-year-old Viking Fishamble Street site in Dublin was assessed for the presence of surviving mitochondrial DNA (mtDNA). Eleven of these bones gave amplifiable mtDNA and a portion of the hypervariable control region was determined for each specimen. A comparative analysis was performed with control region sequences from five extant Nordic and Irish cattle breeds. The medieval population displayed similar levels of mtDNA diversity to modern European breeds. However, a number of novel mtDNA haplotypes were also detected in these bone samples. In addition, the presence of a putative ancestral sequence at high frequency in the medieval population supports an early post-domestication expansion of cattle in Europe.

Keywords: ancient DNA; cattle; medieval Dublin; biomolecular archaeology; mitochondrial DNA; polymerase chain reaction

1. INTRODUCTION

(a) *Domesticated cattle and biomolecular archaeology*

The origins and history of domesticated cattle have always been very active areas of biological systematics. Genetics has been at the forefront of these studies and the availability of a large repository of protein and blood group polymorphisms from many breeds through collection of data for parentage verification has greatly enhanced our knowledge of breed relationships (for review, see Baker & Manwell 1991). Recent molecular studies of DNA variation in cattle have increased this knowledge even further. Variation in maternally inherited mitochondrial DNA (mtDNA) has been investigated using various methods and has provided persuasive evidence that humpless taurine cattle (*Bos taurus*) and humped zebu cattle (*Bos indicus*) were domesticated independently by two separate Neolithic cultures (Loftus *et al*. 1994*a*,*b*; Bradley *et al*. 1996). Analyses of autosomal microsatellite DNA markers and Y chromosome-specific polymorphisms have complemented the mtDNA work and have shed light on additional aspects of the genetic history of cattle including breed structure, phylogeography and sex-mediated gene flow between zebu and taurine breeds in Africa (Bradley *et al*. 1994, 1998; MacHugh *et al*. 1994, 1997, 1998).

The analysis of mtDNA variation in cattle archaeological remains through use of the polymerase chain reaction (PCR) looks set to provide another, possibly chronological, layer of information concerning the biological history of domesticated cattle. A recent study by J. F. Bailey and her colleagues demonstrated the feasibility of obtaining mtDNA sequence information from cattle specimens ranging in age from 12 000 to 500 BP (Bailey *et al*. 1996). Useful archaeological information was apparent in this data, including the observation that the wild European aurochs (*Bos primigenius primigenius*) may have had divergent mtDNA haplotypes compared with modern European populations.

In many respects, cattle archaeological remains provide ideal subject material for studies of biomolecular archaeology. Cattle are relatively big animals with correspondingly large bones that may enhance the survival of DNA. An extensive database of cattle mtDNA control region sequences (the most informative segment of the mtDNA molecule) has become available and this provides an invaluable framework within which genetic information from ancient cattle can be assessed. Artiodactyls are sufficiently divergent from primates such that with judicious choice of PCR primers, it should be possible to avoid the pitfall of contamination from extraneous human DNA—a major problem for researchers working with human remains (Stoneking 1995; Lindahl 1997). Finally, 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*a*).

(b) *The Wood Quay site in Dublin—an early Scandinavian town in Ireland*

Excavations have been conducted by the National Museum of Ireland in the old city of Dublin since 1962. The most famous of these excavations are those that took place in the 1970s and early 1980s at the Wood Quay site on the banks of the river Liffey in central Dublin (Wallace 1985). This 1.8 ha site was initially developed by the Dublin Corporation for a civic office complex. When it was realized that the site contained archaeological remains from a medieval Viking settlement, the building work was halted while a thorough archaeological survey was carried out. The excavations from this survey revealed thousands of artefacts and detritus from the early settlement including domesticated cattle bones. The site was completely waterlogged due to the nearby river and much of the material was preserved under anoxic conditions.

It is believed that Dublin was founded twice by Scandinavian settlers, initially from Norway and then by a mixed group of Norse and Danes. The settlement was established first in 841 AD as a trading-cum-piratical base or *longphort*. These Vikings were defeated and exiled in 902 AD by a successful coalition of Irish kings. They returned circa 917 AD and established a defended town or *dún* downstream from the *longphort*. This resettled site soon became an enclosed town with an extensive network of streets, buildings and plots (Wallace 1992).

The exact nature of the economic relationships between the Viking town and the hinterland is currently unclear. Agricultural produce may have been obtained from indigenous Irish farmers, or alternatively, Scandinavian settlers may have farmed the land themselves. The status of the domesticated cattle present in and around early medieval Dublin is also not known. They may include imported cattle from Scandinavia or England, or alternatively, they may simply have been indigenous cattle herded by Irish farmers. Early accounts have suggested that hornless or polled cattle were introduced to Ireland and Britain from Scandinavia by Viking settlers (Wilson 1909). However, this theory is contradicted by the presence of polled cattle in the Irish archaeological record prior to the appearance of the Vikings (McCormick 1987).

We report here biomolecular archaeological work on a series of cattle bones which have been recovered from Fishamble Street, an excavation that revealed deposits containing material from early Scandinavian dwellings and buildings. Examination of mtDNA variation from the Fishamble Street cattle may help to provide information concerning the origins of the domesticated animals used by the Dublin Vikings. Therefore, in order to provide comparative DNA information, a range of relevant extant populations were also examined. These modern breeds included Kerry cattle, one of the few representative breeds of indigenous Irish cattle still in existence, three native Norwegian cattle breeds and one Icelandic cattle breed.

The medieval Dublin cattle population may also provide a useful genetic yardstick to assess the effects of selective breeding and herdbooks on the genetic composition of modern cattle breeds within the last 200 years. Examination of the pattern and extent of mtDNA sequence variation in the Viking cattle compared with extant European breeds may indicate whether there has been any loss of genetic diversity due to these processes.

