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Independent mitochondrial origin and historical genetic differentiation in North Eastern Asian cattle

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Abstract

In order to clarify the origin and genetic diversity of cattle in North Eastern Asia, this study examined mitochondrial displacement loop sequence variation and frequencies of *Bos taurus* and *Bos indicus* Y chromosome haplotypes in Japanese, Mongolian, and Korean native cattle. In mitochondrial analyses, 20% of Mongolian cattle carried *B. indicus* mitochondrial haplotypes, but Japanese and Korean cattle carried only *B. taurus* haplotypes. In contrast, all samples revealed *B. taurus* Y chromosome haplotypes. This may be due to the import of zebu and other cattle during the Mongol Empire era with subsequent crossing with native taurine cattle. *B. taurus* mtDNA sequences fall into several geographically distributed haplogroups and one of these, termed here T4, is described in each of the test samples, but has not been observed in Near Eastern, European or African cattle. This may have been locally domesticated from an East Eurasian strain of *Bos primigenius*.

Keywords: Cattle; Mitochondrial DNA; Displacement loop; North Eastern Asia; Phylogenetic analysis

1. Introduction

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 years (yrs) B.P.; several thousand years after primary aurochs domestication in West Asia (Payne and Hodges, 1997). The domestic cattle in the region may have been domesticated from local wild cattle or perhaps had an origin in migrants from the early domestic center of the Near East.

Previous study of mitochondrial DNA (mtDNA) diversity of Japanese Black cattle in concert with that of European, African, and Indian cattle has suggested that multiple strains of ancestral aurochs were adopted at geographically and temporally separate stages of the domestication process (Mannen et al., 1998b). One interesting aspect of these data were that a family of Japanese mtDNA haplotypes were described which were distinct from those encountered in the other regions and which suggested an input from the wild which was specific to East Asia. The Japanese native cattle are representative of the Asian "Turano-Mongolian" type (Felius, 1995). However, Japan is an archipelago and cattle were probably imported at quite a late stage in the second century AD. Consequently there is a need for a wider survey of native cattle in North Eastern Asia to examine origins and diversity in the region. Description of biodiversity in cattle is important as an aid to conservation of animal

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genetic resources and national heritage. However, the available information on livestock of North Eastern Asia is sparse (Kim et al., 2002; Mannen et al., 1998b).

In this study we present analyses of mtDNA D-loop sequences for Mongolian and Korean native cattle. We analyze these in concert with published sequences of European, African, Japanese, and Indian cattle and have assessed the relationships among them. In addition, we analyze gene frequencies of *B. taurus* and *Bos indicus* Y chromosome haplotypes within Japanese, Mongolian, and Korean cattle in order to estimate the frequency of *B. indicus* Y chromosome introgression into North Eastern Asian cattle.

2. Materials and methods

2.1. Animals

Genomic DNA was extracted from blood of 57 Mongolian and 48 Korean pure indigenous cattle. Genomic DNA of 99 Japanese Black cattle also used in previous studies were employed for Y chromosome analysis (Mannen et al., 1998a,b, 2003). Complete D-loop sequences of European, African, and Japanese cattle have been previously reported (GenBank/EMBL/DDBJ Accession Nos. L27712–L27735, U87633–U87650, and U87893–U87905).

2.2. Sequencing

We amplified the complete D-loop region of mtDNA using the primers constructed from the cytochrome *b* (5′-ACAACTAACCTCCCTAAGACTC-3′) and 12S rRNA (5′-GATTATAGAACAGGCTCCTC-3′) gene sequences. The PCRs were done with 50 ng genomic DNA as a template in a volume of 50 μl of 1× reaction buffer; 25 μM dNTPs; 0.5 μM of each primer; and 2.5 U of EX *Taq* polymerase (Takara Shuzo, Tokyo, Japan). Amplification of PCR products was carried out using a standard PCR program with 2-min denaturation at 94 °C, 30 cycles for 30 s at 94 °C, 30 s annealing at 57 °C, 90 s extension at 72 °C, and final extension for 7 min at 72 °C. After purification of PCR product using GENEMate DNA purification kit (ISC BioExpress, Kaysville, UT), standard double-strand DNA cycle sequencing was performed using SequiTherm EXCEL II DNA sequencing kits (Epicentre Technologies, Madison, WI) with dye primer (5′-CTCCCTAAGACTCA AGGAAG-3′) and analyzed on a LI-COR automatic sequencer (Model 4200L, Lincoln, NE). Variations in the D-loop region of Japanese Black were defined by comparison with the reference bovine mtDNA sequence (Accession No. V00654) published by Anderson et al. (1982).

