

Inter- and intra-population genetic divergence of East Asian cattle populations: focusing on Korean cattle

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Abstract In order to facilitate the on-going cattle conservation and improvement programs in Korea, we examined genetic relationships among East Asian cattle, focusing on Korean native cattle, using complete mitochondrial DNA control region sequences. In total, 67 haplotypes were discriminated by 97 variable sites in East Asian cattle. Of the variable sites, 35 represented singleton variable sites and 62 were parsimony informative sites. For Korean cattle, 30 haplotypes were discriminated by 40 variable sites. The variable sites identified in this study correspond to transition or transversion point mutations. Inter-populations genetic distance varied from 0.004 to 0.052 for East Asian cattle populations. The genetic divergence observed between Korean Brown and Jeju Black (0.004) was among the lowest. The Mongolian cattle were slightly divergent from other East Asian cattle populations studied. The network analysis uncovered that Jeju

Black and Yunbian samples represented the possible ancestral Haplotype within T3 *Bos taurus* main lineage. Thus, we hypothesized that the Jeju and Yunbian cattle may represent one of the original native North East Asian cattle in the region.

Keywords Korean Brown cattle · Jeju Black cattle · mtDNA · Genetic divergence · Haplotype relationships

Introduction

The earliest domestic cattle in North East Asia were possibly humpless in morphology (Payne and Hodges 1997). Today, the majority of the cattle populations found in this region are *Bos taurus*. Using mitochondrial DNA (mtDNA), several studies provided clues about the origin, evolutionary history and genetic relationships of these cattle populations (Mannen et al. 1998, 2004; Kim et al. 2003; Lai et al. 2006). Based on the previously defined main lineages of *B. taurus*, T, T1, T2, T3 and T4 (Troy et al. 2001; Mannen et al. 2004), the Korean cattle mtDNA correspond to T2, T3 and T4. Among the lineages identified T3 is predominant in Korean cattle.

The Korean indigenous cattle generally called “Hanwoo”. And Hanwoo has four different populations/strains; Korean Brown cattle, Korean bridle cattle, Korean Black cattle and Jeju Black cattle (Ministry of Agriculture, Forestry 2004). According to the report of Ministry of Agriculture, Forestry (2004), the word Hanwoo is referred to Korean Brown cattle. The remaining three populations are considered as endangered breeds. An endangerment was resulted from the efforts of selecting Hanwoo cattle for uniform Brown coat color for many years while excluding

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those three strains from registration as pure breeds for breed improvement. In recent times, government, universities and farmers are continuously making efforts to re-establish these endangered cattle populations in the republic of Korea (Ministry of Agriculture, Forestry 2004). Among the endangered populations, Jeju Black cattle are characterized by quality meat and high unsaturated fat, superior even to Korea's other native cattle (MIFAFF, <http://english.mifaff.go.kr/main.jsp>). Traits related to meat quality and fatty acid compositions are fundamental to livestock industry from consumers' point of view at present and the future.

In addition to the already existing information on the Korean cattle populations (Mannen et al. 1998; Kim et al. 2003), more genetic information is still required to assist the current on-going conservation endeavors and improvement programs. Therefore, in the present study we used DNA sequences of complete mitochondrial DNA control region to assess the genetic divergence of Korean cattle from other Asian cattle populations.

Methods

Samples

Table 1 presents information on sequences included in this study. Cattle mtDNA control region sequences of Chinese accession nos. AY521076-83 (Anxi), AY521112-6 (Sanjiang), AY521100-2 and AY902394 (Jinnan), AY521103-6 (Montbeliard), AY115525-7 (Nanyang), AY119667-8 and AY119672 (Jiaxian), AY902393 and AY521096-8 (Huangpi), AY521130-36 and AY902404-5 (Zaobei), AY521121-6 (Wannan) and Mongolian accession nos. AB11088-AB117092 (Mongolian cattle) were obtained from GenBank (Lai et al. 2006). Samples of Japanese accession nos. U87633–U87640 reported by Mannen et al. (1998) and Korean cattle accession nos. AF499238–AF499264 (Lee et al. 2002) and AF409046–AF409049 (Kim et al. 2003) were also obtained from GenBank database.