2. METHODS

(a) *Collection of material from the Fishamble Street excavations*

Cattle skeletal material in the form of calcaneus (ankle) bones were obtained from the archaeological collections taken from the Fishamble Street excavations which took place in central Dublin during the period 1975–80 (Wallace 1985). These bones were taken from a vertical series of houses on the site and are presumed to represent food refuse discarded by the inhabitants of these dwellings. The material from this series of levels has been concisely dated to between 920 AD and 1020 AD through examination of a large number of coins found in the deposits, the earliest of which dates from 925 AD. These dates are also supported by tree-ring analysis of timbers used in the construction of the dwellings (Wallace 1992). The Fishamble Street excavations revealed a waterlogged and sealed site with little or no disturbance. Consequently, most of the organic material, including the timber used in dwelling construction, has been well preserved due to the anoxic conditions of the deposits. According to the gross morphological criteria outlined by Hagelberg *et al.* (1991), the bovine calcaneus bones obtained from the Fishamble Street could be considered well preserved; the bone material was dense and the external surfaces were undamaged.

(b) *Archaeological bone preparation, DNA extraction and PCR amplification*

A dedicated biomolecular archaeology laboratory located in a separate building from the main laboratory was available for the first stages of ancient DNA work—those involving sample extraction and preparation of PCR reactions. This laboratory has never been used for any previous DNA-based procedures. All the DNA extractions and PCR set-ups were carried out in laminar airflow hoods which were exposed to shortwave UV (254 nm) between use.

In total, 17 bones from the Fishamble Street excavation were subjected to DNA extraction and analysis (see table 1). The surface layer was removed from each bone using an industrial micro shot-blaster in an enclosed hood (Guyson Corp.). The abraded bones were then washed in a 2% sodium hypochlorite solution and irradiated with shortwave UV in a DNA cross-linker (UVP) for 10 min. A flame-sterilized hacksaw blade was used to bisect each bone and bone powder was collected using an electric hand-drill and a clean flame-sterilized dental drill bit. Approximately 2 g of powder was collected from each bone sample. Between 100 and 200 mg of bone powder was used for each DNA extraction with the following protocol, a modified version of the procedure detailed by Richards *et al.* (1995) and Bailey *et al.* (1996). Each sample of bone powder was incubated at 37 °C for 48 h with 1 ml of 0.5 M EDTA pH 8.0, proteinase K (100 μg ml^{-1}), 0.5% Sarkosyl detergent (BDH) and phage λ carrier DNA at 5 μg ml^{-1}. The DNA was then extracted with equal volumes of phenol, phenol–chloroform–isoamyl alcohol 25:24:1 and chloroform–isoamyl alcohol 24:1. Centrifugal dialysis with a Centricon-30 microconcentrator (Amicon) was used to dilute the EDTA 100-fold and to concentrate each DNA sample. Guanidine thiocyanate–glass fibre High-Pure PCR product purification kits (Boehringer

Table 1. *Cattle calcaneus bones analysed from Fishamble Street excavations with laboratory codes, PCR amplification results and mitochondrial control region sequence lengths*

			PCR amplification		
sample code	lab. code	no. of extractions	218 bp product	375 bp product	length (bp)
II:1:132 (a)	WQ01a	2	no	no	—
II:1:132 (b)	WQ01b	3	yes	no	206
II:1:248	WQ02	2	no	no	—
II:1:251	WQ03	3	yes	no	157
II:1:255 (a)	WQ04a	3	no	no	—
II:1:255 (b)	WQ04b	3	yes	no	213
II:1:256	WQ05	3	no	no	—
II:1:269	WQ06	3	no	no	—
II:1:325 (a)	WQ07a	3	yes	no	213
II:1:325 (b)	WQ07b	3	yes	no	213
II:1:330	WQ08	3	yes	no	138
II:1:343	WQ09	3	yes	no	170
II:1:359	WQ10	3	yes	no	134
II:1:400	WQ11	3	yes	no	134
II:1:467	WQ12	3	no	no	—
II:1:226	WQ13	2	yes	no	172
II:1:260	WQ14	2	yes	no	150

Mannheim) were used to remove inhibiting substances, such as humic and/or fulvic acids. The kits were used according to manufacturer's instructions except that isopropanol was used instead of ethanol for the wash buffer (Hanni *et al*. 1995). The number of DNA extractions performed for each bone sample are indicated in table 1.

Possible extraneous and cross-contamination was monitored at the DNA extraction stage using two different methods. A modern Nile crocodile bone (*Crocodylus niloticus*; obtained from the Zoology Department, Trinity College, Dublin) was powdered and extracted in parallel with every batch of extractions from ancient bones. In addition, an empty microcentrifuge tube was left open in the laminar airflow cabinet during bone powder collection for each set of samples. This tube was then used for a blank extraction containing no bone powder.

The most variable segment of the bovine mtDNA control region was targeted for PCR amplification. This segment has been previously identified and characterized through surveys of mtDNA sequence variation in a range of cattle populations (Loftus *et al*. 1994*a*; Bradley *et al*. 1996). Primers were designed using the reference GenBank bovine mitochondrial genome sequence (BOVMT, Anderson *et al*. 1982). The primers were BOV-AN1 (ACG CGG CAT GGT AAT TAA GC sites 16158–16177 reverse), BOV-AN3 (CGA GAT GTC TTA TTT AAG AGG sites 16314–16334 reverse), BOV-AN4 (GGT AAT GTA CAT AAC ATT AAT G sites 15960–15981 forward). These primers specifically target fragments of size 218 bp (BOV-AN1:BOV-AN4) and 375 bp (BOV-AN3:BOV-AN4). Extracts were amplified in 40 µl reactions comprising: 10 mM Tris-HCl pH 8.8, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, deoxyribonucleotides at 200 µM each, 0.5 µM each primer, 1.0 unit of PrimeZyme DNA polymerase (Biometra). Amplifications were performed without an oil overlay in an M-J Research PTC-200 DNA Engine using a heated lid. A 3 min denaturation step at 94 °C was followed by 50 cycles of 40 s denaturation at 93 °C, 40 s annealing at 55 °C, 40 s extension at 72 °C and then a 4 min final extension step at 72 °C. The two extraction controls for each set of samples, two negative water controls and a positive modern control were also amplified. The PCR amplifications were visualized on an agarose gel and positive PCR products were prepared for sequencing using High-Pure PCR product purification kits (Boehringer Mannheim) following the manufacturer's instructions. No modern contamination was evident for either the DNA extraction controls or the PCR amplification controls. A further indication of the authenticity of the results was the lack of amplification of the larger 375 bp fragment in any of the Fishamble Street bones (table 1).

