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Genetic characterization of Northeast Asian cattle based on sequence polymorphisms in the complete mitochondrial genome

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ABSTRACT

In this study, we analyzed complete mtDNA sequences variation and genetic relationship among taurine, indicine and *Bison* groups. In total, 107 sequences from different breeds, 45 European (45 Italian), 16 Middle East Asian (seven Iranian and nine Iraqi), 41 Northeast Asian (34 Korean and seven Japanese), two Nellore (*Bos indicus*) and two *American Bison bison* (*Ame. bison*) were obtained from Gen-Bank database. One Korean Hanwoo (*Bos taurus*) sequence was generated using the SOLiD™ System. In total, 1370 polymorphic sites, representing 8.39% of the complete 107 mtDNA sequences (16,338 bp) were detected and of these, 1186 parsimony informative polymorphic sites were identified. Neighbor-joining tree indicated that Korean, Japanese, Iranian, Iraqi, and Italian cattle were closely related to one another, but are separated from *B. bison*. The *B. taurus* mtDNA polymorphism was greater in the D-loop than in the other regions. The ATP8, ND3, ND5, and ND6 regions were also quite parsimony informative, similar to *Cyt b*. In addition, this study revealed a distinct genetic difference between Korean cattle and *B. indicus*.

Keywords: *Bos taurus*; Complete Mitochondrial DNA Genome; Phylogeny; Polymorphism

1. INTRODUCTION

Cattle are distributed worldwide and are extremely important domestic animals in Korea and other parts of the world. Due to their high economic and social values, this species has received significant attention in evolutionary and genetic diversity studies for a long time. However, there is still incomplete understanding of their association with early human civilization, and research on their origins may enlighten us on hitherto unknown aspects of prehistory.

In Northeast Asia (Mongolia, North China, Korea, and Japan) most cattle lack humps and are classified as *Bos taurus* [1]. Kim and Lee [2] reported that Korean cattle originated from crossbreeding between European *Bos primigenius* and Indian *Bos indicus*. These cattle subsequently moved south and east to become the ancestors of modern-day Japanese cattle, and interactions might have occurred between Japanese cattle and the cattle of other Northeast Asian countries as described by Kim *et al.* [3]. Moreover, over several decades introgression of several European breeds into East Asian cattle as well as interbreeding among regional breeds has occurred for upgrading native breeds in many countries of Northeast Asia [4].

To reveal their phylogenetic relationships based on DNA sequence polymorphisms, several studies have been performed on bovine mitochondrial DNA (mtDNA) [5,6]. Among them, 237 polymorphisms in two complete bovine mtDNA sequences (one *B. taurus*, Simmental, and one *B. indicus*, Dwarf Zebu) and 393 in 36 (34 *B. taurus*, Korean cattle/Japanese Black/Holstein/Fleckvich, and two *B. indicus*, Nellore/Zwergzebu) were reported by Hiendleder *et al*. [5] and Kim *et al*. [6], respectively. However, the large-scale identification of polymerphisms in complete mtDNA sequences for regional cattle breeds has not been reported. Here, we present the analysis of a large-scale data set of complete mtDNA sequences from various cattle breeds. We used complete mtDNA sequences to examine the phylogenetic rela-

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tionships and sequences polymorphism among Northeast Asian cattle as well as various other regional cattle breeds.