In addition to our previous report on Korean cattle mtDNA sequences (Dadi et al. 2012), eight Yunbian cattle (mtDNA control region) samples were sequenced. The complete control region of mtDNA was amplified using forward primer L15737: 5'-CTGCAGTCTCACCAT-CAACC-3' and reverse primer H992: 5'-GATTATAGAA CAGGCTCCTC-3' (Anderson et al. 1982). The reactions were carried out using 0.2 µg of mtDNA, 0.4 µM of each primer, 0.25 mM dNTP, 10× reaction buffer contains Tris-HCl (pH 9.0), PCR enhancers, (NH₄)₂SO₄, 20 mM MgCl₂ and 1 U *Taq* DNA polymerase in a 25 µl reaction volume. Amplifications were carried out as follows: 3 min at 94 °C for initial denaturation followed by 35 cycles each of 1 min

at 94 °C, 30 s at 58 °C, 90 s at 72 °C and a final extension step of 5 min at 72 °C. The PCR products were purified using SolGent™ PCR Purification Kit, following the manufacturer's instructions. The PCR products were then sequenced using both PCR primers and an internal primer L16161:5'-ATTACCATGCCGCGTGA-3'. Sequencing was performed using a BigDye Cycle Sequencing Kit on an ABI-3730XL DNA Analyzer.

Statistical analysis

The complete mtDNA control region sequences were aligned using CLUSTALW (Thompson et al. 1994) as implemented in MEGA (Tamura et al. 2011). All positions containing gaps and missing data were excluded from the analysis. Haplotype frequencies and variable sites were analyzed using DnaSP 5.0 computer program (Rozas et al. 2003). To clarify the genetic relationship of Korean cattle with other East Asian cattle populations, we computed average evolutionary divergence over sequence pairs between populations using MEGA. Analyses were conducted using the Kimura 2-parameter model. To examine the relationships among the haplotypes of cattle populations, median-joining networks were constructed, in conjunction with other sequences from East Asian cattle populations using NETWORK 4.6 (Bandelt et al. 1999).

Results

Figure S1 shows sequence variation observed among haplotypes of East Asian cattle populations mtDNA control region of 884 base pairs in length. In total, 67 haplotypes were discriminated by 97 variable sites. Of the variable sites, 35 represented singleton variable sites and 62 were parsimony informative sites. Haplotype H₃₄ (Sanjiang, Huangpi, Zaobei, Wannan and Mongolian), H₅₀₋₅₃ (Mongolian), H₄₁ (Nanyang), H₄₂ (Jiaxian), H₄₈ (Wannan) and H₅₆ Yunbian cattle populations revealed more variable sites as compared to H₁, the Bovine reference sequence (Anderson et al. 1982), suggesting that these populations are slightly distant from *B. taurus* although the genetic distance estimates among East Asian cattle populations were generally low. For Korean cattle, 30 haplotypes were discriminated by 40 variable sites in comparison with the Bovine reference sequence.

Table 2 shows estimates of evolutionary divergence over sequence pairs for inter- and intra-populations. Inter-populations genetic distance varied from 0.002 to 0.037 for East Asian cattle populations. The estimates of average genetic distance between populations were considerably low. As shown in Table 2, the Mongolian cattle relatively divergent from the Korean, Chinese and Japanese cattle.

Table 1 Information on sequences of GenBank and generated by our study

Breed	Region	Accession number	Number of sequences	Reference
Anxi	China	AY521076-83	7	Lai et al. (2006)
Sanjiang	China	AY521112-6	5	Lai et al. (2006)
Jinnan	China	AY521100-2 and AY902394	4	Lai et al. (2006)
Montbeliard	China	AY521103-6	4	Lai et al. (2006)
Nanyang	China	AY115525-7	3	Lai et al. (2006)
Jiaxian	China	AY119667-8 and AY119672	3	Lai et al. (2006)
Huangpi	China	AY902393 and AY521096-8	4	Lai et al. (2006)
Zaobei	China	AY521130-36 and AY902404-5	7	Lai et al. (2006)
Wannan	China	AY521121-6	6	Lai et al. (2006)
Mongolian	Mongolia	AB11088–AB117092	5	Lai et al. (2006)
Japanese Black	Japan	U87633–U87640	8	Mannen et al. (1998)
Korean Brown	Korea	AF499238–AF499264	27	Lee et al. (2002)
Jeju Black	Korean	AF409046–AF409049	4	Kim et al. (2003)
Yunbian	Korea		8	This study
Jeju Black	Korean		19	Dadi et al. (2012)
Korean Black	Korean		18	Dadi et al. (2012)
Korean Brindle	Korean		19	Dadi et al. (2012)