The 218 bp PCR products were direct-sequenced using a modified Sequenase Version 2.0 (Amersham) protocol developed in our laboratory by D. Bancroft. For each strand, 20 pmol of the appropriate PCR primer was mixed with 100–400 ng of purified template, 2 µl of Sequenase buffer and 1 µl 50% (v/v) DMSO and adjusted to a final volume of 11 µl. This mixture was denatured at 95 °C for 3 min and then rapidly snap-cooled in liquid nitrogen to anneal the primer to the denatured template. A standard Sequenase version 2.0 protocol was then followed with a concentration of 5% DMSO maintained throughout each step. Sequencing reactions were fractionated and visualized using standard polyacrylamide gel electrophoresis and autoradiography. Each medieval bone sample was extracted a number of times (see table 1) and verified DNA sequence was determined at least twice from two or more separate extractions.

(c) *PCR amplification and DNA sequencing of modern cattle samples*

Control region hypervariable sequences were determined for 50 cattle samples representing five distinct indigenous breeds from Iceland, Norway and Ireland. Table 2 summarizes the origins and breed status for these samples. Whole genomic DNA was isolated from blood and semen straws using previously described methods (MacHugh *et al*. 1997). The 375 bp BOV-AN3:BOV-AN4 control region segment was amplified using identical PCR conditions to those detailed above. To facilitate direct-PCR sequencing, the BOV-AN4 primer was biotinylated

Table 2. *Contemporary Icelandic, Scandinavian and Irish cattle breeds analysed for this study*

breed	no. of samples	geographical origin	breed status	other information
Icelandic	11	Iceland	authentic breed	
Kerry	10	south-west Ireland	authentic breed	endangered[a]
Norwegian Red	12	Norway	synthetic derived breed	
Telemark	5	Telemark, Norway	authentic breed	endangered[a]
Westland Fjord	12	south-west Norway	authentic breed	endangered[a]

[a]Scherf (1995).

at the 5′ end. This enabled the product to be purified into single-stranded template using biomagnetic separation technology. Streptavidin-coated M-280 Dynabeads (Dynal) were used for this purification step according to the manufacturer's instructions. The single-stranded DNA bound to the magnetic beads was sequenced using the non-biotinylated BOV-AN3 primer and the Sequenase Version 2.0 kit (Amersham). A modified Sequenase protocol was used such that a 5% (v/v) concentration of DMSO was maintained throughout the procedure. Sequencing reactions were fractionated and visualized as described above.

(d) *Statistical and phylogenetic methods*

The mitochondrial control region segments for all the Fishamble Street cattle bones were aligned with the modern cattle control region sequences from Scandinavia, Iceland and Ireland using the CLUSTALW computer program (Thompson *et al*. 1994). A number of additional European cattle sequences were also included in the analysis. These supplementary samples encompass seven control region segments from each of the following breeds: Aberdeen Angus, Charolais, Friesian, Hereford, Jersey and Simmental. These sequences have been previously described and analysed by Bradley *et al*. (1996).

The ARLEQUIN computer program (version 1.1, Schneider *et al*. 1997) was used to estimate a range of genetic parameters and distributions. Mean nucleotide diversity values for each population were calculated from the maximum length of shared sequence according to Tajima (1983). Mean pairwise sequence differences and mismatch distributions were produced for each population using the methods detailed by Rogers & Harpending (1992) and Rogers (1995). Estimates of genetic diversity among populations in terms of pairwise F_{ST} genetic distances were also calculated. The pairwise F_{ST} genetic distance is considered the most appropriate metric for surveys of genetic variation among populations where genetic drift has been the primary determinant of differentiation (Reynolds *et al*. 1983; Slatkin 1995). An unrooted neighbor-joining dendrogram (Saitou & Nei 1987) was constructed from a matrix of pairwise F_{ST} genetic distances using the NEIGHBOR module incorporated in the PHYLIP version 3.572 software package (Felsenstein 1993).

An alternative method of phylogenetic analysis was performed by constructing a reduced median network of the control region haplotypes present in the Nordic and Fishamble Street populations according to the algorithm described by Bandelt *et al*. (1995). Reduced median networks display the principal character relationships among the haplotypes present in the data. Networks of sequence relationships are considered to be particularly appropriate for the shallow genetic differentiation observed in intraspecific studies of molecular variation (Bailey *et al*. 1996; Richards *et al*. 1996).

3. RESULTS

(a) *Mitochondrial control region amplification from medieval cattle bones*

As detailed in table 1, it was possible to obtain positive amplification products using the 218 bp BOV-AN1:BOV-AN4 amplicon in 11 out of 17 of the Fishamble Street cattle bones. This success rate of approximately 65% is in broad agreement with Richards *et al*. (1995), who suggest that more than 50% of skeletal remains from the past 2000 years are likely to contain amplifiable endogenous DNA. The veracity of these results is also supported by the complete lack of amplification of the larger 375 bp BOV-AN3:BOV-AN4 control region product. This observation would indicate that there is an inverse relationship between amplification efficiency and amplicon length for these samples; an important characteristic of authentic ancient DNA (Austin *et al*. 1997*b*). In addition, multiple extractions were performed on all of the bones and verified sequences for each sample were obtained from at least two independent DNA extractions. As outlined in § 2, sample, extraction and PCR controls were used for all DNA isolation, purification and amplification steps.