2.3. Sequence analysis

Alignment of sequences was achieved using the CLUSTAL W 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 Γ distribution parameter value $\alpha = 0.13$, which was estimated from all D-loop sequences examined in this study using the PUZZLE program (Strimmer and von Haeseler, 1996). Using this distance, an unrooted neighbor-joining phylogeny was constructed. The distance computation and phylogenetic tree construction are incorporated in the MEGA package Ver. 2.1 (Kumar et al., 2001). Reduced median networks were constructed by hand after the recommendations of Bandelt et al. (1995).

2.4. SRY gene analysis

Tanaka et al. (2000) reported a polymorphism in the bovine SRY gene (causal of a replacement Cys to Phe at position 214) which distinguishes *B. taurus* and *B. indicus* haplotypes. As the substitution position contained a cleavage site by *Mse*I, PCR-RFLP method was carried out. PCR primers were 5′-TTAGAACGCTTACACCG CATATTACTTC-3′ and 5′-TAGTAAAATTGAGA TAAAGAGCGCCTTTGTTAG-3′. The PCRs used 50 ng genomic DNA as a template in a volume of 20 μl of 1× reaction buffer; 25 μM dNTPs; 0.5 μM of each primer; and 1 U of EX *Taq* polymerase (Takara Shuzo, Tokyo, Japan). Amplification of the PCR products were carried out using a standard PCR program with 1-min denaturation at 94 °C, 35 cycles for 1 min at 94 °C, 1-min annealing at 53 °C, 1-min extension at 72 °C, and final extension for 7 min at 72 °C. To detect the substitutions, amplified PCR products were digested with *Mse*I and followed by electrophoresis in a 3% MetaPhor agarose gel (BMA, ME).

3. Results

3.1. Variation in the mtDNA D-loop of North Eastern Asia

Fifty-six complete bovine mtDNA D-loop sequences with information of population genetic frequencies had been reported previously (Loftus et al., 1994; Mannen et al., 1998a,b). Here, we have analyzed complete D-loop sequences of 44 Mongolian and 30 Korean native cattle (Accession Nos. AB117037–AB117092). The Korean and Mongolian samples showed 23 and 32 distinct *Bos* haplotypes, respectively. In 23 Korean haplotypes, one was represented five times, and three

Table 1
D-loop sequence variations of *Bos indicus* haplotypes in Mongolian cattle

Base Position[a]	15951	16022	16074	103	166	173	297
Sahiwal[b]	C	A	C	A	G	G	C
M374 (3)[c]	-	-	-	-	-	-	-
M382 (3)	-	T	-	-	-	-	-
M348 (1)	-	-	-	-	-	-	T
M369 (1)	-	G	T	-	-	-	T
M372 (1)	T	-	-	G	A	A	T

Hyphen indicates the identical nucleotide with reference sequence.

[a] Numbers written vertically show nucleotide base positions of mtDNA genome.

[b] Reference sequence (L27733) determined by Loftus et al. (1994).

[c] Numbers in parentheses indicate number of animals observed in Mongolian cattle.

were represented in duplicate. In 32 Mongolian samples, one was represented four times, three were three times, and three were represented in duplicate. Interestingly, nine mtDNA chromosomes in Mongolian cattle were of *B. indicus* type comprising of five mtDNA haplotypes (Table 1).

3.2. Phylogenetic analysis

In order to focus on the relationships among North Eastern Asian cattle, two types of phylogenetic tree were constructed using individual sequences and excluding *B. indicus* haplotypes; first, a neighbor-joining tree with all sequences and second, reduced median networks within each North East Asian country.

