



Mitochondrial DNA Variation and Genetic Relationships in Japanese and Korean Cattle

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ABSTRACT : The complete mtDNA D-loop regions of Japanese and Korean cattle were analyzed for their mtDNA variations and genetic relationships. Sequencing the 30 Higo substrain and 30 Tosa substrain of Japanese Brown, respectively 12 and 17 distinct *Bos haplotypes* were identified from 77 polymorphic nucleotide sites. In order to focus on the relationships among Japanese and Korean cattle, two types of phylogenetic tree were constructed using individual sequences; first, a neighbor-joining tree with all sequences and second, reduced median networks within each Japanese and Korean cattle group. The trees revealed that two major mtDNA haplotype groups, T3 and T4, were represented in Japanese and Korean cattle. The T4 haplogroup predominated in Japanese Black and Japanese Brown cattle (frequency of 43.3-66.7%), while the T3 haplogroup was predominant (83.3%) and T4 was represented only twice in the Korean cattle. The results suggested that the mitochondrial origins of Japanese Brown were Japanese ancient cattle as well as Japanese Black in despite of the considerable introgression of Korean and European cattle into Japanese Brown. (**Key Words :** mtDNA, Cattle, Japanese Black, Japanese Brown, Korean Cattle Phylogenetic Tree)

INTRODUCTION

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. They have been used as Japanese native cattle for breeding. Nowadays, there are four kinds of beef cattle (Japanese Black, Japanese Brown, Japanese Polled and Japanese Shorthorn) in Japan. Japanese Black is the main breed and famous for its ability to produce high-quality meat. Japanese Brown, which has two different kinds of substrains (Tosa and Higo), was created by the adaptation of Korean native cattle to Japanese native cattle approximately a hundred years ago. Therefore, both breeds have quite similar phenotype in body shape and coat color. However, it is reported that Simmental with brown coat color had been introduced to improve Japanese Brown for several years since 1906 (Mizuma et al., 1982).

In Japanese cattle, 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). In addition, Mannen et al. (1998; 2004) reported the origin and genetic diversity of cattle in North Eastern Asia including Japan and Korea using mtDNA sequence variation. However, no further genetic information is available concerning the origins of Japanese Brown and the genetic relationship with Korean cattle.

Mitochondrial DNA sequence data for polymorphic sites have been used for studies of molecular evolution and genetic relationships between and within species (Liu et al., 2004; Jeon et al., 2005). In this study we present analyses of mtDNA D-loop sequences for two substrains of Japanese Brown cattle. These sequences were compared with published sequences of Japanese Black and Korean cattle in order to assess the genetic relationship between them.

MATERIALS AND METHODS

Samples

Blood samples of 30 Japanese Brown (Higo) and 30 Japanese Brown (Tosa), which have lower genetic relationship between individuals on maternal lines, were collected from diverse areas in Kumamoto (Higo) and Kochi (Tosa) Prefecture, respectively. Genomic DNA was extracted from blood samples according to standard protocols. Complete D-loop sequences of Japanese Black and Korean cattle have been available (GenBank/EMBL/

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Table 1. D-loop sequence variations of *Bos. taurus* haplotypes in Japanese Brown cattle (A) Higo (B) Tosa