Table 2 Estimates of average evolutionary divergence between populations (below diagonal) and within population (on-diagonal), standard errors are shown above the diagonal (884 bp in length, 151 sequences)

Population	KB	JB	JAB	ANX	SAN	JIN	MONT	NAN	JIA	HUA	ZAO	WAN	MON	YUN	KBL	BRI
KB	0.005	0.001	0.002	0.001	0.002	0.001	0.001	0.003	0.003	0.004	0.002	0.003	0.007	0.001	0.001	0.001
JB	0.004	0.004	0.002	0.001	0.002	0.001	0.002	0.003	0.003	0.004	0.002	0.003	0.007	0.001	0.002	0.002
JAB	0.006	0.004	0.002	0.002	0.002	0.002	0.002	0.003	0.003	0.004	0.002	0.003	0.007	0.002	0.001	0.001
ANX	0.005	0.005	0.006	0.005	0.002	0.001	0.001	0.003	0.003	0.004	0.002	0.003	0.007	0.001	0.002	0.002
SAN	0.014	0.013	0.014	0.014	0.023	0.002	0.002	0.003	0.003	0.004	0.002	0.003	0.006	0.002	0.001	0.001
JIN	0.005	0.004	0.005	0.005	0.013	0.004	0.002	0.003	0.003	0.004	0.002	0.003	0.007	0.002	0.002	0.001
MONT	0.006	0.006	0.008	0.006	0.015	0.006	0.006	0.003	0.003	0.004	0.002	0.003	0.007	0.002	0.003	0.003
NAN	0.021	0.020	0.021	0.020	0.023	0.021	0.022	0.037	0.004	0.004	0.003	0.003	0.005	0.003	0.003	0.003
JIA	0.021	0.020	0.022	0.020	0.023	0.020	0.021	0.025	0.034	0.004	0.003	0.003	0.005	0.003	0.004	0.004
HUA	0.029	0.028	0.031	0.028	0.028	0.029	0.029	0.028	0.028	0.037	0.004	0.004	0.004	0.004	0.002	0.002
ZAO	0.011	0.011	0.012	0.011	0.018	0.011	0.013	0.023	0.023	0.028	0.018	0.003	0.006	0.002	0.003	0.003
WAN	0.021	0.020	0.021	0.021	0.023	0.021	0.022	0.026	0.026	0.029	0.023	0.031	0.005	0.003	0.008	0.008
MON	0.049	0.049	0.052	0.048	0.039	0.049	0.049	0.034	0.034	0.027	0.043	0.035	0.004	0.007	0.001	0.001
YUN	0.009	0.008	0.010	0.009	0.017	0.009	0.010	0.023	0.023	0.030	0.015	0.023	0.048	0.010	0.001	0.001
KBL	0.004	0.005	0.003	0.012	0.003	0.005	0.019	0.019	0.027	0.010	0.020	0.046	0.007	0.004	0.004	0.001
BRI	0.005	0.006	0.005	0.014	0.005	0.006	0.021	0.028	0.012	0.021	0.021	0.048	0.009	0.005	0.004	0.005

