Genetic Relationships of Cattle Breeds Assessed by PCR-RFLP of the Bovine Mitochondrial DNA D-loop Region

Du Hak Yoon[1], Hak Kyo Lee[2], Sung Jung Oh[1], Ki Chang Hong[3], Gwang Joo Jeon[2,*]
Hong Sik Kong[4] and Jun Heon Lee[5]

[1] Division of Animal Genomics and Bioinformatics, National Livestock Research Institute, Suwon 441-350, Korea

ABSTRACT : To investigate the genetic relationships among various cattle breeds, bovine mtDNA D-loop region was used in 411 animals of 18 cattle breeds, including 8 Asian *Bos taurus*, 7 European *Bos taurus*, 1 Asian *Bos indicus*, and 2 African *Bos indicus*. The size of amplified PCR products from mtDNA D-loop region was 964 bp and the products were digested by 15 different restriction enzymes. Two different band patterns were identified in eight restriction enzymes (*Bst*XI, *Hae* III, *Msp* I, *Apa* I, *Taq* I, *Alu* I, *Bam*H I, *Eco*N I) and the rest of restriction enzymes showed more than 3 different band patterns among which *Apo* I and *Msp*R9 resulted in 7 different restriction patterns. The genotypes, number of haplotype, effective number of haplotype, and degree of heterozygosity were analyzed. Based on all the PCR-RFLP data, different haplotypes were constructed and analyzed for calculating genetic distances between these breeds using Nei's unbiased method and constructing a phylogenetic tree. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 10 : 1368-1374)***

Key Words : Bos Taurus, Bos Indicus, Mitochondrial DNA, PCR-RFLP, Genetic Distance

INTRODUCTION

With the fast growth of molecular techniques, evolutionary and phylogenetic studies have been growing dramatically, mainly using the mitochondrial DNA (mtDNA) variations. The mtDNA is maternally inherited and its substitution rate is higher than that of nuclear DNA (Brown et al., 1979; Giles et al., 1980). Also, there are few important proteins that are synthesized from the mtDNA (Hutchison et al., 1974). Because of these characteristics, mtDNA variation was used to identify the genetic relationships between and within species of mammals including human. Also, there is absence of crossovers between mtDNA and therefore the mtDNA is the most efficient material to investigate the genetic diversity of the related groups (Brown et al., 1979)

Anderson et al. (1981) initially determined the human mtDNA sequence of 16,569 bp and found 13 protein coding regions including 12S, 16S rRNAs, 22 tRNAs, Cytochrome c oxidase subunits I, II, and III, ATPase subunit 6, and Cytochrome b. Also they found bovine mtDNA was consisted of 16,338 bp and there was similarity of protein coding regions between human and bovine but low similarity in D-loop region between them (Anderson et al., 1982). Bob et al. (1982) also found the total length of mouse mtDNA to be 16,295 bp, which is similar in size with human mtDNA but the translational start codon was AUN, which means any of A, G, C, and T could be in the third base of the codon.

Regarding domestication of cattle, Kikkawa et al. (1995) insisted the multiple domestication processesoccurred but the theory lacks any supporting evidence. Loftus et al. (1994a, b) also gave a controversial opinion for the domestication of cattle breeds. They reported that the divergence between the Zebu and Taurine breeds was traced back to 200,000 years ago, or possibly a million years ago. Through mtDNA D-loop analysis, Indian Bos indicus was totally different from African Bos taurus (Bradley et al., 1996) indicating two different ancestors. Based on the mtDNA D-loop variation,Troy et al. (2001) indicated that European cattle breeds were totally different from African breeds and showed more similarity with middle east breeds.

Although Kikkawa et al. (1995) and Mannen et al. (1998b) reported phylogenetic relationships of Japanesecattle with other breeds, not much have been published for the origin of Asian cattle. However, Mannen et al. (1998b) reported that the Japanese black cattle are less related with European cattle breeds, indicating that Japanese black cattle are very important population for investigation of the origin of East Asian cattle.

