Ancient DNA suggests a recent expansion of European cattle from a diverse wild progenitor species

JILLIAN F. BAILEY[1], MARTIN B. RICHARDS[1], VINCENT A. MACAULAY[1], ISABELLE B. COLSON[1], I. TIM JAMES[2], DANIEL G. BRADLEY[3], ROBERT E. M. HEDGES[4] AND BRYAN C. SYKES[1]

[1]*University of Oxford, Department of Cellular Science, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU, U. K.*
[2]*Department of Biological Sciences, University of Durham, Durham DH1 3LE, U. K.*
[3]*Department of Genetics, Trinity College, Dublin 2, Ireland*
[4]*University of Oxford, Research Laboratory for Archaeology and the History of Art, 6 Keble Road, Oxford OX1 3QJ, U. K.*

SUMMARY

A total of 11 *Bos primigenius* and *Bos taurus* bones from archaeological sites between 500 and 12 000 years old were examined for the presence of DNA. It was possible to amplify and sequence mitochondrial control region DNA extracted from seven of the 11 samples, including two Pleistocene *B. primigenius* samples. We compared the results with published data by constructing phylogenetic networks.

The two *B. primigenius* samples clustered with the extant *B. taurus* samples in the networks. The similarity between *B. primigenius* and modern taurine cattle confirms that these should be considered members of a single species.

The sequences obtained from the *B. taurus* specimens were either identical to the reference sequence for modern European cattle or closely related to it. They included two sequences not previously documented.

The network analysis of the ancient data highlights the intermediary nature of the *B. primigenius* sequences between modern European and African *B. taurus* and the proximity of the ancient DNA *B. taurus* sequences to modern European *B. taurus*. Further analysis of the extant data in the light of the ancient DNA results suggests that a degree of Pleistocene diversity survives in the extant European *Bos* population that is mainly derived from a more recent population expansion.

1. INTRODUCTION

It is thought that modern cattle were domesticated from a single wide-ranging Pleistocene species, the wild aurochs *B. primigenius*, which was distributed throughout Europe, Asia and North Africa (Clutton-Brock 1987). However the details of domestication have been hard to discern, since it is difficult to distinguish the domestic breeds (*B. taurus* in Africa and Europe and *B. indicus* in Asia) from the wild progenitor species in the archaeological record. For example, it is not known whether *B. taurus* was derived from local *B. primigenius* populations in Northern Europe and North Africa or whether, as was the case with ovicaprids (sheep and goats) and certain cereals, it was introduced from Western Asia.

Unfortunately, the wild aurochs population of Europe has been extinct for nearly 400 years. Nevertheless, considerable progress has been made by means of the analysis of mitochondrial DNA (mtDNA) control region sequences from modern breeds. Phylogenetic analysis has indicated not only a very deep split between an Asian and an African-European cluster, but also a shallow split between African and European cattle, suggesting three separate domestication events (Loftus *et al.* 1994; Bradley *et al.* 1996). In spite of this, it remains unclear whether or not all European cattle are derived from stock domesticated in Western Asia.

We have therefore begun to explore the possibility that ancient DNA may be able to make a contribution. This paper reports the extraction, amplification and sequencing of mitochondrial DNA from two samples of British aurochsen dating to more than 6000 years prior to the onset of the Neolithic in Britain. These are compared with sequences from a Bronze-Age Egyptian sample and four medieval samples from Britain and Ireland, and previous data from extant breeds from Europe and Africa (Loftus *et al.* 1994; Bradley *et al.* 1996). Sequences have been submitted to the GenBank–EMBL DNA sequence data libary (accession nos. U50940–U50946).

2. MATERIALS AND METHODS

(a) *Sample preparation and DNA analysis*

The samples used are shown in table 1. A total of four British Pleistocene *B. primigenius* samples, radiometrically

Table 1. *Samples tested and results*

(Radiocarbon dates are calibrated and the associated standard errors given. The OxA-no. refers to the dating number. Other dates are based on archaeological context (indicated by ~) or archaeological evidence. Histology scores were according to the scale of Hedges *et al.* (1995). Nitrogen content was scored as a percentage; modern bone typically scores roughly 5%. N. A. = not available. – = not tested.)