Bold values represent measurements within each population

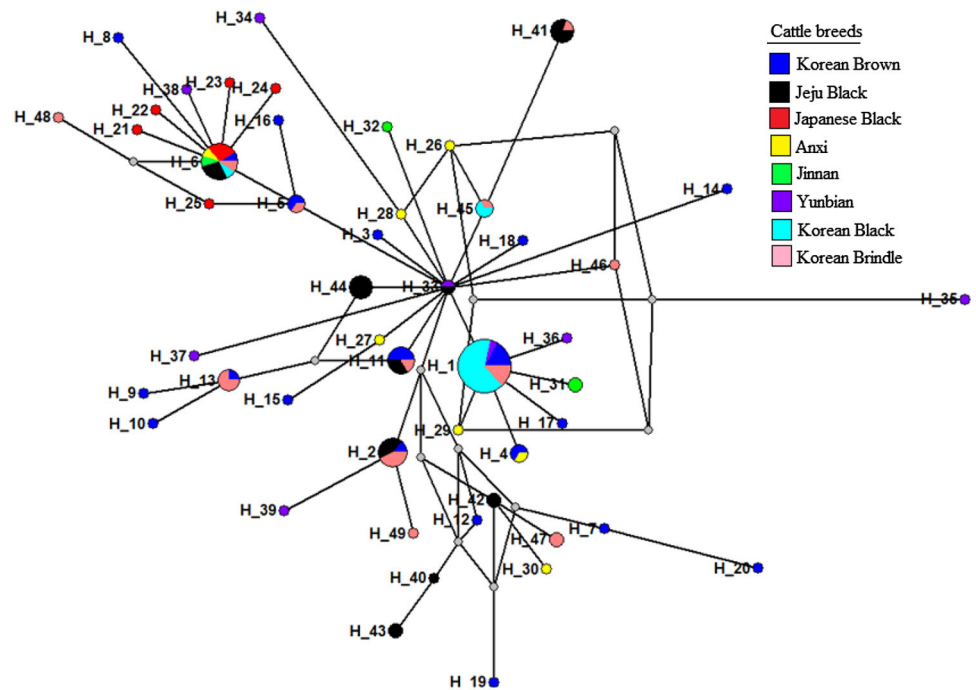
KB Korean Brown, JB Jeju Black, JAB Japanese Black, ANX Anxi, SAN Sanjiang, JIN Jinnan, MONT Montbeliard, NAN Nanyang, JIA Jiaxian, HUA Huangpi, ZAO Zaobei, WAN Wannan, MON Mongolian, YUN Yunbian, KBL Korean Black, BRI Korean Brindle

The genetic distance detected among the Korean cattle populations was the lowest as expected. Generally, apart from their phenotypic heterogeneity, the North East Asian cattle populations are closely related in their matrilineal genetic ancestry.

The median-joining network for mtDNA sequences of eight cattle populations from different regions is presented

in Fig. 1. The network analysis lack resolution when we include all breeds in the network analysis, thus we select different populations from different regions to see the haplotype relationships. The Network analysis depicted possible ancestral haplotype (H_33) within the T3 lineage defined by a transition mutation A–G of Bovine Reference Sequence (BRS) for cattle. Haplotype H_33 and BRS belong to a T3

Fig. 1 The median-joining networks for mtDNA sequences of eight cattle populations. The haplotype frequency is proportional to the area of its circle and the length of the branch is approximate. Median vectors (mv) are denoted by small gray circle



sub-lineage (T3b) that is defined by a transition at nucleotide position 169. It is the oldest lineage in the cattle samples and root of the genealogy. This ancestral haplotype was found in Jeju Black and Yunbian cattle. Haplotype H6 seems common haplotype that have been identified in five cattle breeds, except Yunbian cattle. In the median-joining analysis, 10 median vectors were observed which represented unsampled sequences or extinct ancestral haplotype in the cattle populations studied.

Discussion

The North East Asian extant cattle are presumably descendants of the Near East *Bos primigenius* populations. In previous study, independent mtDNA was also reported tracing separate domestication of an East Eurasian strain of *B. primigenius* in this region (Mannen et al. 2004). The origin, evolutionary history and phylogenetic relationships were well resolved for East Asian cattle (Kim et al. 2003; Mannen et al. 2004; Sasazaki et al. 2006). However, more genetic data are still needed to make informed decision-making for conservation and breed improvement programs.

To elucidate the magnitude of genetic divergences which exists between populations, we estimated evolutionary divergence over sequence pairs for inter- and intra-populations. Generally, inter- and intra-population genetic distances of East Asian cattle populations were minimal. This is not surprising in a sense that these populations were descended from a common ancestor. Another reason for

low genetic differences is that under traditional breeding practices gene flow via breeding female among the populations could appreciably further homogenize the populations. The genetic distance estimates observed in this study is not much different from the regional pair-wise genetic distances previously reported (Mannen et al. 1998; Kim et al. 2003).

The Median-joining network analysis revealed that H-33 represented the possible ancestral Haplotype within T3 *B. taurus* main lineage. The indigenous Jeju Black cattle are characterized by its black fur, a breed unique to Jeju Island and have been raised exclusively on the island since the prehistoric times (MIFAFF, <http://english.mifaff.go.kr/main.jsp>). Considering the prehistoric information which states the earliest domestic bovine found in North East Asia dated to 5,000 years before present (Payne and Hodges 1997) and this molecular data, we hypothesized that the Jeju cattle may represent one of the original native Korean or North East Asian cattle. In general, the low levels of genetic distances observed between the cattle populations suggest that all East Asian cattle are descendants of the Near East *B. primigenius* population.

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