Mitochondrial DNA Variation and Evolution of Japanese Black Cattle (Bos taurus)

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ABSTRACT

This article describes complete mitochondrial DNA displacement loop sequences from 32 Japanese Black cattle and the analysis of these data in conjunction with previously published sequences from African, European, and Indian subjects. The origins of North East Asian domesticated cattle are unclear. The earliest domestic cattle in the region were Bos taurus and may have been domesticated from local wild cattle (aurochs; B. primigenius), or perhaps had an origin in migrants from the early domestic center of the Near East. In phylogenetic analyses, taurine sequences form a dense tree with a center consisting of intermingled European and Japanese sequences with one group of Japanese and another of all African sequences, each forming distinct clusters at extremes of the phylogeny. This topology and calibrated levels of sequence divergence suggest that the clusters may represent three different strains of ancestral aurochs, adopted at geographically and temporally separate stages of the domestication process. Unlike Africa, half of Japanese cattle sequences are topologically intermingled with the European variants. This suggests an interchange of variants that may be ancient, perhaps a legacy of the first introduction of domesticates to East Asia.

CATTLE have had an important but incompletely understood association with early human civilization, and study of their origins may enlighten us on hitherto unknown aspects of prehistory. All modern cattle have been considered to have the same roots in captured aurochs from the primary domestication centers of Anatolia and the Fertile Crescent (Perkins 1969; Epstein 1971; Epstein and Mason 1984; Payne 1991). However, this is an opinion that may be an artifact of the history of archaeology (Meadow 1993). For example, Loftus et al. (1994) have provided molecular evidence for a predomestic divergence between zebu, or humped cattle (Bos indicus), and taurine, or humpless cattle (B. taurus), using mtDNA displacement loop (D-loop) variation. A subsequent study has also argued that modern mtDNA sequence distributions are suggestive of biologically distinct origins for the indigenous B. taurus populations of Africa and Europe (Bradley et al. 1996).

In North Eastern Asia (Mongolia, North China, Korea, and Japan), most cattle lack humps and are classified as Bos taurus (Phillips 1961). The earliest domestic cattle in the region were probably of this type and appear between 5000 and 4000 YBP, several thousand years after primary aurochs domestication in West Asia (Payne and Hodges 1997). The extinct wild ox (B. primigenius) is thought to have been distributed locally, but whether its domestication led to North East Asian cattle, or if these were introduced from older exotic domestic stocks, is unknown.

Around the second century A.D., cattle migrated from North China via the Korean peninsula to Japan. This cattle movement was accompanied by the introduction of rice cultivation. Both genetic (Namikawa 1980) and morphological (Ogawa et al. 1989) studies have illustrated that native Japanese cattle are B. taurus and are representatives of the "Turano-Mongolian" type (Felius 1995). However, no further genetic information is available concerning the origins of the B. taurus populations of North East Asia. In addition, several European breeds have been used for upgrading native breeds in many countries of North East Asia. Japanese Black cattle, which is the majority of beef breed in Japan, is less influenced by European breeds than other breeds in Japan and other countries. Therefore, Japanese Black is a useful population to understand the origins of cattle in North East Asia.

In this study, we present an analysis of a large data set comprising mtDNA D-loop sequences for Japanese Black cattle. We analyze these in conjunction with published sequences of bison and of European, African, and India cattle and have assessed the relationships among them. The patterns of genetic variation suggest ancient population expansion and illustrate distinct taurine mtDNA lineages, which may have separate, predomestic ancestral origins. This study also indicates that Japanese cattle have been subjected to the introgression of mtDNA that are similar to European types and that
may reflect either historical importation or a more ancient interplay between the cattle of Asia and Europe.

MATERIALS AND METHODS

Animals: We obtained 32 DNA samples of Japanese Black fattening cattle from 30 prefectures in Japan. DNA was extracted from lymphonoduli of the kidney knob. Complete D-loop sequences of European, African, and Indian cattle have been previously reported in GenBank (accession no. L27712-L27737 and U51806-U51842). Samples of the outgroup species (Bison bison) were also obtained from GenBank (accession no. BBU12936, BBU12946, BBU12948, BBU12954, and BBU12959).

Sequencing: The D-loop region of mtDNA was amplified using PCR with primers constructed from the published proline tRNA (5'-CTGCAGTCTCACCACTACAACC-3') and 12S rRNA (5'-CTCCTCGACACAGATTAG-3') gene sequences (Loftus et al. 1994). Amplification and purification of product were carried out according to the method of Loftus et al. (1994). Standard double-strand DNA sequencing was performed using ~200–500 ng of the amplified product. Variations in the D-loop region of Japanese Black cattle were defined by comparison with the standard bovine mtDNA sequence (accession numbers V00654) published by Anderson et al. (1982).