no.	species	provenance	age	OxA-no.	PCR amp. 156bp	177bp	220bp	388bp	histology (arbitrary units)	nitrogen (percentage)
D740	*B. primigenius*	Bob's Cave U.K.	12,290±90 BP	5861	yes	yes	yes	no	5	3.46
D812	*B. primigenius*	Gough's New Cave U.K.	11,900±140 BP	813	yes	yes	yes	no	N.A.	4.45
D813	*B. primigenius*	Kent's Cavern U.K.	11,880±120 BP	1203	no	–	–	–	N.A.	N.A.
D814	*B. primigenius*	Pin Hole Cavern U.K.	10,970±110 BP	1937	no	–	–	–	N.A.	N.A.
D757	*B. taurus*	Caythorp U.K.	~4000 BP	N.A.	no	no	no	no	1	0.91
D822	*B. taurus*	Kaft Hassan Dawood Egypt	~2000–3000 BP	N.A.	yes	yes	yes	no	3	0.25
D553	*B. taurus*	Italy	~2000 BP	N.A.	no	–	no	–	0	1.50
D815	*B. taurus*	Ireland	~1200 BP	N.A.	yes	–	–	no	4	4.62
D816	*B. taurus*	Ireland	~1200 BP	N.A.	yes	–	–	no	4	4.10
D630	*B. taurus*	Medieval Pit U.K.	~500 BP	N.A.	yes	yes	yes	no	5	4.27
D355	*B. taurus*	Mary Rose U.K.	~1545 AD	N.A.	yes	yes	yes	no	5	4.68
D738	*Equus caballus*	Kitley No Name Cave U.K.	~500 BP	N.A.	no	–	–	–	1	0.95
D555	*Ovis aries*	Italy	~2000 BP	N.A.	no	–	–	–	4	3.25

dated to 11000–12300 years, were extracted, alongside four medieval *B. taurus* samples, one from the Bronze Age, one from the Roman period and one from the British Neolithic dated on the basis of archaeological context. In addition, one *Equus* and one *Ovis* sample 500 and 1900 years old were also extracted as negative controls for the *Bos* specific primer set. Although some museum curation procedures involve soaking specimens in glue, a potential source of contamination, none of the samples had been conserved in this way.

Histology and collagen survival were assessed as per Hedges *et al.* (1995). The samples were then treated as follows. The total surface area was abraded using a shotblaster; drill bits were also cleaned in this way to remove any surface contamination. A new drill bit was used to obtain the powder subsequently used for the DNA extractions. In the case of D812 this treatment had been done by the staff at the Radiocarbon Accelerator Unit in Oxford, 5 years previously.

Strict geographical isolation was maintained between the pre- and post-amplification stages of the experimental procedures. The sample extraction procedure was a phenol-chloroform method scaled down by a factor of ten from the method described previously (Richards *et al.* 1995) with the addition of phage lambda carrier DNA (1.35 μg ml^{-1} of initial extraction volume). EDTA was then removed using Microcon C-30 concentrators (Amicon) and the samples purified to remove any humic–fulvic acids using the silica–guanidine thiocyanate columns (Wizard DNA clean-up system, Promega) and eluted in sterile water.

The extracts were amplified as in Richards *et al.* (1995) at three different concentrations in duplicate with extraction blanks and several polymerase chain reaction (PCR) blanks, none of which indicated any contamination. Primer pairs were designed to cover the most informative region of a 370 bp fragment previously studied (regions 16042–16157 and 16228–16325). The primers were An1 (ACG CGG CAT GGT AAT AAG C sites 16158–16177 reverse), An2 (GCC CCA TGC ATA TAA GCA AAG sites 16022–16041 forward), An3 (GGG GTA ATG TAC ATA ACA TTA sites 15958–15979 forward), An5 (ATG CCG CGT GAA ACC AGC AA sites 16169–16188 forward) and An6 (CTG TAG AGC TAC CTG ATT AC sites 16325–00007 reverse). These specifically target fragments sized 156 bp (An1–An2), 220 bp (An1–An3), 177 bp (An5–An6) and 388 bp (An3–An6) from the bovine mitochondrial control region. All the primer pairs were amplified under the following conditions: denaturation at 94° C for 4 min, followed by 40 cycles of annealing at 55° C for 1 min, extension at 72° C for 1 min and denaturation at 94° C for 1 min, with a final extension of 8 min, except for An5–An6 which had an annealing temperature of 57° C.

The PCR products were visualized on an agarose gel, and a diluted aliquot re-amplified for 20 cycles under the same conditions to obtain a sufficiently concentrated fragment for sequencing. They were sequenced directly using the Dynal system for isolating single-stranded DNA and the Sequenase Version 2.0 DNA sequencing kit (USB). In the case of samples D815 and D740 the PCR products were also sequenced with an ABI automated sequencer. All samples sequenced gave reproducible results between both PCR products and extracts of the same sample.