(A)																												
Base	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1											
Position ^a	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6											
	8	0	0	0	0	1	1	1	2	2	3		1	1	1	2												
	0	4	5	8	9	2	4	4	3	5	0		0	6	7	2												
	7	2	7	5	3	2	1	6	2	5	2	8	6	9	4	3												
F ^b	G	T	G	G	G	T	T	A	C	T	G	G	T	A	C	-												
BrH1	A	-	C	-	-	-	-	-	-	C	-	-	-	G	T	-												
BrH2 (10) ^c	-	C	-	-	A	-	-	-	-	-	A	-	-	G	-	-												
BrH3 (3)	-	C	-	-	A	-	-	-	-	-	A	-	-	G	-	C												
BrH4 (5)	-	C	-	-	A	-	C	-	-	-	A	-	-	G	-	-												
BrH5	-	C	-	-	A	-	-	G	-	-	A	-	-	G	-	-												
BrH6	-	-	-	A	-	-	-	-	-	-	-	A	-	G	-	C												
BrH7	-	-	-	-	-	C	-	-	-	-	-	-	-	G	-	C												
BrH8	-	-	-	-	-	C	-	-	-	-	-	-	C	G	-	C												
BrH9	-	-	-	-	-	C	-	-	T	-	-	-	C	G	-	C												
BrH10 (2)	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	C												
BrH11 (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-												
BrH12 (2)	-	-	-	-	A	-	-	-	-	-	-	-	-	G	-	-												
(B)																												
Base	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1								
Position	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6								
	9	0	0	0	0	0	0	1	1	1	1	1	1	1	1	2	2	2	2	3	1	2						
	1	4	4	5	5	5	8	9	0	1	1	1	1	2	6	6	8	9	0	3	5	6	0	6	2			
	0	2	9	0	5	7	6	3	4	2	3	8	9	2	4	7	5	7	9	1	5	4	2	8	9	3		
F	T	T	C	C	T	G	G	G	C	T	T	A	T	T	T	C	G	G	C	C	T	G	G	G	A	-		
BrT1	-	C	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-		
BrT2	-	C	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	C		
BrT3 (8)	-	C	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	G	-	
BrT4	-	C	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	G	C	
BrT5	-	C	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	G	C	
BrT6	-	C	T	-	-	A	-	A	T	-	-	-	-	C	-	-	-	-	-	-	-	-	-	A	-	G	-	
BrT7	-	-	T	-	-	-	-	-	-	C	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	
BrT8	C	-	-	-	C	-	-	-	-	C	-	-	-	-	-	T	-	-	-	T	-	A	-	-	-	G	-	
BrT9	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	
BrT10	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	G	-	
BrT11 (4)	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	
BrT12	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	A	-	G	-	
BrT13	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	A	-	T	-	C	-	-	-	-	-	G	-	
BrT14	-	-	-	-	-	-	-	-	-	-	G	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-
BrT15	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-
BrT16 (4)	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	G	C
BrT17	-	-	-	T	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-

^aNumbers written vertically show nucleotide base positions of mtDNA genome.

^bReference sequence (V00654) determined in Friesian by Anderson et al. (1982).

^cNumber in parentheses indicate number of animals observed in Japanese Brown cattle.

DDBJ Accession No. U87893-U87905 and AB117037-AB117092).

Sequencing

The PCR reactions were performed in 10 µl reaction volumes with 20 ng genomic DNA as a template, 2.0 µl reaction buffer (100 mM Tris-HCl, 15 mM MgCl, 500 mM KCl, pH 8.6), 1.6 µl dNTP Mix (2.5 mM), 0.13 µl of each primer (20 nmol/ml) and 1.0 U of EX Taq polymerase (Takara Shuzo Co., Tokyo, Japan). The sequences of

primers were previously described by Mannen et al. (2004). Amplification of PCR products was carried out using a standard PCR program with 5-min denaturation at 94°C, 30 cycles for 1-min at 94°C, 1-min annealing at 57°C, 1-min extension at 72°C, and final extension for 7-min at 72°C. After purification of PCR product using the GENEMate DNA purification kit (ISC BioExpress, Kaysville, UT), standard double-strand DNA cycle sequencing was performed with approximately 200-500 ng of amplified product using the ABI PRISM® BigDye® Terminator v3.1