2. MATERIALS AND METHODS

2.1. Sampling and Sequencing of Hanwoo Proven Bull mtDNA

Blood sample of Hanwoo proven bull 27,223 was obtained from the Hanwoo Experiment Station of the National Institute of Animal Science, South Korea. Genomic DNA was extracted from the blood with a QIAamp DNA Blood Maxi kit according to the manufacturer's instructions (Qiagen, Valencia, CA, USA). The DNA was fragmented with a Covaris S2 and HydroShear (Genomic Solutions, Chelmsford, MA, USA) using the proper settings for the targeted sizes. Libraries were prepared according to the SOLiD™ System Mate-paired Library Preparation protocol for the Applied Biosystems SOLiD™ System: Library Preparation Guide (02/2009 and 10/2009 editions; Applied Biosystems, Foster City, CA, USA). Four paired-end libraries with three different size ranges (600 - 700 bp, 1 - 2 kb, and 0.6 - 2.2 kb) were constructed. Sequencing was performed according to the Applied Biosystems SOLiD™ system. Templated beads were deposited onto two full-scale slides per library, and sequencing was conducted on 50 bases using SOLiD v3.0 chemistry, with one exception: the library prepared from the 0.6 - 2.2 kb DNA fragments was used for four full-scale slides with sequencing performed on 50 bases using SOLiD v3 plus chemistry. The UMD3 mitochondrial DNA sequence (ftp://ftp.cbcb.umd.edu/pub/data/Bos_taurus/Bos_taurus_UMD_3.0/, NC 006853) was used as a reference.

2.2. Data Analysis

Complete *B. taurus* mtDNA sequences for 45 European (45 Italian), 16 Middle East Asian (seven Iranian and nine Iraqi), and 41 Northeast Asian (34 Korean and seven Japanese) cattle previously deposited into GenBank were used. A hundred and three *B. taurus*, two *B. indicus* (Nellore), and two *Ame. bison* (outgroup) *complete mtDNA* sequences were aligned using ClustalW version 1.8.3. [7] as implemented in MEGA 5.03 [8]. All positions containing gaps and missing data were eliminated using the complete-deletion option [8,9]. For the *B. taurus* mtDNA sequences, the nucleotide composition, pattern of nucleotide substitutions, and site variations were examined using MEGA5.03 [9]. A phylogenetic analysis was also performed using MEGA5.03. A neighbor-joining tree [10] was constructed using 107 sequences based on Tamura-Nei distances. The statistical confidence of each node in the neighbor-joining tree topology [10] was estimated using 1000 random bootstrap replications. The NIAS_BT_Hanwoo mtDNA that was newly sequenced in this study has been deposited into GenBank under Accession No. HQ025805.

3. RESULTS AND DISCUSSION

3.1. Nucleotide Composition and Sequence Variation

An analysis of the 103 *B. taurus* complete mtDNA sequences displayed 621 polymorphic sites representing 3.8% of the mtDNA genome. Similarly, four complete mtDNA sequences (two *B. indicus* and two *Ame. bison*) revealed 1370 polymorphic sites, accounted for 8.3% of the complete mtDNA sequences. From these polymerphic site comparisons, 383 (*B. taurus*) and 1186 (*B. indicus* and *Ame. bison*) parsimony informative polymerphic sites were identified, respectively. The average nucleotide frequencies of A, T, C, and G were 33.4%, 27.2%, 13.4% and 26.0% for most *B. taurus* cattle, respectively (**Table 1**). In **Table 1**, each entry shows the probability of an instantaneous substitution from one base (row) to another (column). When only entries within a row are compared, rates of different transitional substitutions are shown in bold, while those of transversional substitutions are shown in italics. The ratios of transition/transversion mutation rate were k_1 = 34.14 (purines) and k_2 = 34.71 (pyrimidines) for the *B. taurus* mtDNA sequences, and k_1 = 33.66 (purines) and k_2 = 30.71 (pyrimidines) for the Korean *B. taurus* sequences. The overall transition/transversion bias (R) was 15.99 for the *B. taurus* mtDNA sequences and 14.77 for the Korean *B. taurus* mtDNA sequences, with

$$R = \left[A*G*k_1 + T*C*k_2\right] \Big/ \left[\left(A+G\right)*\left(T+C\right)\right].$$

These results indicate that the transition bias for *B. taurus* mtDNA genomic sequences was a bit higher than for the Korean *B. taurus* sequences. While for other breeds, the transition bias for *B. taurus* mtDNA genomic sequences was a little bit lower than for the each of *B. taurus* breed sequences as shown in **Table 1**. The strong transitional bias detected in this study accorded with previous studies on Mesolithic Wild Aurochs (*Bos primigenius*) [11] and the ancient and modern bovine populations [12]. **Table 2** shows the number of polymorphic sites for different mtDNA regions and regional variation among the 103 *B. taurus* mtDNA sequences. The D-loop (mtDNA control region) and cytochrome *b* (*Cyt b*) gene regions contain abundant phylogenetic information and are appeared to be good markers for studying genetic differentiation and phylogenetic relationships among species within the same genus or family [3,13-16]. Of 922 (D-loop) and 1140 (*Cyt b*) DNA sequences in the 103 individual *B.*

Table 1. Nucleotide composition and maximum composite likelihood estimate of the pattern of nucleotide substitution in the complete *B. taurus* mtDNA sequence.