For the evolutionary and phylogenic studies in cattle, many researchers have worked on mtDNA (Watanabe et al., 1985, 1989; Amano et al., 1994; Kikkawa et al., 1995; Jung et al., 1996; Lee et al., 1998; Jung et al., 2002).Loftus et al. (1994a, b) also proposed a theory for cattle domestication which was controversial. However, phylogenetic studies of Hanwoo (Korean cattle) have not been widely conducted

* Corresponding Author: G. J. Jeon. Tel: +82-31-670-5330, Fax: +82-31-675-5331, E-mail: gj_jh@hotmail.com
[2] Department of Genomic Engineering, Genomic Informatics Center, Hankyong National University, Ansung 456-749, Korea.
[3] College of Agricultural and Environmental Science, Korea University, Seoul, Korea.
[4] Animal Products Grading Service, Korea.
[5] Chungnam National University, Korea.
Received October 23, 2004; Accepted April 12, 2005

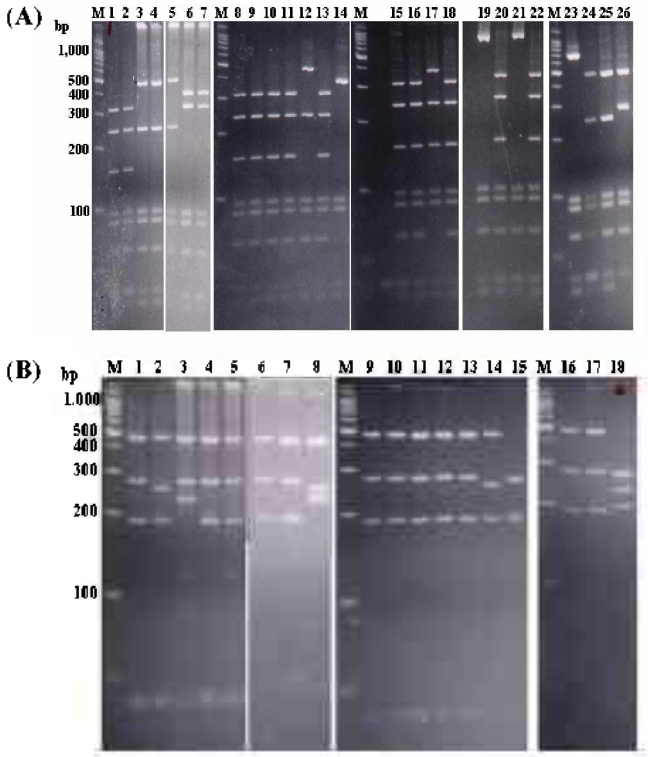

Figure 1. PCR-RFLP patterns of bovine mtDNA D-loop region. M: 100 bp DNA ladder (Promega). (A) mtDNA D-loop/*Apo* I, A haplotype; lane 1, 2, 8, 9, 10, 11, 13, 15, 16, 18, 20, 22 (315, 234, 147, 94, 82, 60, 32 bp), B type; lane 3, 4, 5, 12, 24, 25 (462, 234, 94, 82, 60, 32 bp), C type; lane 6, 7 (381, 315, 94, 82, 60, 32 bp), D type; lane 19, 21, 23 (696, 94, 82, 60, 32 bp), E type; lane 14 (381, 375, 94, 82, 32 bp), F type; lane 17 (375, 234, 147, 94, 82, 32 bp), G type; lane 26 (462, 266, 94, 82, 60 bp). (B) mtDNA D-loop/*Msp*R9 I, A type; lane 1, 4, 5, 6, 7, 9, 10, 11, 12, 13, 16, 17 (460, 275, 187, 42 bp), B type; lane 2, 14 (460, 250, 187, 42, 25 bp), C type; lane 3 (460, 275, 229 bp), D type; lane 8 (460, 250, 229, 25 bp), E type; lane 15 (275, 260, 200, 187, 42 bp), F type; lane 18 (275, 260, 229, 200 bp).

due to lack of experimental sample size. Therefore, the main purpose of our study was to investigate the haplotype frequencies using PCR-RFLP polymorphisms of mtDNA D-loop region and to estimate the origin of Hanwoo by measuring the genetic distances in comparison with other cattle breeds.