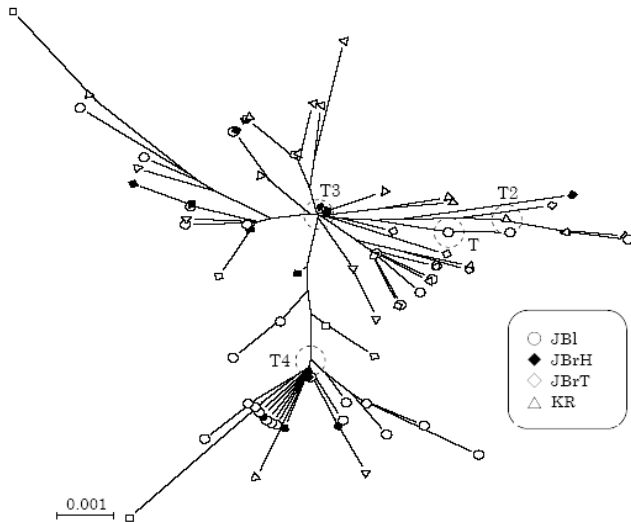


Figure 1. Unrooted neighbor-joining tree constructed from Japanese and Korean mtDNA sequences. Open circles by dashed line indicate branching point of primary *B. taurus* haplotypes defined in previous study (Mannen et al., 2004). JBl: Japanese Black, JBrH: Japanese brown (Higo), JBrT: Japanese brown (Tosa), KR: Korean cattle.

Cycle Sequencing Kit (Applied Biosystems, CA, USA) on a ABI PRISM® 3100 Genetic Analyzer.

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). 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 the NETWORK program Ver.4.1 (Bandelt et al., 1999).

RESULTS

Variation in the mtDNA D-loop of Japanese Brown

Complete mtDNA D-loop sequences had been reported and analyzed previously in Japanese Black (Mannen et al., 1998) and Korean cattle (Mannen et al., 2004). Here, we have sequenced the complete D-loop region of 30 Japanese Brown (Higo) and 30 Japanese Brown (Tosa) (Accession No. AB244486-AB244514). The nucleotide substitutions found in the mtDNA D-loop region are shown in Table 1. When we aligned our sequences with the reference sequence of Friesian reported by Anderson et al. (1982), Higo and Tosa samples showed 12 and 17 distinct *Bos* haplotypes, respectively, by 77 polymorphic sites. In 12 Higo haplotypes, BrH2 was represented ten times, BrH4 was represented five times, BrH3 was represented three times, and three (BrH10, 11, 12) were represented in duplicate (Table 1A). In 17 Tosa haplotypes, BrT3 was represented eight times, and two (BrT11, 16) were represented four times (Table 1B).

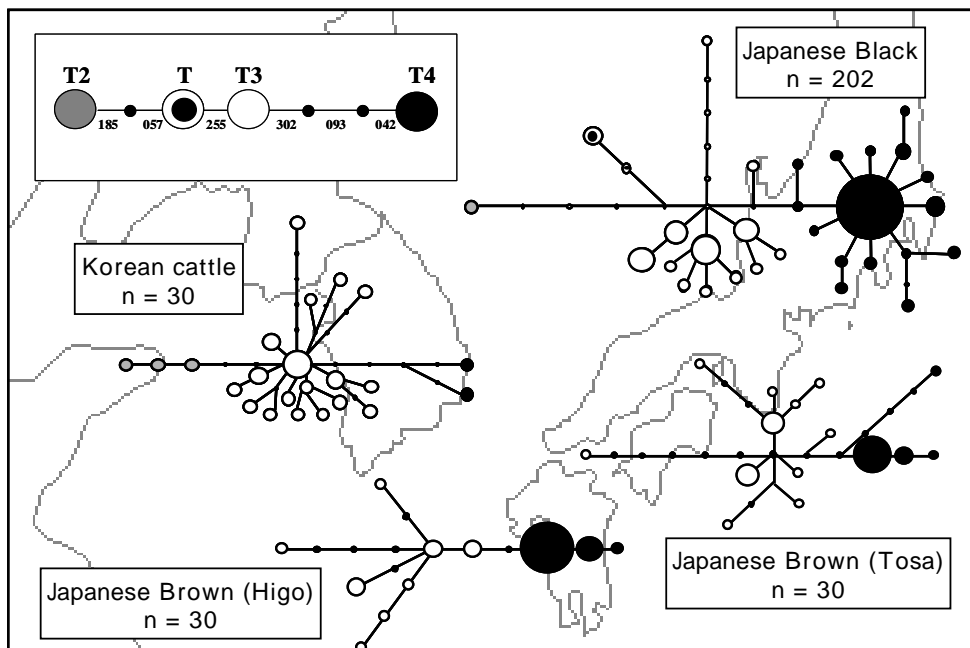


Figure 2. Reduced median network of *B. taurus* mitochondrial haplotypes in Japan and Korea. The inset of the figure shows the relationships of the four primary *B. taurus* haplotypes based on previous study (Troy et al., 2001). T3 and T4 are identical to the reference sequence (Anderson et al., 1982) and (Mannen et al., 2004), respectively.