Varying lengths of control region sequence were obtained by direct-sequencing from the 11 positive medieval cattle bones. Verified sequence length varied from 134 bp in samples WQ10 and WQ11 to 213 bp in WQ4a, WQ7a and WQ7b. Seven of the 11 sequences were identical to the corresponding segment of the European cattle consensus control region sequence (Bradley *et al*. 1996). In total, six nucleotide transitions were observed within the Fishamble Street sequences and four haplotypes distinct from the European consensus were evident (WQ3 and WQ7a, one transition; WQ8 and WQ14, two transitions). The length of shared sequence among all of the medieval samples was 132 bp (16041–16172) and this segment was used for all subsequent analyses involving these ancient sequences.

(b) *Genetic diversity within and among medieval and extant cattle populations*

Alignment of 42 cattle sequences previously described (Bradley *et al*. 1996) with the 61 samples, both contemporary and medieval, from the present study, revealed 32 distinct control region haplotypes (figure 1). Fourteen of these haplotypes are new and were not evident in the previous survey. The modern Icelandic, Scandinavian and Irish breeds accounted for 11 of these new sequence types while the medieval population included three novel haplotypes. As indicated above, a fourth novel haplotype was also observed in the medieval population (WQ7a)

	042	047	049	050	051	053	056	057	058	062	068	069	074	076	084	085	108	110	113	119	122	127	130	131	133	135	137	138	139	143	164	185	195	229	231	247	250	255	260
AN1	T	G	C	C	T	T	A	G	C	A	T	A	T	A	C	T	T	C	T	T	T	C	T	T	T	T	T	T	C	A	T	G	A	A	C	C	A	T	C
AN2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AN5	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HE1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HE7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-
HE8	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	C	-	-
JE1	C	A	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T
JE2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-
JE3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JE4	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	G	-	-	-	-	-	-
JE5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-
JE6	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FR3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-
FR6	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-
SI4	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SI5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SI6	-	-	-	-	C	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-
SI7	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IC1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-
IC8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	T	-	-	-
KR22	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WE2	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WE4	-	-	-	-	-	-	G	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-
WE9	C	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NO1	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-
NO2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NO7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NO8	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-
NO12	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-
WQ1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	*	*	*	*	*	*	*
WQ3	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	*	*	*	*	*	*	*
WQ8	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	*	*	*	*	*	*	*	*
WQ14	-	-	-	-	-	-	-	-	T	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	*	*	*	*	*	*	*

Figure 1. Mitochondrial control region sequence variations observed for 42 European cattle previously analysed by Bradley *et al.* (1996), 50 Irish, Scandinavian and Icelandic cattle, and 11 medieval samples from Fishamble Street at the Wood Quay site. Sequence population codes and numbers are given in the first column. Breed abbreviations are as follows: AN, Aberdeen Angus; FR, Friesian; HE, Hereford; JE, Jersey; SI, Simmental; IC, Iceland; KR, Kerry; WE, Westland Fjord; NO, Norwegian Red; WQ, Fishamble Street. Only variable sites are shown. The sequence positions from the BOVMT GenBank sequence are given above each column (minus 16000). Identity with the first sequence (AN1, the most common European type) is denoted by a dash (–). The missing bases from the medieval sequences are indicated with asterisks (*).

when the total sequence is taken into consideration. However, this haplotype was due to a single substitution at 16022, a site which was not sequenced in the modern breeds. It was therefore not included in figure 1. In terms of haplotypic representation, the Fishamble Street population is relatively diverse. Although 7 out of the 11 sequences match the European consensus, the other four are novel and have not been observed in any previously analysed European cattle populations.

Table 3 shows mean nucleotide diversities and pairwise differences estimated for both the medieval cattle population and the modern Scandinavian, Icelandic and Kerry breeds. Also shown are the corresponding values calculated for the six European breeds analysed in the previous study (Bradley *et al.* 1996). In terms of these diversity metrics, the most diverse populations are the Jersey and Simmental, while the Telemark, Charolais, Friesian and Kerry breeds display the least variation. This may be a consequence of the relatively small sample sizes. However, the Telemark and Kerry populations are considered to be endangered and each breed currently only numbers in the hundreds (Scherf 1995). Therefore, the lack of mitochondrial diversity noted for these populations may reflect low effective population sizes and in the case of the Telemark, an overrepresentation of a single mitochondrial haplotype. There were only two control region haplotypes observed in the sample of ten Kerry cattle and these two variants differed by only a single substitution. The medieval Fishamble Street population also displays a relatively low nucleotide diversity value (6.61×10^{-3}). The mean pairwise sequence difference value for this population is not directly comparable to the other breeds because a smaller region of sequence was analysed.

Figure 2 summarizes the data in table 3 graphically and shows a comparison of the control region nucleotide diversities observed in the present study with a boxplot distribution of nucleotide diversities from a much larger sample of contemporary European cattle breeds (Bradley *et al.* 1996; Troy 1998). It is clear from figure 2 that the medieval Dublin cattle population represented by the Fishamble Street bones is just within the normal range of European mitochondrial diversity. Conversely, the endangered Kerry and Telemark breeds fall completely outside the distribution of extant European diversity. The Westland Fjord breed, which is also considered to be under threat, displays a moderate level of control region diversity and this may reflect a larger effective population of breeding females.

Rogers and co-workers have demonstrated that the demographic history of populations is reflected in the analysis of frequency distributions of pairwise sequence

Table 3. *Mean nucleotide diversities and pairwise sequence differences for the medieval cattle population and 11 contemporary breeds*

cattle population	no. of samples	sequence analysed (bp)	mean nucleotide diversity[a]	mean number of pairwise differences[b]
Fishamble Street	11	132	6.61	0.87
Icelandic	11	240	6.77	1.86
Kerry	10	240	3.54	0.47
Norwegian Red	12	240	14.80	2.12
Telemark	5	240	0.00	0.00
Westland Fjord	12	240	8.15	1.24
Aberdeen Angus	7	240	17.32	2.29
Charolais	7	240	0.00	0.29
Friesian	7	240	2.17	0.86
Hereford	7	240	4.33	1.33
Jersey	7	240	20.92	3.62
Simmental	7	240	18.76	2.76

[a]Nucleotide diversities were calculated for each modern breed using only the 132 bp segment shared with the medieval Fishamble Street population.
[b]Mean number of pairwise differences were calculated using the total available sequence for each breed.