MATERIAL AND METHODS

Genomic DNA extraction and PCR amplication of mtDNA D-loop

DNA samples were extracted from blood or semen by some modification of the Miller et al. (1988) and Sambrook et al. (1989). We examined 411 individuals from 18 cattle breeds: 8 Asian cattle breeds: Korean cattle (Hanwoo), Korean Black cattle, Japanese Black cattle, Japanese Brown cattle, Chinese Yanbian cattle, Chinese Luxi cattle, Chinese Nanyang cattle and Cheju mixed breeds (Hanwoo, Brahman and Charolais), 7 European cattle; Angus, Charolais, Simmental, Hereford, Holstein Friesian, Limousin and Brown Swiss, 1 Indian cattle breed: Sahiwal and, 2 African cattle breeds: Kavirondo zebu and White Fulani. The D-Loop region of mtDNA was amplified using PCR with the following primers: Forward, 5'-CCCAAAGCTGAAGTTC TATT-3' (15758-15777 nucleotides in the sequences on Anderson et al. (1982: GenBank Accession. No. V00654, J01394). Reverse, 5'-TTGGGTTAAGCTACATCAAC-3' (364-383 nucleotides).

The Polymerase Chain Reaction was conducted in 50 μl volumes, each containing 100 ng of genomic DNA, 10× PCR buffer (100 mM Tris pH 8.9, 50 mM KCl, 15 mM MgCl, 0.01% gelatin, 0.1% Triton X-100, 10 mg/ml BSA), 10 pmole of each primer, 200 μM of dNTPs and 1 unit Taq DNA polymerase (Promega, USA). The cyclic condition of PCR includes a first denaturation step of 5 min at 94°C followed by 30 cycles, each consisting of 1 min at 94°C, 2 min at 56°C, and 90 second at 72°C and then, a final extension step of 10 min at 72°C using PTC 200 peltier thermal cycler (MJ Research, USA).

Restriction endonuclease digestion and gel electrophoresis

Fifteen restriction endonucleases (Hsp92 II, BstX I, Hae III, Mbo I, Msp I, Sau96 I, Apa I, Taq I, Alu I, Dde I, Hinf I, BamH I, Apo I, MspR9 I, EcoN I) were used for this study. After the PCR experiments, each products were completely digested in a total volume of 15 μl including 3 μl of the PCR products, 2 units of each endonucleases and the appropriate buffers for 3-6 h at the recommended digestion temperature. The resultants of digestion were electrophoresed in a 3% Metaphore agarose gel or 5% Polyacrylamide gel, and then visualized with ethidium bromide for the genotyping of each sample.

Phylogenetic analysis

Using the records of genotypes of individuals from various breeds, POPGENE package (Yeh FC et al., 1997) was used to analyze the haplotype frequency at different loci for the various breeds and genetic diversity was determined. The genetic distance among the breeds was calculated by the method of Nei (1978). Based on the calculated genetic distance, phylogenetic and molecular evolutionary analyses were conducted using MEGA version 2.1 (Kumar et al., 2001).

RESULTS AND DISCUSSION

PCR-RFLP of bovine mtDNA D-loop region

A 964 bp of mtDNA D-loop region was amplified by PCR analysis. Fifteen different restriction enzymes were used in this study for identifying different restriction enzyme digestion patterns (Figure 1). Haplotype frequencies

Table 1. Haplotype frequencies of each restriction enzyme by the multiple-population descriptive statistics with 18 cattle breeds

Enzyme haplotype	*Hsp*92 II	*Bst*X I	*Hae* III	*Mbo* I	*Msp* I	*Sau*96 I	*Apa* I	*Taq* I
A	0.195	0.975	0.979	0.923	0.691	0.833	0.784	0.997
B	0.730	0.025	0.021	0.070	0.309	0.148	0.216	0.003
C	0.010			0.007		0.014		
D	0.063					0.006		
E	0.002							
Enzyme haplotype	*Alu* I	*Dde* I	*Hinf* I	*Bam*H I	*Apo* I	*Msp*R9 I	*Eco*N I	
A	0.988	0.688	0.960	0.271	0.668	0.617	0.917	
B	0.012	0.216	0.011	0.729	0.236	0.298	0.084	
C		0.065	0.014		0.016	0.019		
D		0.031	0.006		0.019	0.009		
E			0.009		0.054	0.045		
F					0.005	0.009		
G					0.002	0.002		