(*b*) *Phylogenetic and diversity analysis*

Control region sequence polymorphism was scored as variants from the published bovine reference sequence (Anderson *et al.* 1982). Phylogenetic networks were constructed using the median algorithm of Bandelt *et al.* (1995). Median networks include all most parsimonious trees supported by the data. Reduced networks simplify median networks by uncovering mutations that are likely to have occurred more than once at the same site, in a manner similar to maximum parsimony, resolving closed paths in the network into separate mutation events (which can be labelled a, b, c etc). Any remaining closed paths (for example, the

cubes and squares in figure 1) indicate ambiguity in the data (in that one sometimes cannot infer which of two variable sites has mutated more than once). Networks are particularly appropriate for the low resolution encountered in intraspecific data sets. We also constructed a neighbour-joining tree (not shown) using the Indian consensus as an outgroup and in this *B. primigenius* sequences cluster with the *B. taurus* sequences. However their position is unstable with respect to the African and European clusters because of recurrent mutations in *B. indicus*.

Divergence times were calculated from mean pairwise differences using MacPairwise (Macaulay & Micklem 1995). Standard errors in the pairwise means were calculated from 200 bootstrap replications of the data. In estimating the mutation rate, two estimates of the transition/transversion ratio were used, namely, 57:1 (Bradley *et al.* 1996) and 41:1 incorporating the data presented here. The *Bison–Bos* divergence is considered to be around 1 Ma BP and the four transversions observed in the 213 bp region between the *Bison–Bos* groups constitute the equivalent of 228 and 164 transitions respectively. The rates were calculated as 107.0% and 77.6% per Ma or one substitution in 213 bp per 4390 and 6050 years, giving an average rate of one substitution in 5000 ± 800 years. This range of values is higher than the one described previously (Bradley *et al.* 1996) which is expected as the 213 bp region was chosen to contain the most variation. The overall fractional standard error was calculated by summing the fractional errors for the pairwise mean and the mutation rate, although this does not allow for errors inherent in the rate estimation. Population differentiation was assessed using the permutation test with the Ks* statistic of Hudson *et al.* (1992).

3. RESULTS

Table 1 shows results for the eleven samples examined for histological preservation and estimated collagen survival, indicated by nitrogen content, and DNA sequence information. DNA survival did not correlate with age, but was associated with good histological preservation and with the exception of D822 also high nitrogen content. This is consistent with the analysis of an extended data set by Colson *et al.* (1996).

Table 2 shows the variable positions in control region sequences of archaeological samples aligned to the European consensus AN1 haplotype (which is identical, for this region, to the reference sequence of Anderson *et al.* 1982). For reference, the African consensus ND4 (Bradley *et al.* 1996) the Indian consensus SA2 (Loftus *et al.* 1994) and a bison sequence (R. N. Beech, J. Sheraton, R. Polziehn and C. Strobeck, personal communication: Bbu12955) are also included in this table. Although African and European control region sequences are similar to each other in comparison with those of Asian cattle, sharing what may be described as 'taurine' mtDNA haplotypes, the modern breed data of Loftus *et al.* (1994) and Bradley *et al.* (1996) nevertheless clearly fall into two main clusters. These clusters may not necessarily be supported by bootstrapping, which arbitrarily demands that they be separated by three characters without recurrent mutation to achieve a 95% confidence level (Felsenstein 1985), whereas (as is clear from figure 1) there is a high rate of recurrent mutation in the bovine control region. By indicating likely recurrent events, without arbitrarily resolving areas of genuine uncertainty in the phylogeny as would any most parsimonious tree, the reduced median network allows one to identify plausible clusters of lineages in a qualitative fashion (Richards *et al.* 1996).

Figure 1 clearly shows a principal split, or separation, at three positions (16050, 16113 and 16255) between European and African domesticates. Of these positions, 16113 splits the European and African domesticates most cleanly, with only a single African haplotype on the European side of the split. At position 16050, all but one European haplotype fall on one side of the split and all but two Africans on the other, and at position 16255 all of the Africans fall on one side and all but two of the Europeans on the other. This supports the conclusion of Bradley *et al.* (1996) who used a number of different analytical methods to show that European and African domesticates had separate origins with different ancestral haplotypes.

Genetic sub-division within the modern European cattle was tested statistically using the permutation test of Hudson *et al.* (1992), applying the Ks* statistic. We found no distinction between beef and dairy cattle (p-value of Ks* = 0.26). However when a division of the breeds is made on a geographical basis, i.e. between European mainland and European insular (British and Jersey), the distinction between the two groups is statistically significant (p-value of Ks* = 0.03). We also compared diversity values among the modern British and Continental breeds of cattle. The mean pairwise difference π among insular cattle (Aberdeen Angus, Hereford and Jersey) is twice as great as that of the Continental breeds (Charolais, Simmental and Friesian), with values of 3.15 and 1.55 respectively.