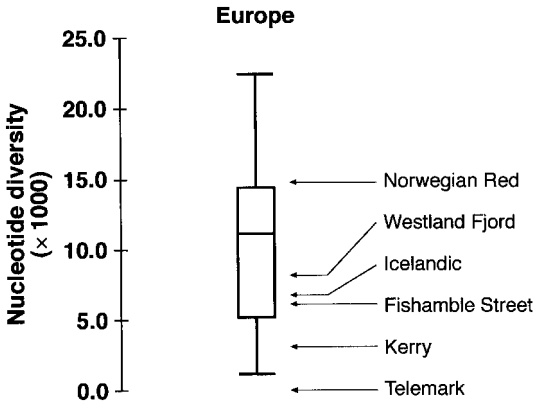

Figure 2. A boxplot of the distribution of control region nucleotide diversities in 13 extant European cattle populations (Bradley *et al*. 1996; Troy 1998). Also shown are the corresponding nucleotide diversities for the medieval Fishamble Street cattle bones and the extant Nordic and Irish cattle breeds analysed in the present study. The interquartile range of the European nucleotide diversity is represented by the length of the box and the median is shown as a horizontal line within the box. The vertical lines outside the box extend to the smallest and largest observations.

mismatches for control region sequences (Rogers & Harpending 1992; Rogers 1995). Figure 3 shows histograms of control region mismatch frequencies for the medieval cattle and the five extant breeds surveyed. The qualitative shape of the distributions may underline aspects of the demographic history and status of the various populations. The mismatch curves for the Icelandic and Norwegian Red breeds are again indicative of stable populations with moderate levels of mitochondrial diversity. On the other hand, the distributions for the Telemark and Kerry cattle breeds reflect historical population bottlenecks. The mismatch curve for the Westland Fjord breed may also represent attenuated mitochondrial diversity due to a restricted effective population size. The shape of the curve, with a peak at 1–2 mismatches and a tailed distribution of higher-order mismatches would suggest that the breed may be in the process of losing mitochondrial diversity. It is more difficult to interpret the distribution for the medieval Dublin population because it is based on a smaller segment of control region sequence. However, the signature of a population restriction is not immediately apparent for this sample.

(c) *Phylogenetic analysis of control region sequences*

The relationships among the 61 mtDNA haplotypes sequenced in this study are illustrated in the reduced median network shown in figure 4. The central node, containing the largest number of observed haplotypes (25 modern and eight medieval), corresponds to the putative ancestral European haplotype AN1 identified by Bradley and colleagues (Bradley *et al*. 1996). This control region haplotype also matches the GenBank BOVMT reference sequence. The relatively high frequency of the AN1 haplotype in the Fishamble Street cattle sample and the modern cattle from Iceland, Scandinavia and Ireland would suggest that these populations form part of the normal European radiation. As shown in figure 4, most of the non-AN1 sequences present in these populations differ by only a single substitution from the core sequence and none differs by more than three transitions from this consensus. It is therefore unlikely that indigenous European female aurochs (*Bos primigenius*) from Scandinavia or elsewhere contributed to the genetic history of these populations.

The range of novel haplotypes observed in the 11 medieval Dublin cattle is noteworthy because it implies that mitochondrial diversity may have declined in European populations since the tenth century. The most obvious process that could account for such an attenuation of diversity is genetic manipulation due to selective breeding, breed formation and, more recently, the introduction of artificial insemination (AI).

An unrooted neighbor-joining phylogenetic tree was constructed from pairwise F_{ST} genetic distances among all European populations and the Fishamble Street cattle (figure 5). There is very little biogeographical clustering evident in figure 5, a consequence of the low levels of

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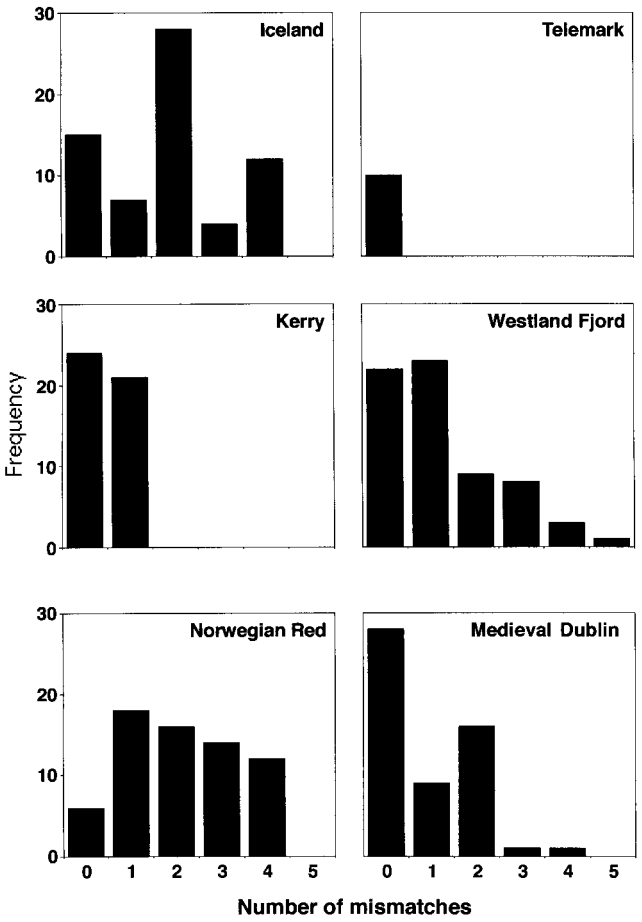

Figure 3. Frequency distributions of the number of sequence mismatches observed in pairwise comparisons within populations. The histograms correspond to control region sequence comparisons and the number of pairwise differences are given on the horizontal axis with the absolute number of each category represented on the vertical scale.

breed partitioning for mtDNA noted in previous surveys of intracontinental variation (Loftus *et al.* 1994*a*,*b*; Bradley *et al.* 1996). Those populations with sequences closest to the AN1 core haplotype emerge close to the centre of the tree. The populations with long branches at the extremities of the tree (Aberdeen Angus, Kerry, Westland Fjord) may be distinct because of a low effective mtDNA population size. This is because the F_{ST} genetic distance between two populations is known to be inversely proportional to population size for short divergence times (Reynolds *et al.* 1983).