Table 2. Genetic variation of each population

Breeds	N	Na*	Ne[1]	H[2]	Polymorphic loci (%)
Asian Bos taurus					
Hanwoo	29	2.067	1.385	0.224	73.3
Korean black cattle	20	1.800	1.459	0.245	66.7
Japanese black cattle	24	1.600	1.311	0.163	46.7
Japanese brown cattle	11	1.867	1.299	0.175	66.7
Yanbian	25	2.200	1.369	0.219	80.0
Luxi	21	2.000	1.713	0.292	60.0
Nanyang	10	1.533	1.275	0.148	46.7
CBK	20	2.000	1.385	0.215	66.7
European Bos taurus					
Angus	24	1.867	1.237	0.157	66.7
Brown Swiss	29	1.400	1.218	0.118	40.0
Charolais	23	1.867	1.334	0.212	73.3
Simmental	29	1.600	1.173	0.113	53.3
Hereford	18	1.786	1.311	0.186	60.0
Holstein	23	1.867	1.356	0.193	60.0
Limousin	28	1.533	1.152	0.102	46.7
Asian Bos indicus					
Sahiwal	20	2.000	1.223	0.148	66.7
African Bos indicus					
Kavirondo Zebu	22	1.800	1.265	0.138	60.0
White Fulani	20	1.600	1.260	0.136	40.0

* Na = Observed number of haplotypes. [1]Ne = Effective number of haplotypes. [2]H = Nei's (1973) gene diversity.

for different restriction enzymes from a total of 411 animals belonging to 18 different breeds were presented in Table 1.

Two different restriction enzyme digestion patterns were identified in eight restriction enzymes including BstXI, Hae III, Msp I, Apa I, Taq I, Alu I, BamHI, and EcoNI and the rest of restriction enzymes showed more than 3 different digestion patterns among which Apo I and MspR9 resulted in 7 different digestion patterns. These results indicate that more restriction enzyme digestion patterns were identified in this study compared with the published results by Jung et al. (1996) and Lee et al. (1998).

Variation of mtDNA D-loop region

Based on the PCR-RFLP analysis of mtDNA D-loop region, observed number of haplotypes (Na), effective number of haplotypes (Ne), heterozygosities (H) were calculated (Table 2). The percentages of polymorphic loci, which show polymorphism within breed, were also calculated. For the calculated Ne, Limousin has the lowest value as 1.152 and Luxi has the highest value as 1.713. The H value and percentages of polymorphic loci were relatively high in Asian breeds as compared to European and Bos indicus breeds. These high H value and percentages of polymorphic loci were explained by Watanabe et al. (1985). They indicate that Japanese black cattle and Japanese Shorthorn breeds have higher within breed variations because they are crossed with European breeds such as Simmental, Ayrshire, Devon, Brown Swiss, and Shorthorn. These results also supported by the mtDNA D-loop sequence analysis among breeds (Jung et al., 2002).

Table 3. Summary of the statistical analysis on the genetic variation for all loci among 411 individuals of 18 cattle breeds

Locus	Na*	Ne[1]	Ht	Hs	Gst	Nm[2]
*Hsp*92 II	5	1.739	0.447	0.334	0.252	1.482
*Bst*X I	2	1.051	0.051	0.048	0.046	10.357
Hae III	2	1.042	0.048	0.045	0.066	7.052
Mbo I	3	1.167	0.179	0.107	0.403	0.741
Msp I	2	1.745	0.443	0.337	0.239	1.595
*Sau*96 I	4	1.397	0.269	0.189	0.296	1.189
Apa I	2	1.513	0.332	0.200	0.398	0.757
Taq I	2	1.005	0.004	0.004	0.040	12.139
Alu I	2	1.025	0.024	0.022	0.076	6.088
Dde I	4	1.903	0.535	0.351	0.345	0.950
Hinf I	5	1.084	0.074	0.068	0.083	5.511
*Bam*H I	2	1.654	0.428	0.107	0.751	0.166
Apo I	7	1.977	0.529	0.276	0.479	0.543
*Msp*R9 I	7	2.116	0.530	0.433	0.184	2.218
*Eco*N I	2	1.181	0.193	0.098	0.493	0.514
Mean	3.400	1.440	0.273	0.175	0.359	0.892

* Na = Observed number of haplotypes. [1]Ne = Effective number of haplotyes. [2]Nm = estimate of gene flow from Gst. e.g., Nm = 0.5(1-Gst)/Gst.