A total of five of the ancient samples were sequenced for both regions 16042–16157 and 16228–16325 and two (D815 and D816) for region 16074–16157. The five ancient DNA sequences (D355, D630, D822, D740 and D812) were compared with previously published *Bos* sequences from Africa and Europe (Loftus *et al.* 1994; Bradley *et al.* 1996) for 213 bp from the regions 16042–16157 and 16228–16325. The results are included in the reduced median network presented in figure 1. A median network of the European, African, Indian and *B. primigenius* consensus sequences is shown in figure 2. The *B. primigenius* samples fall closest to the extant European consensus, and from figure 1 it is clear that they are approximately equidistant from the extant African and European clusters.

Although our *B. primigenius* samples share two of the diagnostic variants (16050 and 16113) with Europeans and are closest to the European extant group in the network of consensus sequences (figure 2), they are nevertheless quite a distance from the modern European consensus sequence. When a reduced median network is constructed (figure 1), the node at which the two *B. primigenius* sequences branch from the extant sequences is two transitional steps distant from the European AN5 and SI4–JE4 branching nodes, and also from HE7, whereas it is three steps away from the African BU4 and BU1 branching nodes. The node from which the *B. primigenius* sequences branch in the network is three and four steps away from the European

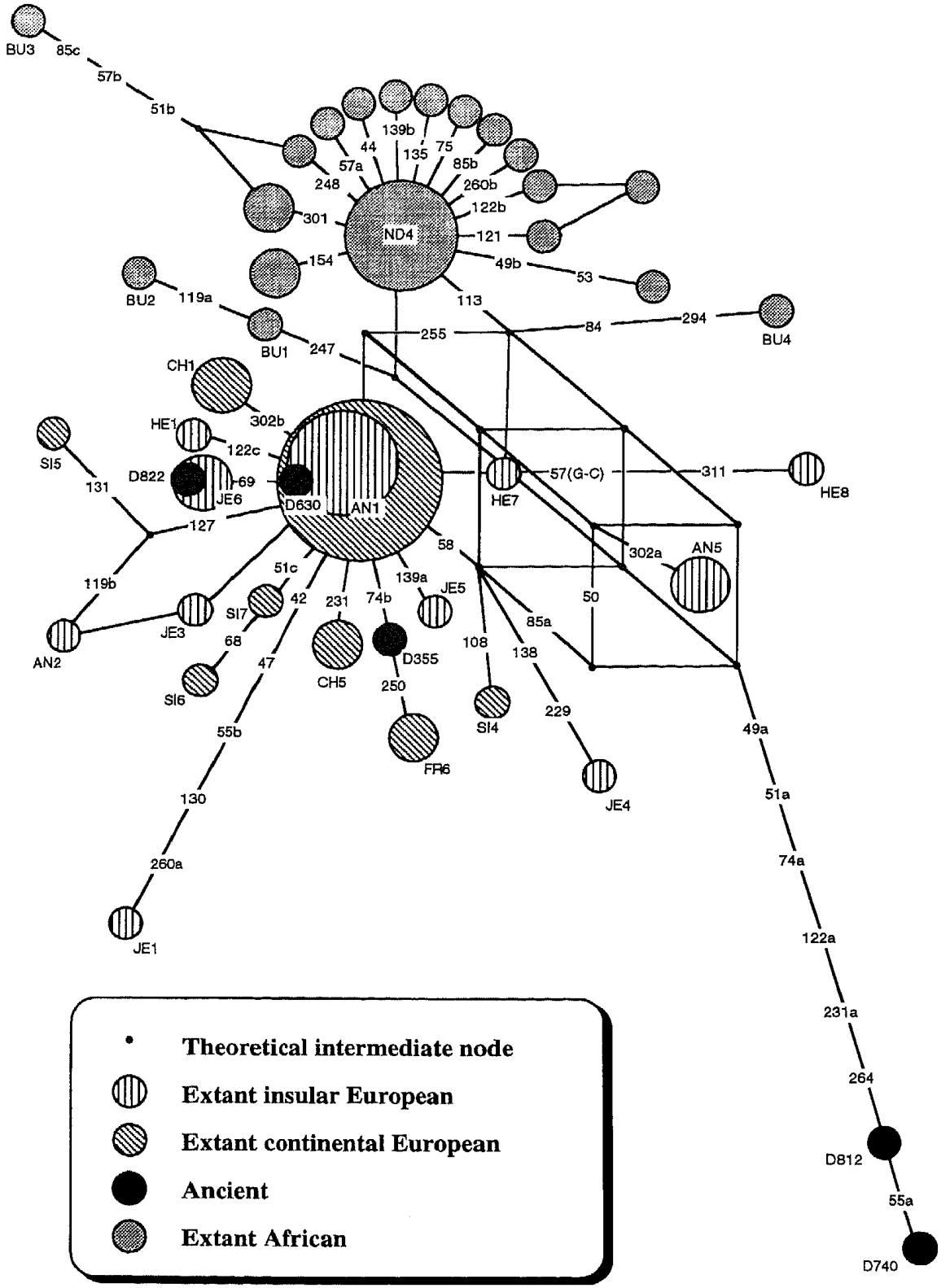

Figure 1. A reduced median network (Bandelt *et al.* 1995) with extant *Bos* lineages from Africa and Europe (Loftus *et al.* 1994; Bradley *et al.* 1996), including five ancient lineages, for both regions 16042–16157 and 16228–16325 (213 bp). Circles represent sequence haplotypes; the area is proportional to the frequency of the haplotype. The extant European samples are labelled: AN = Aberdeen Angus, HE = Hereford, JE = Jersey, CH = Charolais, SI = Simmental and FR = Friesian. ND = N'Dama, BU = Butana; other unlabelled haplotypes are also African. The numbers after the letter denote the haplotypes as per Bradley *et al.* (1996). AN1 is composed of six insular and ten continental individuals as well as two with haplotypes AN4 and JE2 which have collapsed into AN1 in the 213 bp analysis. Archaeological samples are numbered as per table 1. Black points are theoretical intermediate nodes introduced by the median algorithm. Branches between haplotypes represent mutations; they are labelled by position as per Anderson *et al.* (1982) less 16000. Transversions are specified but transitions do not need further definition. Insertion/deletion events are excluded. Recurrent mutations are indicated by a, b, c etc., designating putative distinct mutation events at the same position (e.g. 139a and 139b indicates two separate transition events at position 16139). Positions 057 and 085 have been reduced further to avoid the introduction of a four-dimensional cube; the order of mutations on the path leading to BU3 should therefore be regarded as uncertain.