Fig. 1 shows a phylogenetic reconstruction of African, European, Japanese, Korean, and Mongolian cattle mtDNA haplotypes. The major feature of this tree is a central starburst including intermingled European, Japanese, Mongolian, and Korean sequences. The other three distinct groups cluster separately and distantly from center. T, T1, T2, T3, and T4 are shown as branching point of primary *B. taurus* haplotypes defined by Troy et al. (2001) and subsequent analysis of reduced median networks (Fig. 2). Four main clusters are observed in the basis of branching point of T1–4. In contrast to African and European cattle, haplotypes of the North Eastern Asian cattle appeared within each cluster (excepting the predominantly African cluster, T1).

Reduced median networks illustrate the mitochondrial diversity within each North East Asian sample (Fig. 2). The haplogroups designated as T, T1, T2, and T3 were defined as haplogroups with different geographical distributions by Troy et al. (2001) and the East Asian haplogroup first described by Mannen et al. (1998b) is here designated as T4. The T4 haplogroup consists of a common, phylogenetically central haplotype with derivative sequences differing by only one or two substitutions. This cluster has not been observed in Africa, Europe or the Near East. The Mongolian and Japanese networks are composed of four haplogroups: T, T2, T3, and T4, while the Korean network comprises T2, T3, and T4. Haplogroup T1, which is dominant in Africa, is not observed in North Eastern Asia. The T4 haplogroup predominates in the Japanese network (frequency of 65.4%) with T3 next most common (31.5%). Within the Korean network the T3 haplogroup is predominant (83.3%) and T4 is represented only twice. In the Mongolian network, four haplogroups appeared with moderate frequencies, suggesting that Mongolian cattle have higher mitochondrial diversity. Haplogroup T2, which has been observed previously within Anatolian and Near Eastern samples (Troy et al., 2001), occurred at a relatively high frequency (34.3%) in Mongolian samples.

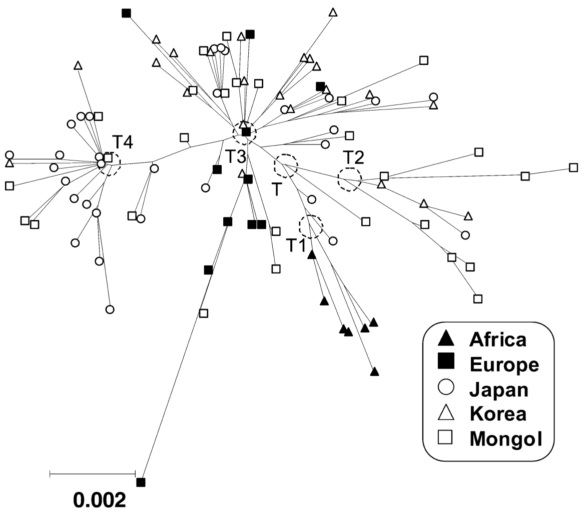

Fig. 1. Unrooted neighbor-joining tree constructed from African, European, Japanese, Korean, and Mongolian mtDNA sequences. Open circles by dashed line indicate branching point of primary *B. taurus* haplotypes defined in Fig. 2.

3.3. Population expansions

Mismatch distribution analysis revealed genetic signatures consistent with population expansion; albeit with different estimates of expansion date for each of the four taurine mtDNA lineages T1–T4 (Fig. 3). Lineages T1 and T3 illustrated bell-shaped (unimodal) mismatch distribution, while T2 gave a multimodal distribution. T4 lineage revealed linearly decreasing mismatch distribution. Average mismatch differences were similar for T1 (3.3), T2 (3.5), and T3 (2.7) but were markedly lower within the East Asian cluster, T4 (1.0). The linearly decreasing T4 shape is caused by the low number of substitutions, while the multimodal T2 shape would be