4. DISCUSSION

(a) *The genetic status of the medieval Dublin cattle population*

The results of this survey of mtDNA control region sequence variation suggest that the cattle used by the Vikings of early medieval Dublin were similar to modern cattle populations found in northern Europe. Measures of intrapopulation variation, such as mean nucleotide diversity and pairwise differences (table 3), show that the Fishamble Street population had levels of mtDNA diversity comparable to modern breeds. Unfortunately, even though appropriate reference breeds were analysed, the shallow levels of mtDNA diversity present in European taurine cattle populations would preclude the identification of these 1000-year-old animals as being either Scandinavian in origin or native Irish cattle. However, the presence of three novel control region variants in the Fishamble Street cattle is noteworthy. These medieval sequences have yet to be observed in extant European cattle, including the 50 Nordic and Irish cattle analysed here. These sequences may therefore represent mtDNA haplotypes which have been lost from extant European cattle populations during the intervening period. Mitochondrial DNA, with an effective population size fourfold smaller than autosomal genetic loci is very sensitive to demographic processes such as periodic population constrictions (Avise 1994).

A note of caution is required when interpreting haplotypic diversity in these medieval samples however. The exact age of each bone is, of course, unknown. It is only clear that this material came from deposits that range in age between approximately 920 and 1020 AD. This time-span covers approximately 20 cattle generations and the composition of the local cattle herds may have varied appreciably during this time. Cattle may have been imported from other European countries, including Scandinavia, and periodic epizootics could have altered the genetic demography very rapidly. The sample of medieval cattle mtDNA presented here can not strictly speaking be considered as a single panmictic population. It is actually a broad slice of cattle mtDNA diversity at one human settlement over a 100-year period. However, the presence of the putative ancestral AN1 sequence at a high frequency (8 out of 11) in these samples does provide some support for the contention that domesticated European cattle experienced an ancient population expansion—probably during the Neolithic period (Bailey *et al.* 1996; Bradley *et al.* 1996). In other words, the presence of the AN1 and closely related haplotypes in the medieval samples implies that the expansion took place before the medieval period and not as a result of recent increases in cattle numbers.

(b) *Mitochondrial diversity within the extant Nordic and Irish breeds*

As with the Fishamble Street medieval cattle, the Scandinavian, Icelandic and Irish breeds displayed patterns of mtDNA variation that place them within the normal European cattle radiation. The sensitivity of mtDNA to demographic changes in population size was evident in that two of the breeds known to be endangered (Kerry and Telemark) displayed markedly less mtDNA diversity than the other populations. This has implications for genetic conservation and highlights the potential for mtDNA to serve as an 'early warning' when the number of breeding females is perilously low in endangered populations. The lack of diversity in Kerry cattle was also reflected, although not as dramatically, in a survey of microsatellite variation in this breed. The Kerry breed had the lowest mean allelic diversity at 20 microsatellite markers in a survey of 20 breeds from Europe, Africa and Asia (MacHugh *et al.* 1997).

(c) *Future biomolecular work on cattle archaeological remains*

The results presented here and previous work on ancient DNA in cattle and aurochs archaeological

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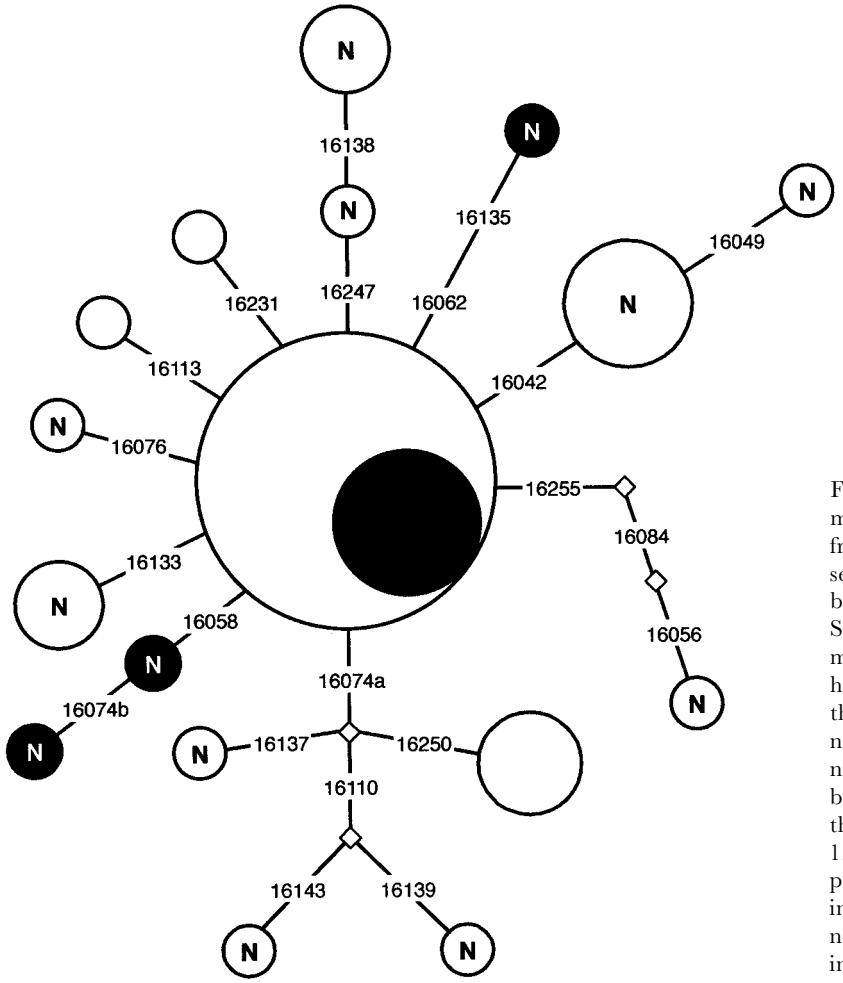