and African consensus sequences respectively. Therefore, the Pleistocene sequences appear to cluster only slightly more closely with European than African domesticates.

The short branches of extant European cattle appear to root directly to AN1 in a star phylogeny. There are also a number of longer extant branches which do not appear to root back to these haplotypes. To distinguish the most recent expansion (the starburst) from the pre-existing diversity (the long branches), we calculated the mean pairwise difference of AN1 (the European consensus) and all haplotypes one step away from it; the value was 0.78. This implies an approximate minimum time for the most recent expansion of around 4000 ± 1500 years. This pattern is mirrored by a star phylogeny in the extant African cattle centred on the ND4 haplotype.

This suggests that there was a diverse population in Africa and Europe prior to the expansions of the AN1 and ND4 haplotypes and their derivatives, which may have been continuous throughout Africa and Europe. To calculate a very approximate estimate of the time of the most recent common ancestor for the European *B. taurus* ancestor, we counted the number of mutations separating the two most distantly related extant European sequences (AN5 and JE1). This branch length was nine transitions. Using the calibration described above, this corresponds to a divergence time of about 46000 ± 22500 years. A second very approximate estimate of the most recent common ancestor for *B. taurus* was calculated from the number of mutations separating the two most distantly related extant sequences (JE1 and BU3). This branch length was 13 transitions, corresponding to a divergence time of about 66500 ± 29000 years. The *B. primigenius* sequences (D812–D740) were 14–15 and 15–16 transitions away from the most distant European and African sequences respectively and therefore fall approximately within the time frame of the Pleistocene (76500 ± 32000 years).

4. DISCUSSION

B. primigenius, the wild aurochs, which is believed to be the ancestor of European domestic cattle, *B. taurus*, was distributed throughout Europe at the end of the last Ice Age. In contrast with ovicaprids, this distribution makes it difficult to clearly assess the domestication process since it is less obvious that the domestic animals were introduced from the Near East (Clutton-Brock 1987). Moreover, the nomenclature of the *Bos* species has become slightly confused. *B. primigenius* remains were considered as two species or sub-species but recently the two forms have been shown merely to be the result of sexual dimorphism, and it is suggested that *B. primigenius* and *B. taurus* form a continuous species (Bohlken 1968; Grigson 1978). Also, the size difference observed between the two has been interpreted as resulting from climatic change in the postglacial rather than the domestication process (Jarman 1969; Barker 1976; Barker 1989).