Nucleotide	mtDNA of *Bos taurus* cattle breeds, excluding Korean cattle breeds					mtDNA of Korean cattle breeds, including the NIAS-BT_Hanwoo sequence				
	Ave. composition	A	T	C	G	Ave. composition	A	T	C	G
A	33.42%	-	0.75	0.71	**12.59**	33.42%	-	0.8	0.76	**13.27**
T	27.19%	0.92	-	**24.72**	0.37	27.19%	0.98	-	**23.37**	0.39
C	13.44%	0.92	**25.9**	-	0.37	13.44%	0.98	**24.49**	-	0.39
G	25.95%	**31.3**	0.75	0.71	-	25.95%	**32.99**	0.8	0.76	-
	k_1 = 34.137, k_2 = 34.712, R = 15.994					k_1 = 33.657, k_2 = 30.712, R = 14.774				

Nucleotide	mtDNA of *Bos taurus* cattle breeds, excluding Japan cattle breeds					mtDNA of Japan *Bos taurus* cattle breeds				
	Ave. composition	A	T	C	G	Ave. composition	A	T	C	G
A	33.42%	-	0.7	0.67	**12.74**	33.41%	-	0.03	0.03	**11.28**
T	27.19%	0.86	-	**24.65**	0.34	27.20%	0.04	-	**29.53**	0.02
C	13.44%	0.86	**25.82**	-	0.34	13.46%	0.04	**30.96**	-	0.02
G	25.95%	**31.66**	0.7	0.67	-	25.93%	**28**	0.03	0.03	-
	k_1 = 36.937, k_2 = 37.018, R = 17.153					k_1 = 736.053, k_2 = 1000, R = 416.135				

Nucleotide	mtDNA of *Bos taurus* cattle breeds, excluding Iraq cattle breeds					mtDNA of Iraq *Bos taurus* cattle breeds				
	Ave. composition	A	T	C	G	Ave. composition	A	T	C	G
A	33.42%	-	0.79	0.75	**12.69**	33.42%	-	0.66	0.63	**12.49**
T	27.20%	0.97	-	**24.39**	0.39	27.18%	0.81	-	**25.22**	0.33
C	13.44%	0.97	**25.57**	-	0.39	13.44%	0.81	**26.4**	-	0.33
G	25.94%	**31.56**	0.79	0.75	-	25.96%	**31.04**	0.66	0.63	-
	k_1 = 32.626, k_2 = 32.485, R = 15.09					k_1 = 38.238, k_2 = 39.993, R = 18.231				

Nucleotide	mtDNA of *Bos taurus* cattle breeds, excluding Iran cattle breeds					mtDNA of Iran *Bos taurus* cattle breeds				
	Ave. composition	A	T	C	G	Ave. composition	A	T	C	G
A	33.42%	-	0.79	0.75	**12.61**	33.42%	-	0.03	0.03	**11.28**
T	27.19%	0.97	-	**24.52**	0.39	27.18%	0.04	-	**29.53**	0.02
C	13.44%	0.97	**25.71**	-	0.39	13.44%	0.04	**30.96**	-	0.02
G	25.94%	**31.34**	0.79	0.75	-	25.96%	**28**	0.03	0.03	-
	k_1 = 32.249, k_2 = 32.504, R = 15.027					k_1 = 56.81, k_2 = 56.206, R = 26.174				

Nucleotide	mtDNA of *Bos taurus* cattle breeds, excluding Italy cattle breeds					mtDNA of Italy *Bos taurus* cattle breeds				
	Ave. composition	A	T	C	G	Ave. composition	A	T	C	G
A	33.42%	-	0.76	0.72	**12.76**	33.42%	-	0.66	0.63	**12.49**
T	27.19%	0.93	-	**24.38**	0.38	27.19%	0.81	-	**25.22**	0.33
C	13.44%	0.93	**25.56**	-	0.38	13.44%	0.81	**26.4**	-	0.33
G	25.94%	**31.72**	0.76	0.72	-	25.95%	**31.04**	0.66	0.63	-
	k_1 = 34.001, k_2 = 33.666, R = 15.673					k_1 = 35.996, k_2 = 36.076, R = 16.716				

Bold letters: rates of different transitional substitutions. Italic letters: rates of different transversional substitutions.

