

## An Unusual Pattern of Ancient Mitochondrial DNA Haplogroups in Northern African Cattle

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**Marina S. Ascunce, Andrew Kitchen, Peter R. Schmidt, Michael M. Miyamoto, and Connie J. Mulligan (2007)** An unusual pattern of ancient mitochondrial DNA haplogroups in Northern African cattle. *Zoological Studies* 46(1): 123-125. In this note, we report on a newly acquired ancient DNA sequence of the mitochondrial control region for cattle (*Bos taurus*), as obtained from a 900-yr-old archaeological sample from Eritrea, Northeastern Africa. In combination with 4 other ancient DNA sequences from Mali, Northwestern Africa, we hypothesize that Northern African cattle were more polymorphic, ca. 900-2000 yr ago, because of the greater frequency of a mitochondrial DNA haplogroup (T/T3) that is presently rare in Africa but otherwise common throughout the greater Mediterranean region. <http://zoolstud.sinica.edu.tw/Journals/46.1/123.pdf>

**Key words:** Ancient DNA, Mitochondrial control region, *Bos taurus*, Northern Africa.

Comparative DNA studies of the control region for mitochondrial DNA (mtDNA) have revealed surprising complexity in the evolutionary history of Old and New World livestock species (Bruford et al. 2003). For the greater Mediterranean area, these analyses have shown that the mitochondrial control region haplotypes for modern cattle (i.e., *Bos taurus*) belong to one of 4 sequence clusters or haplogroups (Fig. 1). Most (94%) modern cattle populations from Northern Africa carry haplogroup T1, which is rarely found outside of Africa (6% in the Near East and absent elsewhere). In contrast, modern populations from mainland Europe carry 2 very similar haplogroups, T and T3 (94%), which decrease in the Middle East (65%-74%) and almost completely disappear in Africa (6%). Haplogroup T2 makes up the remainder of this mtDNA diversity and is present at 6% in Europe and 21%-27% in the Near East, but is absent from Africa. These haplogroup distri-

butions have been interpreted as indicating a Near East origin for European *B. taurus* and the independent domestication of cattle in Africa (Bradley et al. 1996, Troy et al. 2001, Hanotte et al. 2002).

In this note, we report on the analysis of an ancient mtDNA (control region) sequence as obtained from a bovine skeletal sample from an early, first millennium, archaeological site near the community of Adi Nefas, Eritrea in Northeastern Africa (900 yr before the present; YBP; Schmidt and Curtis 2001) (Fig. 1). This newly acquired ancient DNA sequence is combined with data for the same mitochondrial control region as determined for 4 specimens from Mali, Northwestern Africa (ca. 900-2200 YBP; Edwards et al. 2004). In concert with the modern mtDNA data, these 5 ancient DNA sequences raise the possibility that the mtDNA gene pool for Northern African cattle was more diverse ca. 900-2000 yr ago.

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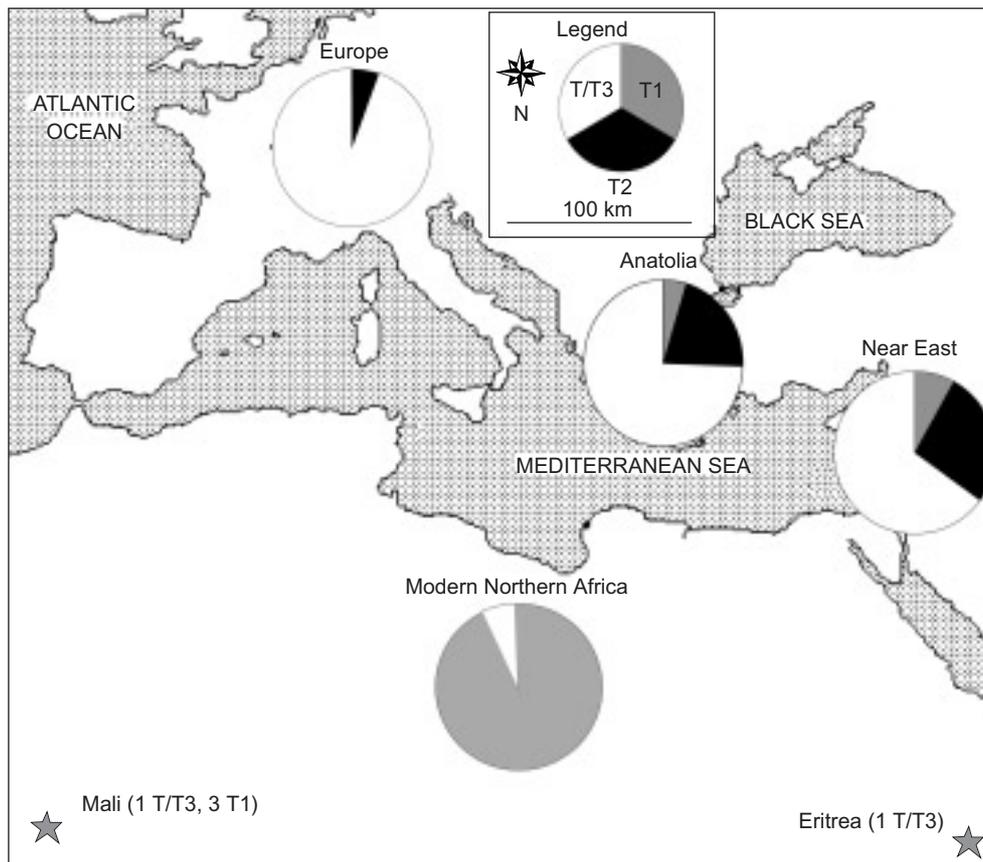
## MATERIALS AND METHODS