Phylogenetic analysis in Japanese and Korean cattle

In order to focus on the relationships among Japanese and Korean cattle, two types of phylogenetic tree were constructed using individual sequences; first, a neighbor-joining tree with all sequences and second, reduced median networks within each Japanese and Korean cattle group.

Figure 1 shows a phylogenetic reconstruction of Japanese and Korean cattle mtDNA haplotypes. T, T2, T3 and T4 were shown as branching points of primary *B. taurus* haplotypes defined by Troy et al. (2001) and Mannen et al. (2004). The major feature of this tree was two distinct groups, T3 and T4.

Reduced median networks illustrated the distribution of mtDNA haplotypes within Japanese and Korean cattle (Figure 2). The haplogroups designed as T, T2 and T3 were defined as haplogroups with different geographical distributions by Troy et al. (2001) and the East Asian haplogroup T4 was first described by Mannen et al. (1998). The T4 haplogroup consisted of a common, phylogenetically central haplotype with derivative sequences differing by a few substitutions. This cluster has not been observed in Africa, Europe or the Near East. The Japanese Black cattle network was composed of four haplogroups; T, T2 T3 and T4, while the Korean network comprised T2, T3 and T4. The two Japanese Brown cattle breeds, Higo and Tosa, were composed of T3 and T4. The T4 haplogroup predominated in the Japanese Black and Higo networks (frequency of 65.4% and 66.7%, respectively) with T3 next common (31.5% and 33.3%, respectively). In the Tosa network, T3 and T4 haplogroups were observed at same level as frequency (56.7% and 43.3%). Within the Korean network the T3 haplogroup was predominant (83.3%) and T4 was represented only twice. Haplogroup T2, which has been observed previously within Anatolian and Near Eastern samples, occurred at a low frequency (only four individuals) in Japanese and Korean samples.

DISCUSSION

In this study we have obtained complete mtDNA D-loop sequences of Japanese Brown, which had been influenced by Korean cattle, and have analyzed these in conjunction with previously published data from Japanese and Korean cattle. Sequence comparisons and phylogenetic analyses revealed that two major mtDNA families were represented in Japanese Brown cattle; one, T4, was detected in Japanese samples, whereas the second, T3, was predominant in Korean. The T4 group of haplotypes showed a typical starlike phylogeny, which center around a numerically and topologically predominant variant in Japanese Black and Japanese Brown. Notably, these haplogroups were geographically distributed between Japan and Korea. In

contrast with the T4 haplogroup frequency of Korean samples (6.7%), both Japanese Brown substrains showed a higher frequency of T4 haplotype (66.7% and 44.3%). The result may reflect historical background for the origin and breeding of the Japanese Brown.

This observation and extent of the T4 cluster in Japanese cattle suggest the result of a domestication-induced expansion. Primary cattle were introduced to the 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. Korean cattle had been introduced for improvement of Japanese Brown cattle, however, most imported cattle used were bulls. A previous work illustrated a striking retardation of maternal lineage introgression in cattle populations (MacHugh et al., 1997). This historical background and our result suggest that Japanese Brown cattle had been constructed based on Japanese native cow, and genetic influence from Korean cattle was low in maternal material.

This study presented the mitochondrial diversity in Japanese Brown cattle. The results suggest that the mitochondrial origin of Japanese Brown were traced to Japanese ancient cattle as well as Japanese Black cattle, despite the introgression of Korean cattle into Japanese Brown. However, additional genetic analyses using a paternal marker on the Y chromosome and autosomal markers will be required to clarify the genetic influence of Korean cattle and the relationship between Japanese and Korean cattle.

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