(*a*) *Pleistocene origins of* Bos *lineages*

The European *B. primigenius* sequences cluster slightly more closely with the European extant cattle in the network of consensus sequences (figure 2), and certainly with taurine cattle rather than *B. indicus*. Extant *B. taurus* and *B. indicus* may be crossed to produce viable fertile offspring. As *B. primigenius* and *B. taurus* are phylogenetically more closely related, it seems likely that they would have been able to interbreed and in this respect should be considered a single species. The two *B. primigenius* sequences are themselves very similar to each other but, because of the small sample size, it is impossible to tell whether or not they represent a frequent Pleistocene lineage which is now extinct in the extant *B. taurus* population. It is noteworthy that D740 was found in association with Late Upper Palaeolithic flint artefacts very similar to those found at Gough's Cave in Cheddar, the provenance of sample D812 (Chamberlain & Ray 1994).

The novel sequences obtained from the Pleistocene samples, in conjunction with the extant data, suggest that there was a highly diverse population of aurochsen in the Pleistocene. This is consistent with the idea that the starburst of lineages centred upon the AN1 haplotype in Europe is the result of a more recent expansion superimposed upon the backdrop of a diverse ancient population.

(*b*) *Neolithic expansion of* Bos

The timing of this subsequent expansion is important. We have estimated a minimum expansion time of 3500–4700 Ma BP, but these values are associated with a large margin of error. An expansion may have been caused by the introduction of modern farming methods, the establishment of cattle breeds, the introduction of farming during the Neolithic or as a result of environmental changes during the post-glacial.

There are a number of indications that the expansion may have occurred before the introduction of modern

In spite of the presence of two cubes generated by the high level of recurrent mutation, the network convincingly shows two distinct starburst phylogenies, one African and one European, in which a central, frequent haplotype has recently expanded producing a number of derivative haplotypes a single mutational step away. In addition, there are a number of more distant outliers, particularly amongst the Europeans, and more particularly amongst insular Europeans. The medieval European and Bronze-Age Egyptian sequences can be seen to fit neatly within the European starburst, whereas the two Pleistocene sequences are located at the end of a long branch emerging from one of the cubes, only slightly closer to the European cluster than to the African cluster.

Table 2. *The variable positions in control region sequences of archaeological bovid samples aligned to the European consensus AN1 haplotype* (*see Bradley* et al. 1996) *for the regions* 16 042–16 157 *and* 16 228–16 325

(For reference, the African consensus ND4, the Indian consensus SA2 and a bison sequence (Bbu12955) are also included in this table. An asterisk refers to a deletion–insertion event and a '?' to a position not sequenced.)

Primers An1/An2

		045	047	048	049	050	051	055	
European consensus	*Bos taurus*	A	G	A	C	C	T	T	*
D355	*Bos taurus*	.	.	.	.	.	.	.	*
D630	*Bos taurus*	.	.	.	.	.	.	.	*
D815	*Bos taurus*	?	?	?	?	?	?	?	?
D816	*Bos taurus*	?	?	?	?	?	?	?	?
D822	*Bos taurus*	.	.	.	.	.	.	.	*
D740	*Bos primigenius*	.	.	.	T	.	C	C	*
D812	*Bos primigenius*	.	.	.	T	.	C	.	*
African consensus	*Bos taurus*	.	.	.	.	T	.	.	*
Indian consensus	*Bos indicus*	.	.	.	T	.	.	.	*
Bison (Bbu12955)	*Bison bison*	T	A	T	.	.	.	.	T

Primers An1/An2 (continued)

	056	057	058	067	069	074	076	082	084	085	094	097	102	109	110	112	113	116	117	118	119	121	122	125	126	127	128	129	130	131	132	133	137	138	143	147
European consensus	A	G	C	A	A	T	A	G	C	T	T	A	G	T	C	T	T	T	G	A	T	G	T	A	T	C	T	A	T	T	A	T	T	T	A	T
D355	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D630	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D815	?	?	?	?	?	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D816	?	?	?	?	?	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D822	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D740	.	.	T	.	.	C	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.
D812	.	.	T	.	.	C	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.
African consensus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Indian consensus	.	A	T	.	.	C	.	A	T	.	.	.	A	C	.	.	C	C	A	.	C	A	C	.	.	.	.	.	C	.	.	.	C	C	*	C
Bison (Bbu12955)	G	A	.	G	.	A	G	A	T	.	C	.	.	C	T	C	C	.	.	G	C	A	C	G	C	*	*	*	*	*	*	*	.	C	*	.