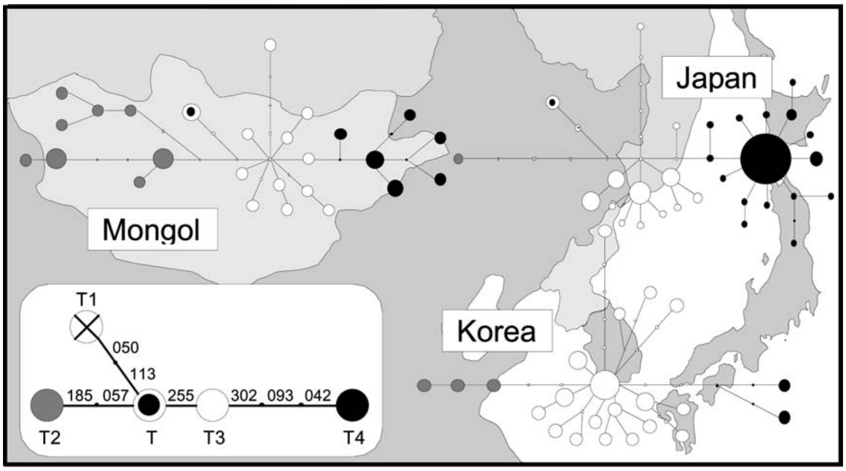

Fig. 2. Reduced median networks of *B. taurus* mitochondrial haplotypes in Japan, Korea, and Mongol. The inset of the figure shows the relationships of the five primary *B. taurus* haplotypes base on previous study (Troy et al., 2001). T3 is identical to the reference sequence (Anderson et al., 1982). The new primary haplotype T4 is defined by three transitions at positions 16,042, 16,093, and 16,302 from T3.

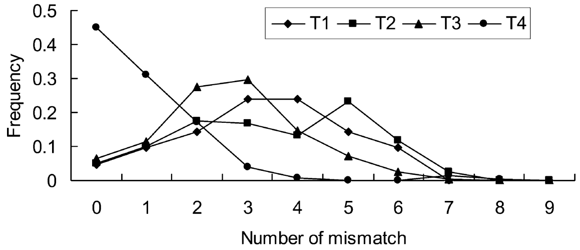

Fig. 3. Mismatch distributions within four mtDNA lineages. Numbers of mismatch were calculated by using Arlequin ver. 2.000 (Schneider et al., 1996) for each of mtDNA lineages. Average mismatch differences were 3.3 (T1), 3.5 (T2), 2.7 (T3), and 1.0 (T4).

explained by highly variation and diverged sublineages within the T2 haplogroup (Fig. 1).

3.4. Frequency of zebu and taurine Y chromosome haplotypes

Following the discovery of *B. indicus* mitochondrial chromosomes in Mongolian cattle, frequencies of *B. taurus* and *B. indicus* Y chromosome haplotypes were assessed in Japanese, Mongolian, and Korean cattle. Table 2 gives the frequencies of zebu Y chromosome and mitochondrial haplotypes in North Eastern Asian cattle. In contrast with mitochondrial haplotypes, all samples revealed *B. taurus* Y chromosome variants.

Table 2
Observed numbers of *B. taurus* (taurine) and *B. indicus* (zebu) genotypes of mtDNA and Y chromosome in Japanese, Korean, and Mongolian native cattle

Population	mtDNA		SRY	
	Taurine	Zebu	Taurine	Zebu
Japan	128	0	99	0
Korea	30	0	48	0
Mongol	35	9	31	0

4. Discussion

In this study we have obtained complete mtDNA D-loop sequences of Mongolian and Korean native cattle, which are representative samples in North Eastern Asia and have analyzed these in conjunction with previously published data from Japanese, Europe, India, and Africa. Subsequently we investigated gene frequencies of *B. taurus* and *B. indicus* Y chromosome haplotypes for North Eastern Asian cattle. We have shown the presence of *B. indicus* mtDNA haplotypes (20%), but an absence of *B. indicus* Y chromosome introgression, in Mongolian cattle.

Mongolian cattle have strictly *B. taurus* morphological features, as do Japanese and Korean cattle. In addition, Mongolia's northern and cooler climate does not offer a selective advantage for the heat-tolerant zebu cattle. The complete absence of zebu mtDNA in both Japanese and Korean samples suggests that the introgression may be a secondary phenomenon, with the earlier cattle in the region being purely *B. taurus.* This introgression is likely to have occurred in recent millennia, given that cattle introduction from the North Asian continent via the Korean peninsula to Japan is likely to have accompanied by the introduction of rice cultivation around the second century AD (Mukai et al., 1989).