The original source of the ancient DNA from Eritrea consisted of a bone section (2 g) that was obtained from a larger piece of fragmented bone. Multiple DNA extractions, amplifications, cloning, and sequencing were performed according to established procedures for ancient DNA (Mulligan 2005). The DNA amplifications and sequencing relied on the primer pair, AN1 (5'-ACGCGGCAT GGTAATTAAGC-3') and AN2 (5'-GCCCAT GCATATAAGCAAG-3'), for an internal segment of the mitochondrial control region (see below). Throughout this study, rigorous safeguards were routinely employed to ensure the authenticity of the final sequence, e.g., the DNA extractions and amplifications were conducted in a separate laboratory and building with positive air pressure and HEPA air filtration where no previous bovid material, either contemporary or ancient, had ever been analyzed. In general, hot arid climates result in poorer organic preservation than colder climates

(Edwards et al. 2004). Thus, the fact that this specimen was associated with a cool highland environment may have facilitated the successful DNA extraction.

## RESULTS AND DISCUSSION

The new ancient DNA sequence from Adi Nefas, Eritrea was 116 base pairs in length (GenBank accession no.: AY524815), corresponding to positions 16,042-16,157 of the bovine mtDNA genome (Anderson et al. 1982). This mtDNA sequence is identical to a known control region haplotype for *B. taurus* (L27727; Loftus et al. 1994). Thus, as a representative of L27727, this ancient DNA sequence belongs to the combined haplogroup T/T3, which is common in modern cattle populations from Europe and the Near East, but rare in those from Northern Africa (Fig. 1). Furthermore, as characteristic of *B. taurus*, it corroborates the initial morphological identification



**Fig. 1.** Map of the greater Mediterranean region summarizing the mtDNA haplogroups for modern *Bos taurus* from mainland Europe, Anatolia, the Near East, and Northern Africa (Troy et al. 2001) and ancient cattle from Eritrea and Mali (current study and Edwards et al. 2004, respectively).

of the original larger piece of bone as cattle remains.

The previously reported ancient mtDNA sequences for the 4 Mali individuals consist of 1 T/T3 and 3 T1 haplotypes (Edwards et al. 2004). Combining the new Eritrean sequence with these 4 Mali orthologues resulted in an ancient mtDNA sample for Northern Africa of 2 T/T3 and 3 T1 haplotypes (Fig. 1). Assuming that these 5 represent a valid random sample (which admittedly is unlikely), a standard binomial test reveals that the probability of drawing by chance 2 or more T/T3 sequences out of five, given the contemporary haplogroup frequencies for Northern African cattle (Fig. 1), is only 3.5%. Minimally, this result highlights the fact that this set of 5 ancient DNA sequences appears “unusual”, because of the greater frequency of the rare T/T3 haplogroups compared to modern populations of Northern African cattle.

In conclusion, our results raise the possibility that the mtDNA gene pool for Northern African cattle ca. 900-2000 yr ago was more polymorphic in terms of the frequencies of the T1 and T/T3 haplogroups that currently predominate in African and European populations, respectively. This older polymorphism in Northern African cattle may reflect a transition from an even more-diverse ancestral gene pool (as characteristic of its Near East progenitor) and/or the later secondary introduction of T/T3 haplotypes into this region by the immigration of European cattle (Hanotte et al. 2002, Bruford et al. 2003). Concomitantly, selective pressures from domestication and breeding efforts and/or genetic drift may have then led to the final homogenization of this older polymorphism into the current situation of essentially only the T1

haplogroup occurring in Northern Africa. These possibilities reemphasize the fact that both ancient and modern DNA data are of value in the ultimate resolution of the complex history of African cattle (Edwards et al. 2004).

## REFERENCES

- Anderson S, MH de Bruijn, AR Coulson, IC Eperon, F Sanger, IG Young. 1982. Complete sequence of bovine mitochondrial DNA. Conserved features of the mammalian mitochondrial genome. *J. Mol. Biol.* **156**: 683-717.
- Bradley DG, DE MacHugh, P Cunningham, RT Loftus. 1996. Mitochondrial diversity and the origins of African and European cattle. *Proc. Natl. Acad. Sci. USA* **93**: 5131-5135.
- Bruford MW, DG Bradley, G Luikart. 2003. DNA markers reveal the complexity of livestock domestication. *Nat. Rev. Genet.* **4**: 900-910.
- Edwards CJ, DE MacHugh, KM Dobney, L Martin, N Russell, LK Horwitz, SK McIntosh, KC MacDonald, D Helmer, A Tresset, JD Vigne, DG Bradley. 2004. Ancient DNA analysis of 101 cattle remains: limits and prospects. *J. Archaeol. Sci.* **31**: 695-710.
- Hanotte O, DG Bradley, JW Ochieng, Y Verjee, EW Hill, JE Rege. 2002. African pastoralism: genetic imprints of origins and migrations. *Science* **296**: 336-339.
- Loftus RT, DE MacHugh, DG Bradley, PM Sharp, P Cunningham. 1994. Evidence for two independent domestications of cattle. *Proc. Natl. Acad. Sci. USA* **91**: 2757-2761.
- Mulligan CJ. 2005. Isolation and analysis of DNA from archaeological, clinical, and natural history specimens. *Method. Enzymol.* **395**: 87-103.
- Schmidt PR, MC Curtis. 2001. Urban precursors in the Horn: early first millennium communities in Eritrea. *Antiquity* **75**: 849-859.
- Troy CS, DE MacHugh, JF Bailey, DA Magee, RT Loftus, P Cunningham, AT Chamberlain, BC Sykes, DG Bradley. 2001. Genetic evidence for Near-Eastern origins of European cattle. *Nature* **410**: 1088-1091.