Sequence analysis: Alignment of sequences was achieved using the CLUSTALW package (Thompson et al. 1994). Sites representing a gap in any of the aligned sequences were excluded from the analysis, and distances between whole D-loop sequences were estimated using the substitution model of Tamura and Nei (1993) with a I distribution parameter value \( \alpha = 0.27 \) (estimated from these data using the method used by Kocher and Wilson (1991)). This distance computation is incorporated in the MEGA package (Kumar et al. 1994). A reduced median network was constructed by hand after the recommendations of Bandelt et al. (1995), and additional phylogenies were constructed using the NEIGHBOR and FITCH programs incorporated in the PHYLIP package (Felsenstein 1993). Analysis of molecular variance (AMOVA) and pairwise F\(_{ST}\)'s were calculated using ARLEQUIN and earlier software (Excoffier et al. 1992; Schneider et al. 1997).

RESULTS

Variation in the bovine mtDNA D-loop: Twenty-five complete bovine mtDNA D-loop sequences had been reported and analyzed previously (Anderson et al. 1982; Loftus et al. 1994). Here, we have sequenced an additional 32 Japanese Black cattle (accession numbers U87633–U87650). Alignment of these 57 complete D-loop sequences illustrated 43 unique Bos haplotypes (Figure 1). In 32 Japanese Black cattle, 18 haplotypes were found, including 1 (J5) that was represented 10 times, 1 (J14) that was represented 3 times, and 3 (J9, J15, J17) that were represented in duplicate. One other haplotype (E12) was shared between two European animals, and one (A5) between two Africans. No identical sequences were shared across continental groups. Seventy-three variable sites included two 1-bp insertion/deletions and one encompassed length variation in a poly(C) tract. Only one newly described substitution was a transversion, confirming a strong transition bias that has been previously noted for bovine mtDNA (Loftus et al. 1994; Bradley et al. 1996). The geographic structure of sequence variation is observed from the large number of consistent nucleotide differences observed between Indian, African, and European haplotypes (Figure 1). Additionally, the first eight Japanese sequences (J1–J8) display some consistent substitutions, which separate them as a group. However, the others (J9–J18) seem similar to European sequences. These two groups are clearly distinct; we refer to the first eight Japanese haplotypes as the "Japan I" group and the remaining as the "Japan II" group.

Global genetic structure: Genetic structure of B. taurus sequence variation was studied using the AMOVA approach (Excoffier et al. 1992). This procedure incorporates both the estimated divergence between sequences and the frequencies. Through estimating variance components, the structure of genetic variation between hierarchical groupings may be assessed. An AMOVA performed after the exclusion of the highly divergent Bison and Indian sequences estimated that only 36.6% of the variance could be accounted for by a partition of the data into the continental African, European, and Japanese groupings. The quality of this partition, when tested by a permutation test, was not quite significant with \( P = 0.059 \). In contrast, when the Japanese cluster was separated into Japan I and Japan II, then considered with Africa and Europe, the percentage of the total variance explained by the divisions of the data was 51.7% with a probability value \( P < 0.010 \) that a more excellent partition would arise by chance.

Genetic distances and divergence times: Table 1 shows mean sequence divergence values between populations and within populations. The cattle populations each displayed similar levels of mean divergence (0.37–0.43%) with the exception of the Japan I sequence cluster, the members of which differed on average by only 0.12%. Japanese groups were related more closely to Europe than to Africa, and when grouped separately, Japan II sequences were related more closely to Europe.

<table>
<thead>
<tr>
<th></th>
<th>Japan</th>
<th>Europe</th>
<th>Africa</th>
<th>India</th>
<th>Bison</th>
</tr>
</thead>
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<td>0.9721</td>
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<tr>
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<td>0.0569</td>
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<tr>
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<td>0.154</td>
<td>0.1508</td>
<td>0.1634</td>
<td>0.0053</td>
</tr>
</tbody>
</table>

Below the diagonal and on the diagonal are the average sequence divergences between populations and within populations, respectively. Above the diagonal (italics) are genetic distances between populations calculated as pairwise \( F_{ST} \) values (Slatkin 1995). All pairwise \( F_{ST} \) values were significant at \( P < 0.01 \).
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Figure 1.—Sequence variation observed among 58 cattle D-loop sequences. Sequence codes and numbers are given in the first column. Their initials show regional codes: A, Africa; E, Europe; J, Japan; and I, India. Breed names examined are as follows: E1, Friesian, sequence published by Ander-son et al. (1982); J1-18, Japanese Black cattle; E2 and 3, Aberdeen Angus; E4, Charolais; E5 and 6, Friesian; E7 and 8, Hereford; E9 and 10, Jersey; E11 and 12, Simmental; A1 and 2, Butana; A3, Fulani; A4 and 5, Kenana; A6 and 7, N’Dama; I1 and 2, Hariana; I3 and 4, Sahiwal; I5 and 6, Tharparkar.