Figure 4. A reduced median network of cattle mtDNA control region sequences constructed from 11 medieval Dublin samples and 50 sequences from five extant Nordic and Irish breeds. Filled circles represent the Fishamble Street samples and unfilled circles represent the modern sequences. The smallest circles are haplotypes which were observed only once and the areas of the circles are proportional to the number of haplotypes present at that particular node of the network. The numbers on the branches denote mutations and correspond to the BOVMT base positions indicated in figure 1. Novel haplotypes which have not been previously observed in European cattle are indicated with a capital 'N'. Empty or virtual nodes where haplotypes were not observed are indicated with small unfilled diamonds.

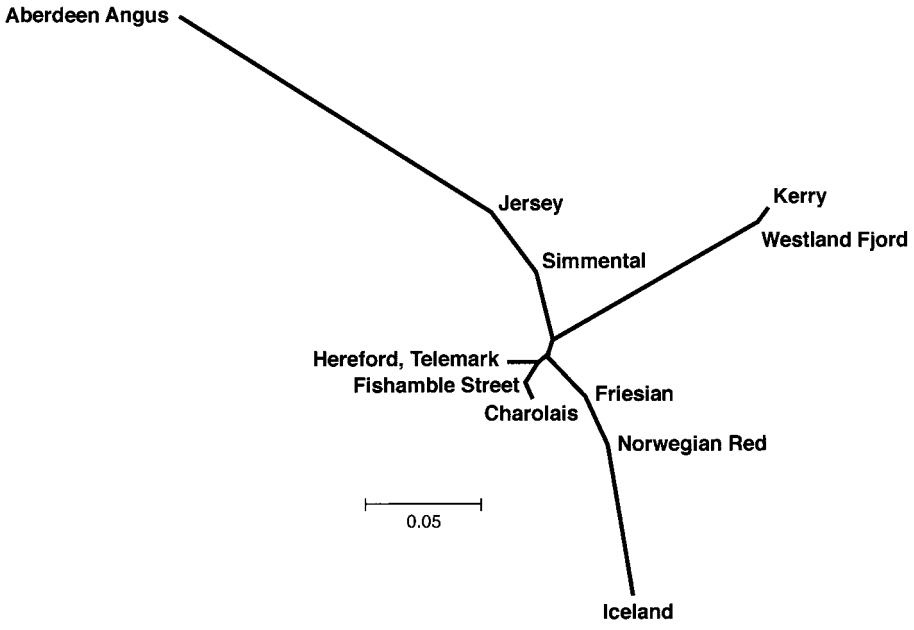

Figure 5. An unrooted neighbor-joining tree summarizing pairwise F_{ST} genetic distances among 11 extant European breeds and the medieval Dublin population. The scale represents arbitrary units of genetic distance and is derived from the original comparison matrix.

material (Bailey *et al.* 1996) suggest that archaeological genetic studies of cattle may be very informative. If DNA from enough specimens is forthcoming from a particular site, mtDNA analysis can provide information of demographic relevance. This may be particularly useful for older sites dating back to the early Neolithic, which may just be at the cusp of the domestication process. One reliable biomolecular signal of domestication in this regard may be a significant reduction in mtDNA diversity when post-domestic deposits are analysed. This attenuation in diversity could mark the beginning of a fully herding lifestyle and the use of a small domesticated population with an essentially closed mtDNA pool. Mitochondrial DNA analysis would also be extremely useful in determining

the Neolithic geography of domestication. For example, are the putative early zebu domesticated cattle from Baluchistan in Pakistan true *Bos indicus* (Meadow 1993)? Are the early Neolithic cattle from Çatal Hüyük in Anatolia more similar to European cattle than Middle Eastern cattle? Does material from the African archaeological record suggest that African taurine cattle were domesticated independently (Wendorf & Schild 1994)? Hopefully, these and other questions concerning the origins of domesticated cattle may be addressed using ancient DNA.

On a different note, the presence of amplifiable DNA in biological material from the 1000-year-old Wood Quay site has implications for Irish archaeology. It suggests that bones from other species, including humans, found at Wood Quay and comparable sites should be amenable to DNA analysis.

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Discussion

A. Millard (*University of Durham, UK*). You showed genetic diversity in a variety of cattle populations and noted that British cattle were more diverse than many others. Is this a sample size effect—more samples given more diversity? (Supplementary question.) If Europe has lost diversity through recent selective breeding, is the occurrence of primitive breeds in the British Isles biasing the diversity patterns?

D. MacHugh. The higher diversity noted for British cattle is not a sample size effect. These figures are actually average nucleotide diversities for each breed with essentially the same sample sizes for each breed. However, the higher diversity in the British Isles was only apparent in mitochondrial DNA data. Generally, for microsatellite data, British breeds display relatively low variation in terms of allelic diversity. The increased mitochondrial diversity is possibly an ancient feature due to introgression of native female aurochs necessitated by the difficulty in replenishing an island population depleted by disease, predation or human exploitation. In addition, 'primitive' breeds such as Kerry, White Park or Highland cattle do not necessarily have divergent mitochondrial haplotypes.

