

Mitochondrial DNA Diversity of Korean Native Goats

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ABSTRACT : Korean native goats have lived on the Korean peninsula for more than 2,000 years and are regarded as a valuable genetic resource for the world. As an initial step to investigate the genetic structures of this breed, phylogenetic analysis and calculation of genetic diversities have been performed using mitochondrial DNA (mtDNA) sequence variations. A total of 19 Korean native goats were grouped into six haplotypes and the large majority of haplotypes were present in 13 animals. All mtDNA of these Korean goats belonged to the mitochondrial (mt) lineage A and revealed remarkably small genetic distances within the population when compared with other Asian goat populations, indicating less genetic variation in the Korean native goats. These results indicate high-inbred status of the Korean native goats and will influence breeding and conservation strategies adopted for this breed. (*Asian-Aust. J. Anim. Sci.* 2006. Vol 19, No. 4 : 482-485)

Key Words : Phylogenetic Analysis, Domestic Goat, Korea, Mitochondrial DNA (mtDNA)

INTRODUCTION

The Fertile Crescent region of Near East is known to be the center of domestication for the number of livestock species and the initial domestication of goats (*Capra hircus*) has been documented in this region for about 10,000 years ago based on the archeological evidence (Zeder and Hesse, 2000). Harris (1962) and Zeuner (1963) suggested that three wild goats species, bezoars (*Capra aegagrus*), markhorns (*C. falconeri*), ibex (*C. ibex*), contributed genetically to the goat domestication process. Among these three wild goats, current goat species were more highly influenced by bezoar (Takada et al., 1997). More recent study indicates that the domestic goats have been affected by two subspecies of bezoar (Mannen et al., 2001).

The mitochondrial DNA (mtDNA) polymorphism, especially the displacement loop (D-loop) region, has been largely applied to understand phylogenetical relationships in many animal species, including cattle (Bradley et al., 1996; Loftus et al., 1994; Mannen et al., 1998; Troy et al., 2001; Mannen et al., 2004), pig (Giuffra et al., 2000), sheep (Hiendleder et al., 1998, 2002), chicken (Lui et al., 2004), horse (Vila et al., 2001), and goat (Luikart et al., 2001; Mannen et al., 2001; Sultana et al., 2003; Joshi et al., 2004; Sultana et al., 2004; Chen et al., 2005). Based on the phylogenetic analysis of caprine mtDNA polymorphism, at least four mtDNA lineages (A to D) has been discovered

and the mt lineage A is the most diverse and widely distributed in comparison to other lineages (Luikart et al., 2001; Sultana et al., 2003; Chen et al., 2005).

Recently, the native livestock genetic resources are become more important and large efforts have been concentrated for maintaining minimum number of animals for each native species (<http://www.fao.org/dad-is/>). Along with mtDNA polymorphism, microsatellite markers and protein polymorphisms were also investigated in Asian animals to investigate the breed structure and phylogenetic relationships with other breeds for the conservation perspectives (Geng et al., 2003; Li et al., 2004; Yang et al., 2004).

Based on the historical evidence, Korean native goats have been being lived in Korean peninsula about 2,000 years ago and show almost identical appearance with black coat color. Because of their low productivity of meat and milk, large number of Korean native goats is being crossbred with high productive exotic breeds such as Saanen (Kim et al., 2002). In this study, the current phylogenetic status and genetic diversities of Korean native goats have been investigated in order to understand the genetic basis of this breed and ultimately contribute to the breeding and conservation strategies for the Korean native goats.

MATERIALS AND METHODS

Animal samples

Blood samples from 19 Korean native goats (*Capra hircus*) containing two different geological locations (15 from Dangjin and 4 from Namwon) were collected. Total DNAs were extracted based on the manufacturer's standard protocol using Magextractor (TOYOBO Ltd, Japan) for investigation of sequence analysis in hyper variable region

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Table 1. Mitochondrial HV1 Sequence variations among Korean native goats

Haplotypes	Base position ¹												
	48	103	135	152	192	212	239	242	274	298	332	434	439
Type 1 (13) ²	T	T	A	T	A	A	T	T	A	C	C	C	C
Type 2 (2)	-	-	-	-	G	G	-	-	-	-	-	-	-
Type 3 (1)	C	C	G	-	-	G	-	-	G	T	T	G	T
Type 4 (1)	C	C	G	-	-	G	-	-	G	T	T	-	T
Type 5 (1)	-	-	-	-	-	-	C	C	-	-	-	-	-
Type 6 (1)	-	-	-	C	-	-	-	-	-	-	-	-	-

¹Numbers indicate nucleotide base position in caprine mtDNA HV1 region and hyphen represents the identical nucleotide with the type 1 sequence.