Primers An5/An6

	228	229	231	247	248	255	260	264	294	298	300	301	302	304
European consensus	A	A	C	C	C	T	C	G	T	A	A	C	G	T
D355	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D630	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D815	?	?	?	?	?	?	?	?	?	?	?	?	?	?
D816	?	?	?	?	?	?	?	?	?	?	?	?	?	?
D822	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D740	.	.	T	.	.	C	.	A	.	.	.	.	.	.
D812	.	.	T	.	.	C	.	A	.	.	.	.	.	.
African consensus	.	.	.	.	.	C	.	.	.	.	.	.	.	.
Indian consensus	.	G	.	T	T	.	.	.	.	.	G	T	.	.
Bison (Bbu12955)	G	.	.	T	T	C	T	A	C	G	.	T	C	C

farming methods. The extant population can be divided in a number of ways to test the most likely origin of the AN1 expansion. First, if the predominance of AN1 was caused by the adoption of modern farming methods then one may expect the haplotypes to cluster with breeds, which is not the case (Loftus *et al.* 1994). Furthermore, there is no distinction between beef and dairy animals, but when a division of the breeds is made on a geographical basis, i.e. between European mainland and European insular, the two groups are distinct. This suggests that the haplotypes pre-date modern farming methods and breed establishment. This would not be unexpected, as bulls are usually the unit of selective breeding and therefore mtDNA, which is maternally inherited, would be less sensitive to this process. Moreover, the calibration of the mutation rate would have to be twenty times greater than currently estimated for the expansion to date to within the last 200 years.

Our ancient DNA results also support this conclusion. The presence of the AN1 and closely related haplotypes in the medieval samples suggests that the expansion took place before the medieval period, and the presence of a closely related haplotype in Bronze-Age Egypt may suggest that the expansion occurred even earlier. Furthermore, the existence of this haplotype is consistent with an origin for the European expansion in the vicinity of the Middle East and indicates that Egyptian cattle at this stage shared elements of the European gene pool. Overall, these results point to a Neolithic origin for the European expansion. An alternative explanation, however, given that the dates are minimum values with high standard errors, is that the expansion was not facilitated by human intervention but by environmental change during the post-glacial.

Whether the occurrence of the AN1 haplotype in all European breeds attests to the spread of cattle across Europe during the Neolithic period or at the end of the Upper Palaeolithic, the occurrence of long-branch lineages predominantly in the European island populations suggests that there may have been inclusion of local aurochsen amongst domesticates in Northern Europe. Support for this interpretation may be found by comparing diversity values amongst modern British and Continental breeds. The diversity among insular cattle is twice as great as that of the Continental breeds, implying a greater time-depth than on the continent. Increasing the sample size of the extant breeds may reveal further rare long-branch lineages in other European regions.

These preliminary results show the power of mitochondrial control region sequences to address questions of animal domestication, especially when data on extant breeds are combined with ancient DNA analysis of wild putative progenitor populations. These conclusions can be tested, strengthened and extended by the examination of mtDNA from further pre-Neolithic samples from Europe and the Near East.

We thank Jonathan Aldridge, Christopher Barnes, Angela Bowles, Caroline Grigson, Jim Hughes, Martin Humm, Gert-Jaap van Klinken, Paul Pettitt, and Charles Shaw for