Diversity within *B. taurus* mtDNA types also suggests additional influence in the Mongolian cattle population. Whereas the Korean and Japanese samples are comprised chiefly of haplogroups T3 (which is also found throughout Europe and the Near East) and T4 (which is specific to East Asia), the Mongolian sequences also contain substantial representatives of T2, a haplogroup only encountered thus far at appreciable frequencies in the Near East. It is likely that the continuity of the Asian and European Steppe facilitated trades or plunders of cattle in ancient times. A hypothesis for a historical influence exposing Mongolian livestock to foreign diversity is that the Mongolian conquest catalyzed the introgression of cattle with a more Occidental origin in the Mongolian population. This was one of the most extensive in history and in the 13th century the conquests of this pastoralist people had extended to Southeast Asia, Southwest Asia and Northern India (Rupen, 1979). The hypothesis could also explain the *B. indicus* introgression in Mongolia. An alternative hypothesis is that by its geographical position Mongolia constitutes a hybrid zone between regions where cattle expansion occurred. Luikart et al. (2001) have also noted unusual diversity within Mongolian domesticates. In their study of goat mtDNA diversity from 44 countries, they described three control region haplogroups, with divergence analogous to that observed between *B. indicus* and *B. taurus*. Their Mongolian sample was the only population in three continents to exhibit all three lineages.

The absence of zebu Y chromosomes contrasts with the substantial *B. indicus* mtDNA influence and points toward a decoupling of the introgression dynamics of the maternal and paternal lineages which has been observed elsewhere in cattle, particularly within Africa (MacHugh et al., 1997). This is perhaps not surprising, given the large variance in reproductive success among males under managed breeding. A local preference of bulls of *B. taurus* morphology, as seems likely here, can profoundly affect Y chromosome distribution while having no influence on mtDNA.

The major feature of phylogenetic analyses in North Eastern Asian cattle (Figs. 1 and 2) is the existence of four major *B. taurus* mitochondrial haplogroups and geographical differentiation. These haplogroups are each comprised of separate starlike clusters of sequences which root to the overall phylogeny through a single predominant sequence. The phylogenetic patterns within each are suggestive of a domestication-induced population expansion and their differing geographical distributions suggest different domestic histories in different regions. The Near East contains members of haplogroups T, T2 and T3 at appreciable frequencies. T3 predominates in Europe and another haplogroup, T1 predominates in Africa. This pattern suggests that European mtDNA diversity is a subset of that resulting from an initial Near Eastern domestication and that African cattle result from a separate event, possibly a domestication of African aurochs (Troy et al., 2001). The data presented here are consistent with this as T1 remains absent in Eurasia and members of the other haplogroups are encountered. Of particular interest, is the North East Asian specific haplogroup T4.

The T4 group of haplotypes shows a typical starlike phylogeny, which center around a numerically and topologically predominant variant. This pattern, coupled with is absence in the Near East, is consistent with the incorporation of East Asian wild ox genetics into the bovine pool, either as a separate domestication or via adoption of female animals into existing herds. Interestingly, an analysis of mismatch distributions using mtDNA D-loop region within four taurine mtDNA lineages revealed diversity patterns implying population expansions, presumably coincident with the domestication process (Fig. 3). However, expansion within the T4 lineage is likely to be a later phenomenon as the average numbers of mismatch differences revealed divergence of about one-third that observed within the other lineages. The later estimate for T4 expansions suggests the incorporation of wild animals in Eastern Asia was a secondary development.

In contrast with Mongolian and Korean samples, one Japanese T4 haplotype appeared as predominant with a frequency of 50%. The result may reflect differences of the historical background. Primary cattle were introduced to Japanese Islands around the second century AD (Mukai et al., 1989). It is likely that limited numbers of animals were introduced at the immigration and subsequently in the course of a short period, primary immigrated cattle spread through several parts of Japan. A pronounced founder effect may have occurred for maternal material.

In conclusion, this study indicated mitochondrial and Y chromosomal divergence in North Eastern Asian cattle. Recently, phylogenetic studies of mtDNA have demonstrated the Asian origin of dog (Savolainen et al., 2002) and pig (Giuffra et al., 2000). Our results also suggested the independent mitochondrial origin in Asian cattle, however, additional molecular studies are required using native cattle from important regions as China or Central Asia to clarify the origin and biodiversity in Asia.

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