²Numbers in parentheses indicate number of animal observed in Korean native goats.

Table 2. Sequence divergence of Asian goat populations¹

	Pakistan	China	Laos	Korea
Pakistan	0.030	<i>0.038</i>	<i>0.050</i>	<i>0.029</i>
China	0.036	0.033	<i>0.039</i>	<i>0.029</i>
Laos	0.046	0.037	0.033	<i>0.038</i>
Korea	0.027	0.028	0.035	0.005

¹Below the diagonal and on the diagonal are the average sequence divergencies between populations and within populations, respectively.

Above the diagonal (*italics*) are genetic distances between populations calculated by Tamura-Nei model in MEGA software.

1 (HV1) in the caprine mitochondrial DNA (mtDNA). We included published mtDNA HV1 sequences of 24 Chinese (GenBank accession nos. AY853278-AY853301), 58 Pakistani (GenBank accession nos. AB110552-AB110574, AB110576-AB110589, AB162196-AB162205, AB162207-AB162217), and 10 Laotian domestic goats (GenBank accession nos. AB044295-AB044304) in our analyses.

Amplification, purification and DNA sequencing

The HV1 mtDNA of 19 Korean native goats were amplified directly from the genomic DNA by polymerase chain reaction (PCR). The primers used are HV1F (5'-CAGTCGAACATCCCTACATTATTATTGG-3') and HV1R (5'-GACCAAACCTATGTGTTTATGGAGTTGG-3') that gave 1,547 bp PCR products containing all HV1 sequences. The PCR reaction consisted of 1×buffer, 1.5 mM MgCl₂, 2.5 mM each of dNTP, 10 pM each primers, and 2.5 unit TAKARA EX Taq polymerase (TAKARA, Japan). The thermal profile included an initial denaturation at 94°C for 2 min following 30 cycles of denaturation at 94°C for 30 sec, annealing at 65°C for 30 sec and extension at 72°C for 1 min with a final extension at 72°C for 7 min using GenAmp 9700 (Applied Biosystems, CA, USA). The PCR products were isolated from 1% agarose gels and purified with UltraClean™ 15 DNA purification kit (MO BIO Inc., CA, USA). The purified DNAs were subjected for the cycle sequencing reactions using the primer HV1seq (5' -TACCCACACAAACGCCAACACC- 3') and analyzed using a ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, CA, USA). The obtained Korean native goat HV1 sequences have been deposited in the GenBank database (DQ217780-DQ217785).

Phylogenetic analyses

Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.0 (Kumar et al., 2004). Alignment of sequences was achieved using the Clustal W program (Thompson et al., 1994), which has been contained in MEGA software. Gaps in any of the aligned sequences were excluded from the analysis and unrooted neighbor-joining (NJ) trees were constructed. Authenticity of the phylogenetic trees was assessed by bootstrap percentages computed after 1,000 replications (Felsenstein, 1985). Genetic distances between HV1 sequences were estimated using the substitution model of Tamura and Nei (1993).

RESULTS AND DISCUSSION

mtDNA variation in Korean native goats

Analysis of 19 mitochondrial sequences from Korean native goats identified total 13 nucleotide changes grouped into six haplotypes. The large majority of haplotype group consisted of 13 individuals and one haplotype group comprised of two individuals, whereas the remaining four haplotypes were represented by a single sequence (Table 1). We found only one bp deletion/insertion event in a Korean native goat. This lower deletion/insertion event indicates that the Korean native goats have less genetic diversity compared with other Asian goats including animals from China, India and Pakistan (Sultana et al., 2003; Joshi et al., 2004; Chen et al., 2005). We initially sampled from two different geographical locations in South Korea (Dangjin and Namwon) and it's very hard that the goats were traveled from one location to another. However, haplotype 1 in Korean native goats represents 68% of the goat samples investigated and this haplotype is predominantly appeared in the two geographical locations. This also supports the hypothesis that the Korean native goats have less genetic diversity compared with those of other Asian countries.