D. R. Harris (*University College London, UK*). Have you and your colleagues studied any of the wild and/or domestic bovids of South and East Asia other than the zebu and the banteng? It would be particularly interesting to know how, if at all, such animals as the gaur, mithan, kouprey and Bali cattle relate to the zebu group, as this has implications for our understanding of the question of which of these 'minor' Asian bovids may have been independently domesticated.

D. MacHugh. It has been previously suggested to us that the divergent 'zebu' mitochondrial haplotype we observed may actually be the result of secondary introgression of an exotic bovid mtDNA. In other words, the archaeologically significant distinction between *Bos taurus* and *Bos indicus* is a biological illusion due to genetic hybridization. This speculation can be effectively countered through the analysis of other genetic systems such as autosomal markers (microsatellites) and Y-chromosome polymorphisms. We have performed extensive surveys of variation using these systems and the pattern observed with these additional genetic elements has always been consistent with a deep split between taurine and zebu cattle. In relation to the other wild and domestic bovids in South and East Asia. Professor Claude Gaillard's laboratory at the University of Berne has assembled a large collection of samples from most of the bovid types in Asia and has included them in a comprehensive survey of microsatellite variation. This data will be published soon.

The mithan and the gaur are actually the same species, the mithan or 'mithun' being a domesticated gaur. Similarly, Bali cattle are actually domesticated banteng. Kikkawa and colleagues have determined a mitochondrial cytochrome *b* phylogeny for taurine, zebu and Bali cattle (Kikkawa *et al.* 1995). The results are in agreement with our data and suggest that all three species last shared a common ancestor approximately 3 million years ago and that the wild ancestors of taurine and zebu cattle diverged about 1 million years ago. Most of the cattle-like species of South and East Asia hybridize with each other to various degrees and natural and human-mediated cross-breeding may have blurred the distinction between many populations. It is quite conceivable that three-way, or even four-way crosses exist among, for example, taurine cattle, zebu cattle, gaur and yak. However, the presence of divergent mtDNA lineages and distinct microsatellite distributions in pure-bred populations from domesticated and wild populations of these species would indicate that they were all domesticated from separate ancestral species.

M. Jones (*University of Cambridge, UK*). You highlighted the potential mismatch between your South African network diagram and the supposed Bantu spread. How far are you from having enough data to effectively challenge the notion of that spread and replace it with a more complex model?

D. MacHugh. The relative complexity of the mtDNA haplotype network in Southern Africa is probably due to a number of factors. I tend to believe that African taurine cattle were domesticated separately from the taurine populations in the Middle East which gave rise to European breeds. If this is the case, the increased diversity in Southern Africa may be due to an influx of taurine cattle with a Middle Eastern mtDNA haplotypic background into Eastern and Southern Africa. These exotic taurine cattle would have found it more difficult to penetrate into the tsetse-infested regions of West Africa because they would not have had the inherited tolerance to tsetse-borne trypanosomiasis which native West African breeds possess. This could explain the disparity between the mtDNA diversity observed in West Africa compared to East and Southern Africa.

An alternative or complementary explanation may be that the reduced diversity observed in West Africa is a consequence of a historical population bottleneck. It is tempting to speculate that this bottleneck could have been associated with selection for resistance to trypanosomiasis. We will have a clearer understanding of these issues and their relationship to human population movements when data from other genetic markers is available from a wider range of African cattle populations.

G. Eglinton (*University of Bristol, UK*). Although you have not yet examined a large suite of ancient samples, are you able to comment on the nature of the damage experienced by the DNA—size of fragments, modification, etc.—in relation to the sample type and burial conditions?

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D. MacHugh. The samples from the Dublin Wood Quay excavations are about 1000 years old and were preserved in water-logged deposits. More than 50% of the samples contained amplifiable endogenous mtDNA. In contrast to this, I have also examined bones from the Çatal Hüyük site in Anatolia. This material is approximately 8000 years old and has been subject to much greater temperature fluctuations. Although these bones were well preserved morphologically, only a small number yielded amplifiable mtDNA and only the smallest PCR products were successfully amplified. Other workers have suggested that bones from colder or temperate areas should retain more preserved DNA than material from arid regions. These observations would support this position.

G. Eglinton (*University of Bristol, UK*). The Dublin group has extensive experience of working with the DNA of living animals. These experimental observations came out of trying to do the same thing with ancient samples. Is the challenge really horrifying? Is there real promise for aDNA [ancient DNA] work on ancient cattle bones?

D. MacHugh. I think the prehistory and archaeology of animal domestication should provide a very informative arena for studies of ancient DNA. In the case of cattle, the relationships among modern populations are well understood and there is a large database of mtDNA sequences for comparison with archaeological material. Also, animal bones do not present the same contamination problems as human material. Unfortunately however, the most interesting sites from the point of view of animal domestication tend to be in regions of the world which may be refractory to the preservation of archaeological DNA. I am thinking in particular of areas such as the Middle East, Anatolia, North Africa and Baluchistan.

C. Renfrew (*University of Cambridge, UK*). Would it be useful to calculate coalescence times for the principal domestic lineages? Some indication of relative time depth could be informative.

D. MacHugh. We have estimated divergence times for European taurine, African taurine and Indian zebu mtDNA. These estimates were calculated in a number of different ways and are detailed in Bradley *et al*. (1996). The estimate for the separation between the taurine and zebu lineage is 117 000–275 000 BP, and that between European and African taurine mtDNA is 22 000–26 000 BP. Estimates based on microsatellite data are substantially higher than these figures (MacHugh *et al*. 1997). We have not calculated coalescence times within each lineage. These values may be informative, but would be strongly influenced by how diverse the respective wild population was, how many different ancestral mtDNA lineages were absorbed into early domesticated populations and also whether subsequent genetic exchange took place with other aurochs populations. However, we have used mismatch analysis of pairwise sequence differences to show that European and African cattle populations may have started expanding about 10 000 and 11 000 BP, respectively (Bradley *et al*. 1996).

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