Table 4. Standard Genetic distance by Nei's unbiased measures (Nei, 1978) among 19 cattle breeds

	HW	KBC	WAG	JBr	YAN	LUXI	NAN	CBK	AG	BS	CHA	SIM	HF	Hol	LM	SA	KZ
KBC	0.0203																
WAG	0.0627	0.0499															
JBr	0.1475	0.0925	0.1234														
YAN	0.0213	0.0231	0.0553	0.1031													
LUXI	0.1180	0.1637	0.2216	0.3377	0.1536												
NAN	0.2742	0.2738	0.3553	0.5053	0.2370	0.0964											
CBK	0.0150	0.0563	0.0847	0.2204	0.0762	0.1027	0.3085										
AG	0.0826	0.0677	0.1367	0.0685	0.0347	0.2722	0.3743	0.1686									
BS	0.0682	0.0428	0.0607	0.1311	0.0427	0.1922	0.3150	0.0960	0.0955								
CHA	0.0672	0.0599	0.1193	0.0716	0.0349	0.2359	0.3965	0.1299	0.0075	0.0661							
SIM	0.0500	0.0488	0.0555	0.1305	0.0248	0.1663	0.2904	0.0840	0.0842	0.0080	0.0586						
HF	0.0954	0.0856	0.0622	0.1308	0.0760	0.2412	0.3964	0.1212	0.1092	0.0424	0.0774	0.0478					
Hol	0.1543	0.1055	0.1151	0.0695	0.0984	0.3324	0.4623	0.2301	0.0522	0.1264	0.0524	0.1374	0.0982				
LM	0.0578	0.0594	0.0682	0.1842	0.0275	0.1870	0.2694	0.0990	0.0869	0.0171	0.0682	0.0112	0.0488	0.1416			
SA	0.0579	0.1174	0.1673	0.2843	0.0938	0.1350	0.2750	0.0880	0.1876	0.1693	0.1786	0.1265	0.1989	0.3198	0.1296		
KZ	0.0567	0.1077	0.1429	0.2621	0.0804	0.1445	0.2774	0.0873	0.1693	0.1557	0.1633	0.1108	0.1829	0.2957	0.1150	0.0025	
WF	0.0655	0.1031	0.1376	0.2693	0.0728	0.1715	0.2603	0.1083	0.1580	0.1433	0.1615	0.1075	0.1723	0.2813	0.0968	0.0111	0.0046

* *Asian Bos taurus:* HW; Hanwoo, KBC; Korean black cattle or Jeju black cattle, KPN; Korean proven bull, Wag; Japanese black Wagyu, JBr; Japanese brown Wagyu, CBK: Charolais–Brahman–Hanwoo, *Chinese yellow cattle:* YAN; Yanbian cattle, NAN: Nanyang cattle, Luxi: Luxi cattle, *European Bos taurus:* AG; Angus, BS; Brown Swiss, CHA: Charolais, SIM: Simmental, HF: Hereford, Hol: Holstein, LM: Limousin, *Asian Bos indicus:* SA: Sahiwal, *African Bos indicus:* KZ: Kavirondo Zebu, WF; White Fulnani.

They explained that the genetic variations in Hanwoo and Japanese black cattle were higher than other European breeds and the reason for the high mtDNA variation in Hanwoo is the introgression of other foreign breeds during past breeding processes. Therefore we assume that Asian breeds have more genetic variation in maternal lines. This also indicates the diverse genetic background in Asian breeds than European and Bos indicus breeds. Within the Asian breeds, Hanwoo and Yanbian cattle shows high percentages of polymorphic loci, indicating that these two breeds have diverse genetic background in maternal lines.

Table 3 shows the genetic variations in each locus for the 18 breeds used in this study. Results shows the lowest Ne value, 1.005, was observed in TaqI locus and the highest value, 2.116, was observed in MspR9I locus. Similar trends were observed in Ht values. Gst value, indicating the level of genetic divergency, was calculated. Form the Gst value, indirect gene flow (Nm) in each generation was presented. The result indicates the locus that has small number of haplotypes were largely affect for the gene flow.

Genetic relationships among breeds based on the D-loop polymorphism

Based on the RFLP haplotype results, genetic distances among cattle breeds were calculated using Nei's unbiased measurement of POPGENE software (Table 4). Results indicate that the Korean cattle (Hanwoo: HW) is closely related with Korean Black cattle (KBC), Yanbien cattle