One Fulani and one Charolais showed identical sequences with Kenana (A5) and Simmental (E12), respectively. Sequences J9, J15, and J17 are each represented twice, J14 3 times, and J5 was encountered 10 times in the Japanese sample. Only variable sites, with sequence positions given above, are shown. Identity with the first sequence is denoted by a dash, substitution by a different base letter, and deletions by asterisks. Positions 16200i and 221i—iii indicate nucleotid insertion relative to the sequence published in Anderson et al. (1982).

than were Japan I. Values above the diagonal show pairwise FST values, which function as short-term genetic distances between populations (Slatkin 1995).

Comparison between bison and cattle sequences and assumption of a 1-myr separation time for the ancestral lineages has allowed an estimation of substitution rate in the bovine mtDNA D-loop at 30.1% per myr (Bradley et al. 1996). When calculated using a different J distribution parameter value (a = 0.27), estimated using these data, the rate becomes 15.7% per myr. It is recognized that any calibration of the rate of substitution in the mtDNA D-loop is an exercise fraught with difficulty and subject to wide errors (Tamura and Nei 1993; Parsons et al. 1997). Nevertheless, it is clear from previous work that the divergence between B. indicus and B. taurus sequences is ancient and must predate domestication (Loftus et al. 1994). The mean divergence within B. taurus lineages, when calibrated, corresponds to 35,700 YBP, and the average divergence times between Japan and Europe and between Japan and Africa yield estimates of 33,100 YBP and 48,400 YBP, respectively. Although somewhat insecure, these estimates point away from a postulated single origin for all taurine cattle breeds within the ~10,000-yr history of animal husbandry.

Phylogenetic analysis: Figure 2 shows a phylogenetic reconstruction featuring Indian, African, European and Japanese cattle populations including bison as a rooting outgroup and using pairwise FST values. All populations were significantly different (P < 0.01) when assessed using an exact test of population differentiation (Raymond and Rousset 1995). This phylogeny illustrates that mtDNA sequences are separated into two major clades: Indian B. indicus and the remaining B. taurus. In accordance with the findings of Bradley et al. (1996), all African mtDNA are assumed to be of taurine origin.
Figure 2.—Phylogenetic relationships among bison, Indian, African, European, and Japanese cattle populations constructed using pairwise FST distances and the neighbor-joining method.

despite the hybrid morphology of many of the animals. The Japan population branches more closely to Europe than to Africa.

To focus on the relationships among African, European, and Japanese cattle, two types of trees were constructed using individual sequences as nodes and excluding the obviously divergent Indian samples; Figure 3 is a neighbor-joining tree, and Figure 4 is a reduced median network.

The major feature of the neighbor-joining tree shown in Figure 3 is a central starburst including intermingled European and Japanese sequences. One distinct group of Japanese sequences (corresponding to the Japan I partition) cluster separately and distantly from the center, as do the African sequences. Limitations with a neighbor-joining tree constructed from many closely related sequences are that this tree may impose only one of many solutions of similar validity and that it usually produces an artificially high level of resolution (Bandelt et al. 1995). In this case, bootstrap support values are low (30–40%) for the important divisions.

A median network is a presentation that includes most parsimonious trees supported by the data; Figure 4 is a reduced network incorporating the same sequences in Figure 1. This network allows extant sequences as internal nodes, and the frequency with which each haplotype occurs is indicated by the area of the circle representing it. Lines connecting sequence nodes denote substitution. Small empty circles represent hypothetical sequences that have not been found in the previous and present study. However, the phylogenetic reconstruction suggests the circles as possible intermediates. Thus A5 is represented in two samples and differs from A7 by one transition in position 16,057. J9 differs from A5 by two transitions and through two possible hypothetical intermediates. Reticulations (ladders and squares) in the network indicate where character conflicts exist as a result of homoplasies (parallel mutations), and the frequency of these is an indication of the uneven distribution of substitution in the D-loop (Wakeley 1993).