Table 2. Sequence variation of *Bos taurus* mtDNA complete genome sequences and variation for each product region and geographical region.

	Position (aligned position)	Sequence length	Total variation (parsimony informative sites variation)	Sequence variation					
				B. taurus (103)	Italy (45)	Iran (7)	Iraq (9)	Korea (35)	Japan (7)
	1-16338 (1-16358)	16,358	525 (3.21%) [347 (2.12%)]	525	264	287	286	178	23
D-loop	15792-16338, 1-363 (15810-16358, 1-367)	922	105 (11.39%) [77 (8.35%)]	105	64	54	54	45	10
s-rRNA	431-1385 (437-1393)	957	18 (1.88%) [7 (0.73%)]	18	6	6	8	4	0
l-rRNA	1453-3023 (1461-3037)	1577	39 (2.47%) [21 (1.33%)]	39	11	19	18	7	1
ND1	3101-4056 (3115-4070)	957	36 (3.76%) [25 (2.61%)]	36	10	22	20	12	0
ND2	4266-5307 (4280-5321)	1044	33 (3.16%) [17 (1.63%)]	33	10	13	13	10	1
COX1	5687-7231 (5703-7247)	1545	47 (3.04%) [30 (1.94%)]	47	22	22	18	13	2
COX2	7374-8057 (7392-8075)	684	19 (2.78%) [10 (1.46%)]	19	7	6	5	8	0
ATP8	8129-8329 (8147-8347)	201	11 (5.47%) [6 (2.99%)]	11	2	6	7	3	0
ATP6	8290-8970 (8308-8988)	681	29 (4.26%) [14 (2.06%)]	29	13	9	10	7	1
COX3	8970-9750 (8988-9768)	784	23 (2.93%) [13 (1.66%)]	23	9	9	10	5	0
ND3	9823-10168 (9841-10186)	357	13 (3.64%) [10 (2.80%)]	13	2	8	9	2	0
ND4L	10239-10535 (10257-10553)	297	8 (2.69%) [4 (1.35%)]	8	2	4	6	0	1
ND4	10529-11906 (10547-11924)	1441	50 (3.47%) [32 (2.22%)]	50	21	25	20	15	2
ND5	12109-13929 (12127-13947)	1821	85 (4.67%) [53 (2.91%)]	85	43	40	41	19	2
ND6	13913-14440 (13931-14458)	528	24 (4.55%) [14 (2.65%)]	24	8	11	12	5	2
CYTB	14514-15653 (14532-15671)	1140	52 (4.56%) [30 (2.63%)]	52	25	20	19	10	2

taurus mtDNA, 105 (11.39% in the D-loop) and 52 (4.56% in *Cyt b*) variable sites were observed, of which 77 and 30 were parsimony informative polymorphic sites, respectively (**Table 2**). Generally, the *B. taurus* mtDNA polymorphic sites were denser in the D-loop than in the other regions. Moreover, this region seemed to be more parsimony informative. The ATP8, ND3, ND5, and ND6 regions were also quite parsimony informative, similar to *Cyt b*. In total, 178 variable sites in the 34 Korean *B. taurus* mtDNA sequences and NIAS_BT_Hanwoo sequence were detected (**Table 3**). These SNPs reported in this study could be used as valuable markers for maternal lineage test.