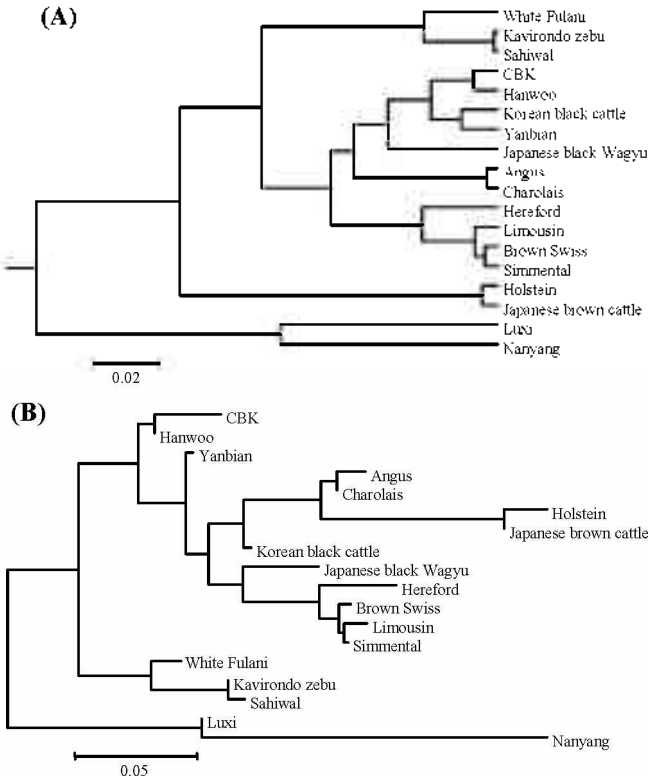

Figure 2. Phylogenetic trees based Nei's genetic distance (1978) by UPGMA (A) and NJ (B) methods of POPGENE package.

(YAN), synthetic breed among Charolais, Brahman, Hanwoo (CBK) with the genetic distance values of 0.0203, 0.0213, and 0.0150, respectively. Also closely related genetic distance, 0.0499, was observed between Japanese Black Wagyu (WAG) and Korean black cattle (KBC). Interestingly, CBK breed was very closely related with Hanwoo with the genetic distance of 0.0150. This is due to the fact that only males from Brahman and Charolais were used in the CBK breed formation. Therefore only Hanwoo mtDNA, inherited as maternal fashion, can be transmitted to the next generation. Previously, Han et al. (1996) indicated that Hanwoo and Yanbian cattle have a common ancestor. In our analysis, the genetic distances from Hanwoo and Yanbian cattle to Bos indicus breeds or European Bos taurus breeds are similar, supporting that Hanwoo and Yanbian cattle breeds evolved from a common ancestor.

Phylogenetic trees were constructed using Nei's genetic distance matrix in POPGENE program (Figure 2), recognizing two major clades, namely Bos indicus breeds versus European and Bos taurus breeds, excluding outgroup of Luxi and Nanyang breeds. Japanese Brown Cattle and Ceju Black Cattle (CBK) show more close relationship with European breeds (Angus, Charolais and Holstein breeds) than Asian breeds (Hanwoo, CBK, Japanese Black Wagyu, Yanbien Cattle). This result was supported by Abe et al. (1975) that Japanese Wagyu breeds were more highly influenced by European breeds based on the milk protein polymorphism. Also, similar resultswere presented by Kikkawa et al. (1995) that genetically close breeds were more likely to cluster in the phylogenetic tree.

Brown et al. (1979) reported mutation of mtDNA was 2-4% per million years. Take this value for the calculation of divergence times among breeds, divergence between European and Zebu breeds was occurred 300,000-500,000 years ago. This divergence was also estimated 2-3 million years ago based on the fossil records. The difference of two above divergence time was based on the different substitution rate in each nucleotide in mtDNA. Before the domestication of Zebu cattle, the divergence between Zebu cattle and European breeds were occurred and this hypothesis was also supported by β-chain structure of hemoglobin. Loftus et al. (1994a, b) also estimated the divergence time between Asian breeds and Asian Zebu was occurred 0.2-1 million years ago.

The study on mtDNA D-loop revealed higher H value and percentage of polymorphic loci in Asian breeds as compared to European and Bos indicus breeds. Based on the D-loop polymorphism, the close genetic relationship of Korean cattle (Hanwoo) was observed with Korean Black cattle, Yanbien cattle, Charolais,and Brahman. The phylogenetic tree analysis showed that the Japanese brown cattle and Ceju Black cattle may have closer relationship with European breeds than Asian breeds.

ACKNOWLEDGEMENT

This work was supported by a grant from BioGreen21 Program, Rural development Administration, Republic of Korea.

REFERENCES

Amano, T., Y. Miyakoshi, T. Takada, Y. Kikkawa and H. Suzuki. 1994. Genetic variants of ribosomal DNA and mitochondrial DNA between swamp and river buffaloes. Anim. Genet. 25:29-36.