For a number of such character conflicts, the network has been reduced using the principle of compatibility (Bandelt et al. 1995). Thus, of the substitutions at sites 16,050, 16,022, and 16,119, eight have been split into two parallel events, and those at 16,042 and 16,122 have been split into three parallel events. As in the neighbor-joining tree, there is a group of Japanese sequences, which are more distinct from Europeans, and these cluster around the most numerous J5. Other Japanese sequences (with the exception of J10) are intermingled with the Europeans. Here also, more numerous sequences tend toward the center of this radiation. Africans are separate and show no topological intermingling with the samples from the other continents.

**DISCUSSION**

In this study, we have obtained mtDNA D-loop sequences of Japanese Black cattle, which represent the first of such data from East Asian domesticates, and we have analyzed these in conjunction with previously published data from Europe, India, and Africa. Sequence comparisons, AMOVA, and phylogenetic analyses reveal that two major mtDNA families are represented in Japanese Black cattle; one has been detected exclusively in Japanese samples, whereas the second is comprised of samples that show sequences and patterns of variability similar to those found in Europe. Two explanations are possible for the presence of the latter group.

First, at the end of the 19th century, cattle of several European breeds were imported into Japan for the pur-
pose of upgrading native breeds. It is possible that some of this exotic genetic material retains an influence in modern Japanese cattle. However, the original importation strategy failed because hybrid cattle required more feed than natives and became less active for draft (Mukai et al. 1989). The implementation of a resulting government policy quickly eliminated crossed cattle from herds to re-create completely pure breeds. Additionally, most of the imported cattle used were bulls, and previous work has illustrated a striking retardation of maternal lineage introgression in cattle populations (Machugh et al. 1997). Specifically, despite the overwhelming and ancient zebu introgression into the originally taurine populations of Africa, no B. indicus mtDNA
variants have been discovered in eight populations (including five zebu) investigated (Bradley et al. 1996; Suzuki et al. 1993). In this study, the frequency of Japanese mtDNA, which are similar to European type, is 48% and thus seems too high to be explained solely by the above hypothesis. In addition, no shared haplotypes are observed between Japan and Europe, despite five haplotypes that are represented more than once within each group. This is suggestive of a nonrecent introgression.

A second hypothesis is that the mixture between Japanese and European cattle may be due to more ancient interchange between the two continental populations before migration from North China to Japan. The Japanese Black are representatives of the Asian “Turano-Mongolian” type of cattle (Fellius 1995). It is likely that the continuity of the Asian and European Steppe facilitated trades or plunder of cattle in ancient times. For example, in 1280 A.D., the Mongol Empire stretched from the Yellow Sea to the Mediterranean.

A development of this hypothesis is that the pattern of Japanese mtDNA variation is a legacy of the early origins of North East Asian cattle. The major feature of the network (Figures 3 and 4) is the existence of three clusters of some phylogenetic and also geographical integrity. One interpretation of the present data is that the central European/Japanese cluster, the African sequences, and the separate Japanese cluster centering on sequence J5 represent the descendants of West Asian, African, and North East Asian strains of wild B. primigenius, respectively. In each population, the numerical predominance and topological centrality (E12, A5, and most strikingly J5; Figure 4) suggest that these haplotypes may represent ancestral mitochondrial types of closely related variants in each region.

The earliest remains of cattle in a domestic context occur in Anatolia from at least 8000 YBP (Perkins 1969). The site of Mehrgarh in Pakistan shows evidence for cattle herding as early as 7000 YBP, and the earliest securely identified African domestic cow dates to only 500 yr later (Meadow 1993; Roubet 1978). The earliest domestic bovine, found in North East Asia, dated only to 5000 YBP (Payne and Hodges 1997). A plausible scenario is that the modern Japanese sequences are descended from a mixture of early migrants from the original domestic centers of West Asia and a locally domesticated strain of wild oxen, represented by sequence J5 and its putative derivatives. A survey of mtDNA variation involving almost a hundred Middle Eastern and Anatolian samples has not revealed haplotypes showing affinity with the Japan cluster (C. S. Troy and D. G. Bradley, unpublished results).

In conclusion, bovine mtDNA D-loop sequences fall into four clusters: a highly divergent B. indicus group sampled in India and three less divergent B. taurus groupings. In phylogenetic analysis, the latter form a starburst tree, with a center consisting of intermingled European and Japanese sequences plus groups of Japanese and African sequences, which each form distinct clusters at extremes of the phylogeny. This topology and calibrated levels of sequence divergence suggest that the clusters may represent different strains of ancestral aurochs, adopted at geographically and temporally separate stages of the domestication process. It is also tempting to speculate that some of the more divergent haplotypes within each continent may be the descendants of minor adoptions of wild stock.

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LITERATURE CITED


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