3.2. Phylogenetic Analysis

The average genetic distance was calculated based on the Tamura-Nei pairwise distance (**Table 4**). The distance between *Ame. bison* and the other genus *Bos* populations (Korean, Japanese, Iraqi, Iranian, Italian, and *B. indicus*) was >0.06. Furthermore, the distance between *B. indicus* and the other *B. taurus* populations was >0.01. However, the average inter-population distance among the *B. taurus* population was ranged from 0.001 to 0.005, while the average intra-population distance was ranged from 0.001 to 0.006. Interestingly, the average distance for the Iraqi intra-population was slightly greater than that for the inter-population, and there was not much difference between the average distances for the intra- and inter-populations with the other cattle populations. From these results, we infer that some Iraqi *B. taurus* cattle originated from other regional cattle populations, and that *B. taurus* cattle populations are closely related based on their mitochondrial genome. All *B. taurus* populations had similar genetic distances from *B. indicus* (0.012 -

Table 3. Sequence variation between NIAS_BT_Hanwoo and each population.

	Korea	Japan	Iraq	Iran	Italy
No. of sequence variations	178	36	299	301	276
No. of parsimony informative sites	51	9	233	11	166
Seq number	34	7	9	7	45
If 100 seq, No. of normalized PI sites	91.18	128.57	2588.89	157.14	368.89

Table 4. Tamura-Nei pairwise distances within and between cattle populations, including *Ame. bison*.

Population	[1]	[2]	[3]	[4]	[5]	[6]	[7]	No. of samples
[1] Korean	0.001	0.000	0.000	0.000	0.000	0.001	0.002	35
[2] Japanese	0.001	0.001	0.000	0.000	0.000	0.001	0.002	7
[3] Iraqi	0.004	0.004	0.006	0.000	0.000	0.001	0.002	9
[4] Iranian	0.003	0.003	0.005	0.005	0.000	0.001	0.002	7
[5] Italian	0.002	0.002	0.005	0.004	0.003	0.001	0.002	45
[6] *B. indicus*	0.015	0.015	0.012	0.014	0.015	0.000	0.002	2
[7] *B. bison*	0.062	0.063	0.062	0.063	0.062	0.062	0.000	2

Note: on diagonal: intrapopulation distance. Below diagonal: interpopulation distance. Above diagonal: standard error of interpopulat.