Anderson, S., A. T. Bankier, B. G. Barrell, M. H. L. de Bruijn, A. R. Coulson, J. Drouin, I. C. Eperon, D. P. Nierlich, B. A. Roe, F. Sanger, P. H. Schreier, A. J. H. Smith, R. Staden and I. G. Young. 1981. Sequence and organization of the human mitochondrial genome. Nature 290:457-465.

Anderson, S., M. H. L. de Bruljn and A. R. Coulson. 1982. Complete Sequence of Bovine Mitochondrial DNA. Conserved Features of the Mammalian Mitochondrial Genome. J. Mol. Biol. 156:683-717.

Attardy, G. 1985. Animal mitochondrial DNA: An extreme example of genetic economy. Int. Rev. Cytol. 93:93-145.

Bhat, P. P., B. P. Mishra and P. N. Bhat. 1990. Polymorphism of Mitochondrial DNA (mtDNA) in Cattle and Buffaloes. Biochemical Genetics 28:311-318.

Bibb, M. J., R. A. van Etten, C. T. Wright, M. W. Walberg and D. A. Clayton. 1981. Sequence and Gene Organization of Mouse Mitochondrial DNA. Cell 26:167-180.

Boettcher, P. J., A. E. Freeman and S. D. Johnston. 1996. Relationships between Polymorphism for mitochondrial Deoxyribonucleic Acid and Yield Traits of Holstein Cows. J. Dairy Sci. 79:647-654.

Bradley, D. G., D. E. MacHugh, P. Cunningham and R. T. Loftus. 1996. Mitochondrial diversity and the origins of African and European cattle. Proc. Natl. Acad. Sci. USA. 93:5131-5135.

Brown, W. M., M. George and A. C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA. 76:1967-1971.

Brown, D. R., C. M. Koehler, G. L. Lindberg, A. E. Freeman, J. E. Mayfield, A. M. Myers, M. M. Schutz and D. C. Beitz. 1989. Molecular analysis of cytoplasmic genetic variation in holstein cows. J. Anim. Sci. 67:1926-1932.

Cao. Y., J. Adachi, A. Janke, S. Paabo and M. Hasegawa. 1994. Phylogenetic relationships among eutherian orders estimated from inferred sequences of mitochondrial proteins: instability of tree based on a single gene. J. Mol. Evol. 39:519-527.

Eledath, F. M. and H. C. Hines. 1996. Detection of nucleotide variations in the D-loop region of bovine mitochondrial DNA using polymerase chain reaction-based methodologies. Anim. Genet. 27:333-336.

Felius, M. 1995. Cattle Breeds- An Encyclopedia. Misset. Doetinchem. The Netherlands.

Gadaleta, G., G. Pete, G. de Candia, C. Quagliariello, E. Sibisa and C. Saccone. 1989. The complete nucleotide sequence of the Rattus norvegicus mitochondrial geneome: Cryptic signals revealed by comparative analysis between vertebrates. J. Mol. Evol. 28:497-516.

Giles, R. E., H. Blanc, H. M. Cann and D. C. Wallace. 1980. Maternal inheritance of human mitochondrial DNA. Proc. Natl. Acad. Sci. USA. 77:6715-6719.

Hiendleder, S., H. Lewalski, R. Wassmuth and A. Janke. 1998. The Complete Mitochondrial DNA Sequence of the Domestic Sheep (Ovis aries) and Comparison with the OtherMajor Ovine Haplotype. J. Mol. Evol. 47:441-448.

Hutchison III, C. A., J. E. Newbold, S. S. Potter and M. H. Edgell. 1974. Maternal inheritance of mammalian mitochondrial DNA. Nature 251:536-538.

Kikkawa, Y., T. Amano and H. Suzuki. 1995. Analysis of Genetic Diversity of Domestic Cattle in East and Southeast Asia in Terms of Variations in Restriction Sites and Sequences of Mitochondrial DNA. Biochemical Genetics 33:51-60.

Kim, K. S., S. E. Lee, H. W. Jeong and J. H. Ha. 1998. The complete Nucleotide Sequence of the Domestic Dog (*Canis familiaris*) Mitochondrial Genome. Mol. Phyl. Evol. 10:210-220.

Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Paabo, F. X. Villablanca and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86:6196-6200.