Genetic distances by comparing with other Asian goat populations

Using previously published Asian goat mtDNA sequence information, mean sequence divergence values between four Asian goat populations (Pakistan, China, Laos, Korea) and within each population were calculated (Table 2). The highest sequence divergence value, 0.046, was

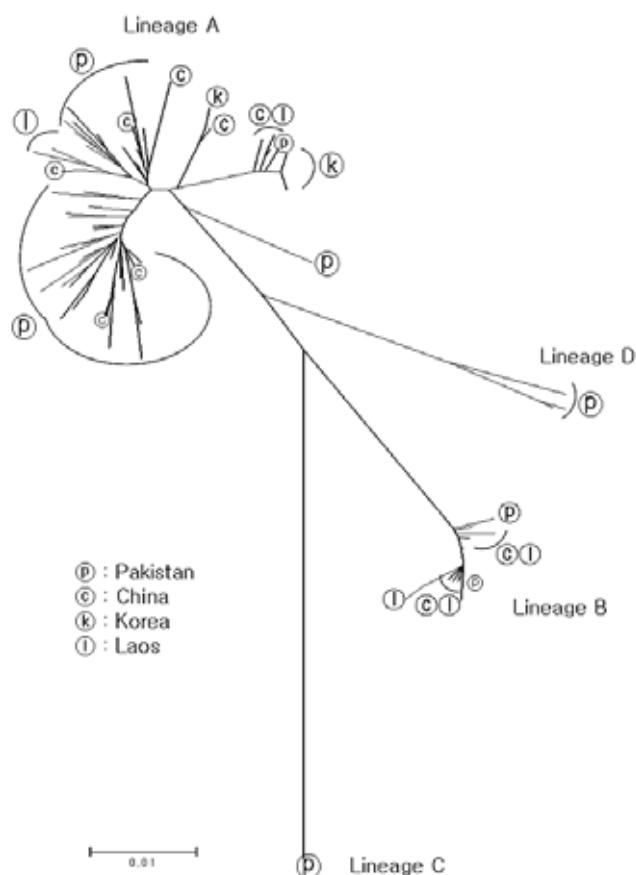


Figure 1. Unrooted neighbor-joining tree constructed from Pakistan, China, Laotian and Korean goat populations. This tree contains all four lineages indicating diversity of the Asian goat populations. The largest lineage A includes all four populations and lineage B includes Pakistan, China and Laotian populations. Also lineage C and D includes only Pakistan goat population. Note that Korean native goat only appeared in lineage A.

observed between Laotian and Pakistani goat populations. The mean sequence divergence values within population displayed similar levels of mean divergence (0.030-0.033) with the exception of the Korean goat population, which showed very low value of 0.005. This also highly supports the evidence of the less variability in the Korean native goats.

When comparing with Korean population, high divergence value of 0.035 was observed with Laotian population. This supported that Korean population was related more closely to Chinese and Pakistani goats than Laotian. The estimated genetic distances between populations also indicated that the Laotian and Pakistani goat populations are most far away (0.046) and Korean goats are closely related with Pakistani (0.028) and Chinese (0.028) goats. The reason of these results would be that Laotian goats include mt lineage B with high frequency, while Pakistani and Chinese goats revealed that mt lineage A is predominant as well as Korean goats.

Phylogenetic analysis for the Asian goats

Phylogenetic tree construction of the Korean native goats was performed with hyper variable region 1 (HV1) in the caprine mtDNA of this study and published sequences from Pakistani, Chinese and Laotian goat populations (Figure 1). The unrooted neighbor-joining tree indicates that all four major goat lineages (A to D) contained Pakistani goats, representing this population have the highest mtDNA sequence diversity in the Asian goat populations investigated. This tree also indicates that the largest lineage A (35 haplotypes) includes all four populations and lineage B (10 haplotypes) includes Pakistani, Chinese and Laotian populations. Also lineage C (1 haplotype) and D (3 haplotypes) includes only Pakistani goats.

All the Korean native goats, representing six haplotypes, were classified into mt lineage A (Figure 1). The tree indicated the Pakistani and Chinese goats were wide-spread all over the mt lineage A. However, Korean native goats located in a specific region of mt lineage A, also indicating the less genetic variation of the Korean native goats.

In this study, we observed less genetic variability of the Korean native goats, compared with other Asian goat populations. These results also support that the Korean native goats were inbred within Korean peninsula for a long time and give some idea for the conservation of this breed. However more detailed molecular studies are required in near future.

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