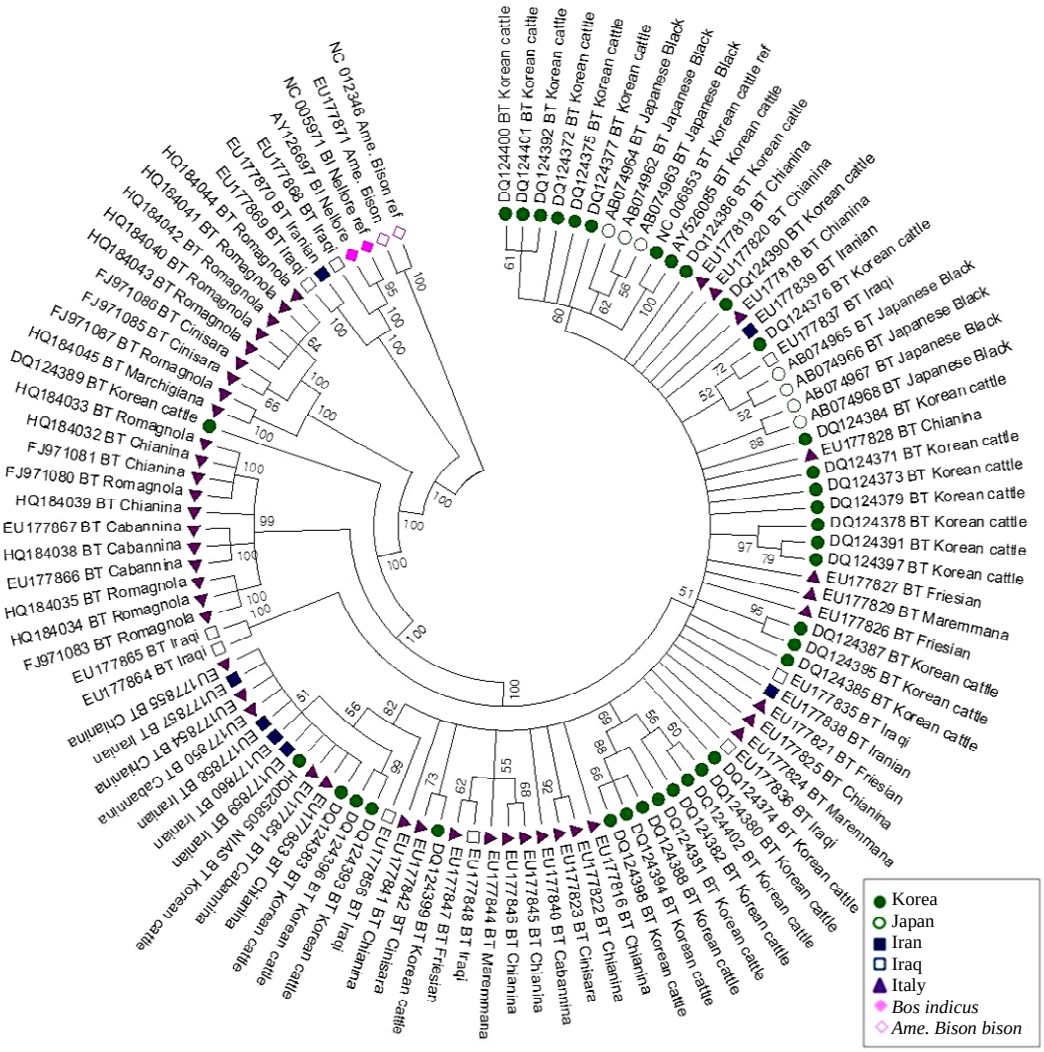

Figure 1. Neighbor-Joining tree of 107 cattle mtDNA complete genome sequences constructed from *Bos taurus* (Korean cattle, Japanese cattle, Iranian cattle, Iraqi cattle, and Italian cattle), *Bos indicus* Nellore, and *Ame. bison* (outgroup) on the basis of Tamura-Nei distances with 1000 bootstrapping.

Table 5. Comparison of polymorphism between *Bos indicus* and each population in *Bos taurus*.

	No. of variable sites (*)	No. of parsimony informative variable sites	No. of singleton sites (*)
Korea	372 (2.27%)	278 (1.70%)	94 (0.57%)
Japan	264 (1.61%)	252 (1.54%)	12 (0.07%)
Iraq	289 (1.77%)	248 (1.52%)	41 (0.25%)
Iran	303 (1.85%)	246 (1.50%)	57 (0.35%)
Italy	431 (2.63%)	338 (2.07%)	93 (0.57%)
Middle East Asia	338 (2.07%)	260 (1.59%)	78 (0.48%)
Northeast Asia	383 (2.34%)	285 (1.74%)	98 (0.60%)

Note: *the percentage for mtDNA complete genome sequence.

0.015) and *Ame. bison* (0.062 - 0.063).

Complete mtDNA sequences from *B. taurus*, *B. indicus*, and *Ame. bison*, together with a Korean cattl mtDNA sequence that was determined newly in this study were used for phylogenetic analysis. The neighbor-joining tree (**Figure 1**) based on Tamura-Nei distances with 1000 bootstrap values demonstrates that the *B. taurus* populations including Northeast Asian (Korean and Japanese), Middle East Asian (Iranian and Iraqi), and European (Italian) cattle were closely related, but that they were separated from *Ame. bison*. Among *B. taurus* populations, two of nine the Iraqi cattle appear to be more closely related to *B. indicus*. We also compared the polymorphism from each population of *B. taurus* respective to *B. indicus* (**Table 5**). If they show any trace of crossbreeding, the inter-population distance is likely close to zero or the number of singleton sites among them will be plentiful, even though there are several variations. As shown in **Tables 4** and **5**, *B. indicus* was closer to the Middle East Asian cattle (Iraqi and Iranian cattle) than to the Near East Asian cattle (Korean and Japanese cattle).

Because we used only two complete *B. indicus* mtDNA sequences, genetic distance and phylogenetic analyses may be insufficient. However, our results reveal a signature for distinctness between Korean cattle and *B. indicus* based on genetic difference (Tamura-Nei pairwise distance of 0.015).

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