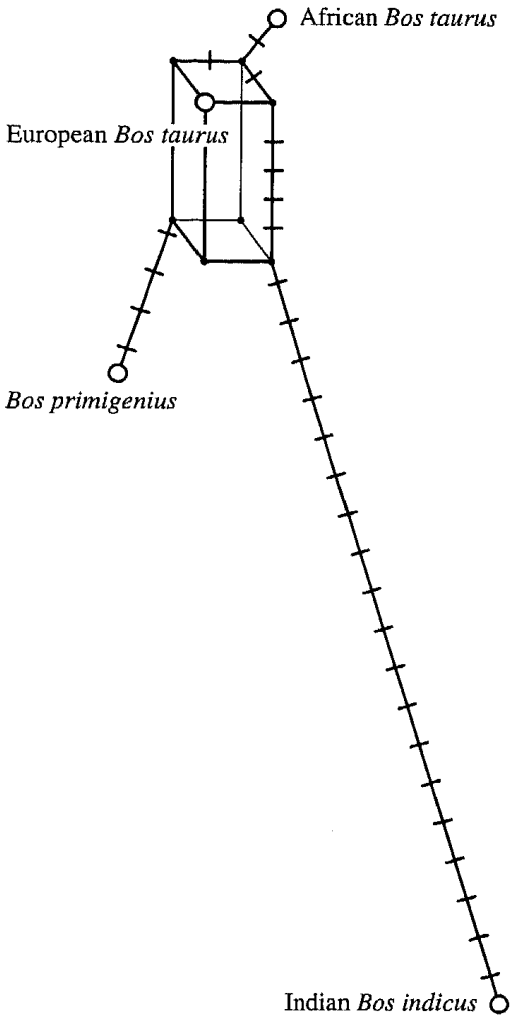

Figure 2. A median network (Bandelt *et al.* 1995) of the *B. taurus* European, African, Indian and *B. primigenius* consensus sequences (labelled unfilled circles). Ticks on the branches represent mutation events. This shows again that the *B. primigenius* sequences are more or less equidistant from the European and African consensus sequences, but also that they are much closer to both than to *B. indicus*.

advice and useful discussions, Andrew Chamberlain, Andrew Currant, Professor Fekri Hassan, Professor Maciej and Renata Henneberg, Rupert Housley, Roger Jacobi, Margaret Rule, Dale Serjeantson and Gordon Turner Walker for samples. This work was supported by the Science-Based Archaeology Committee of SERC–NERC.

REFERENCES

Anderson, S., DeBruijn, M. H. L., Coulson, A. R., Eperon, I. C., Sanger, F. & Young, I. G. 1982 Complete sequence of bovine mitochondrial DNA. *J. molec. Biol.* **156**, 683–717.

Barker, G. 1976 Morphological change and neolithic economies: an example from central Italy. *J. Arch. Sci.* **3**, 71–81.

Barker, G. 1989 *Prehistoric farming in Europe* (New Studies in Archaeology). Cambridge University Press.

Bandelt, H.-J., Forster, P., Sykes, B. C. & Richards, M. B. 1995 Mitochondrial portraits of human populations using median networks. *Genetics* **141**, 743–753.

Bohlken, K. 1962 Probleme der Merkmalsbewertung am Säugetierschadel, dargestellt am Beispiel des *B. primigenius* Bojanus 1827. *Mörphol. Jahrb.* **103**, 509–661.

Bradley, D. G., MacHugh, D. E., Cunningham, P. & Loftus, R. T. 1996 Mitochondrial diversity and the origins of African and European cattle. *Proc. natn. Acad. Sci. U.S.A.* **93**, 5131–5135.

Chamberlain, A. T. & Ray, K. 1994 A catalogue of quaternary fossil-bearing cave sites in the Plymouth area. In *Plymouth Archaeology occasional publication* No. 1. U.K.: Plymouth City Council.

Clutton-Brock, J. 1987 *A natural history of domesticated mammals.* Cambridge University Press.

Colson, I. B., Bailey, J. F., Sykes, B. C. & Hedges, R. E. M. 1996 Diagenesis and the preservation of ancient DNA. *Anc. Biomolec.* (In the press.)

Felsenstein, J. 1985 Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Grigson, C. 1978 The craniology and relationships of four species of *Bos* 4. The relationship between *Bos primigenius* Boj. and *B. taurus* L. and its implications for the phylogeny of the domestic breeds. *J. Arch. Sci.* **5**, 123–152.

Hedges, R. E. M., Millard, A. R. & Pike, A. 1995 Measurements and relationships of diagenetically altered bone from three archaeological sites. *J. Arch. Sci.* **22**, 201–210.

Hudson, R. R., Boos, D. D. & Kaplan, N. L. 1992 A statistical test for detecting geographical subdivision. *Molec. Biol. Evol.* **9**, 138–151.

Jarman, M. R. 1969 The prehistory of Upper Pleistocene and recent cattle. Part 1: East Mediterranean, with reference to northwest Europe. *Proc. Prehistoric Soc.* **35**, 236–266.

Loftus, R. T., MacHugh, D. E., Bradley, D. G., Sharp, P. M. & Cunningham, P. 1994 Evidence for two independent domestications of cattle. *Proc. natn. Acad. Sci. U.S.A.* **91**, 2757–2761.

Macaulay, V. A. & Micklem, K. 1995 *MacPairwise.* Locally developed computer program.

Richards, M., Côrte-Real, H., Forster, P. *et al.* 1996 Palaeolithic and neolithic lineages in the European mitochondrial gene pool. *Am. J. Hum. Genet.* **59**, 185–203.

Richards, M. B., Hedges, R. E. M. & Sykes, B. C. 1995 Authenticating DNA extracted from ancient skeletal remains. *J. Arch. Sci.* **22**, 291–299.

Received 23 May 1996 ; accepted 27 June 1996