Loftus, R. T., D. E. MacHugh, L. O. Ngere, D. S. Balain, A. M. Badi, D. G. Bradley and E. P. Cunningham. 1994a. Mitochondrial genetic variation in European, African and Indian cattle populations. Anim. Genet. 25:265-271.

Loftus, R.T., D. E. MacHugh and D. G. Bradley, P. M. Sharp and P. Cunningham. 1994b. Evidence for two independent domestications of cattle. Proc. Natl. Acad. Sci. USA. 91:2757-2761.

Mannen, H., T. Kojima and K. Oyama. 1998a. Effect of Mitochondrial DNA Variation on Carcass Traits of Japanese Black Cattle. J. Anim. Sci. 76:36-41.

Mannen, H., S. Tsuji, R. T. Loftus and D. G. Bradley. 1998b. Mitochondrial DNA Variation and Evolution of Japanese Black Cattle (*Bos taurus*). Genetics 150:1169-1175.

Mignotte, F., M. Gueride, A. M. Champagne and J. C. Mounolou. 1990. Direct repeats in the non-coding region of rabbit mitochondrial DNA: Involvement in the generation of intra- and inter-individual heterogeneity. Eur. J. Biochem. 194:561-571.

Miller, S. A., D. D. Dykes and H. F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 16:1215.

Nei, M. 1972. Genetic distance between populations. Am. Nat. 106:283-292.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Nei, M., F. Tajima and Y. Tateno. 1983. Accuracy of estimated phylogenetic trees from molecular data. J. Mol. Evol. 19:153-170.

Pegoraro, L., Z. Yang, S. Samaké, F. V. Meirelles, V. Bordingnon, L. Moquin and L. C. Smith. 1996. Sequence comparison of mitochondrial tRNA genes and origin of light strand replication in Bos taurus and Mellore (*Bos indicus*) breeds. Anim. Genet. 27:91-94.

Pesole, G. and E. Sbisa. 1992. The evolution of the mitochondrial D-loop region and the origin of modern man. Mol. Biol. Evol. 9:587-598.

Ron, M., O. Yoffe and J. I. Weller. 1993. Sequence variation in D-loop mtDNA of cow lineages selected for high and low maternal effects on milk production. Anim. Genet. 24:183-186.

Saccone, C., G. Pesole and E. Sbisa. 1991. The main regulatory region of mammalian mitochondrial DNA: Structure-function model and evolutionary pattern. J. Mol. Evol. 33:83-91.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.

Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. <Molecular Cloning: A Laboratory Manual> 2nd Edition. Cold Spring Harbor Laboratory Press.

Sudhir Kumar, Koichiro Tamura, Ingrid B. Jakobsen and Masatoshi Nei. 2001. MEGA2: Molecular Evolutionary Genetics Analysis software, Bioinformatics 17(12):1244-1245.

Takeda, K. and A. Onishi. 1995. SSCP analysis of pig mitochondrial DNA D-loop region polymorphism. Anim. Genet. 26:321-326.

Troy, C. S., D. E. MacHugh, J. F. Bailey, D. A. Magee, R. T. Loftus, P. Cunningham, A. T. Chamberlain, B. C. Sykes and D. G. Bradley. 2001. Genetic evidence for Near-Eastern origins of European cattle. Nature 410:1088-1091.

Watanabe, T., Y. Hayashi, R. Semba and N. Ogasawara. 1985. Bovine Mitochondrial DNA Polymorphism in Restriction Endonuclease Cleavage Patterns and the Location of the

Polymorphic Sites. Biochemical Genetics 23:947-957.

Watanabe, T., J. S. Masangkay, S. Wakana, N. Saitou and T. Tomita. 1989. Mitochondrial DNA Polymorphism in Native Philippine Cattle Based on Restriction Endonuclease Cleavage Patterns. Biochemical Genetics 27:431-438.

Wilkinson, G. S. and A. M. Chapman. 1991. Length and sequence variation in evening bat D-loop mtDNA. Genetics 128:607-617.

Xu, X. and U. Arnason. 1994. The complete mitonchondrial DNA sequence of the horse, Equus caballus: Extensive heteroplasmy of the control region. Gene 148:357-362.

Yeh, F. C., R. C. Yang, T. Boyle, Z. H. Ye and J. X. Mao. 1997. POPGENE, the User-finedly Shareware for Population Genetic Analysis Molecular and Biotechnology Centre University of Alberta, Alberta.