# Genetic Diversity of Goats from Korea and China Using Microsatellite Analysis

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**ABSTRACT :** Nine microsatellite loci were analyzed in 84 random individuals to characterize the genetic variability of three domestic goat breeds found in Korea and China: Korean goat, Chinese goat and Saanen. Allele diversity, heterozygosity, polymorphism information content, F-statistics, indirect estimates of gene flow (Nm) and Nei's standard distances were calculated. Based on the expected mean heterozygosity, the lowest genetic diversity was exhibited in Korean goat ($H_E$=0.381), and the highest in Chinese goat ($H_E$=0.669). After corrections for multiple significance tests, deviations from Hardy-Weinberg equilibrium were statistically significant over all populations and loci, reflecting the deficiencies of heterozygotes (global $F_{IS}$=0.053). Based on pairwise $F_{ST}$ and Nm between different breeds, there was a great genetic differentiation between Korean goat and the other two breeds, indicating that these breeds have been genetically subdivided. Similarly, individual clustering based on the proportion of shared alleles showed that Korean goat individuals formed a single cluster separated from the other two goat breeds. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 4 : 461-465)***

**Key Words :** Microsatellites, Genetic Diversity, Korean Goat, Gene Flow

## INTRODUCTION

Awareness of the value of genetic resources has stimulated the study of the genetic diversity of native breeds. Detailed knowledge of genetic variation within and among different breeds is very important for understanding and developing endogenous economic genetic traits of breeds (Yeo et al., 2000).

Microsatellites have been used successfully to define genetic structures and genetic relationships among different breeds. Microsatellites display higher levels of variation, and consequently, enable population differentiation to be found more efficiently. However, most of the studies using microsatellites have concentrated on cattle, pig, and sheep (Buchanan et al., 1994; MacHugh et al., 1998; Martinez et al., 2000; Hanslik et al., 2000), while not much information is available about the genetic diversity of native goats. Some studies have been performed concerning genetic diversity of 11 indigenous south-east Asian goat populations and the Australian feral population (Barker et al., 2001), five Chinese local goat breeds (Yang et al., 1999), and Swiss goat breeds (Saitbekova et al., 1999), but no further information is available on gene differentiation among different goat breeds from North East Asia. In addition, the population structure and genetic diversity of Korean goats are still not understood.

In this paper, we present the genetic variability and population structures of Korean goat, Chinese goat and Saanen using nine microsatellite markers. We also estimated the gene differentiation and the genetic relationship within and between these three goat breeds.

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## MATERIALS AND METHODS

### Sample collection and DNA extraction

Blood samples were collected from a total of 84 individuals belonging to three different goat breeds: Korean goat (n=30), Chinese goat (n=24) and Saanen (n=30). Fresh blood was taken from individuals from distinct geographical areas who were chosen at random without consideration of the relationship between animals. Korean goat was sampled from the suburbs of Kyongsan, Kyungpook Province, Korea. Chinese goat was sampled from the Yanbian area of North China. Saanen was sampled from Youngdong, Kyungpook Province, Korea. The Saanen goat was imported from Australia for milk production. Genomic DNA was extracted from the EDTA stabilized blood samples of all the goats as described by Maniatis et al. (1982).

### Microsatellite analysis

Five Bovine (*INRA63*, *ETH10*, *ILSTS005*, *TGLA53*, and *INRA005*), two Ovine (*McMA47* and *McMA 49*) (Beh et al., 2000), and two Caprine (*CRSP21* and *CRSP26*) (Yeh et al., 1997) macrosatellite markers were used for the analysis of native goat breeds. The bovine macrosatellites are markers recommended by the EC cattle biodiversity collaborators (www.ri.bbsrc.ac.uk/cdiv_www/markers.htm). The PCR reaction was accomplished in a total volume of 12.5 μl using 25 ng of genomic DNA, 1.5 mM of $MgCl_2$, 200 μM of each dNTP, 4 pmol of each primer, and a 0.5 unit of *Taq* polymerase. The PCR reaction cycle was accomplished by denaturation for 1 min at 94°C, primer annealing for 1 min at the desired temperature, and an extension for 1 min at

72°C and repeated 30 times. PCR products were separated on a 6% denaturing polyacrylamide gel and silver stained according to manufacturer's standard protocol (Promega, WI, USA). The exact allele sizes were determined by direct comparisons with the adjacent PCR bands of the size-known DNA samples and 10 bp ladders.

**Data analysis**

Allele frequency, the mean number of alleles per locus, observed heterozygosity, and heterozygosity expected from Hardy-Weinberg assumptions for each locus, and Nei's standard genetic distances (Nei, 1978) were computed using the GENETIX software package (Belkhir et al., 2000). The two measures of heterozygosity are highly correlated, but this study focused on the expected heterozygosity since it is considered to be a better estimator of the genetic variability present in a population. F-statistics (Weir and Cockerham, 1984) and their significance were estimated using FSTAT program (Goudet, 1995), with the sequential Bonferroni procedure applied over loci in deriving significance levels. For the Hardy-Weinberg equilibrium (HWE) estimation, we followed the probability test approach (Guo and Thomson, 1992) using the GENEPOP program (Raymond and Rousset, 1995). The MICROSAT computer program (Minch, 1998) was used to calculate inter-individual genetic distances, based on the proportion of shared alleles. These distance values were used to construct an UPGMA tree using the NEIGHBOR module of the PHYLIP software package (Felsenstein, 1993).

## RESULTS

**Allele frequencies and genetic variability**

A total of 62 alleles were detected across the nine loci analyzed. The allele frequencies for each of three goat breeds can be obtained from the authors. All the loci were polymorphic and the number of alleles varied between four (*ETH10*) and ten (*McMA47*) with a mean value of 6.9. Bovine and ovine microsatellites were effective for the detection of polymorphisms in the three goat breeds. Although several alleles were found uniquely in each goat breed, they are unlikely to be useful as breed markers due to their low frequencies in the small sample size.

The allele diversity, observed and expected heterozygosity, polymorphism information content per breed, and p-values from the Hardy-Weinberg (HW) expectations are shown in table 1. From all analyses, the lowest genetic diversity was exhibited in Korean goat, and the highest in Chinese goat. From 27 instances (three breeds, nine loci), five deviations significant from HWE were detected at the 5% level. All of these deviations were positive $F_{IS}$ values (data not shown). After corrections for multiple significance tests, deviations over all loci were found to be significant in Korean goat and Saanen. Over all breeds and loci, HW disequilibrium was highly significant (table 1).

**Table 1.** Allele diversity (the mean number of observed alleles per locus), heterozygosity (Het.), polymorphism information content (PIC) and p-value for Hardy-Weinberg expectations averaged over nine microsatellite loci

| Breed | Allele diversity | Het.-obs. | Het.-exp. | PIC | *p*-value[1] (No. of locus)[2] |
|---|---|---|---|---|---|
| Korean goat | 3.4 | 0.356 | 0.381 | 0.35 | <0.05 (3) |
| Chinese goat | 5.8 | 0.671 | 0.669 | 0.62 | 0.054 (0) |
| Saanen | 5.3 | 0.589 | 0.619 | 0.57 | <0.01 (2) |
| Mean | 4.8 | 0.539 | 0.556 | 0.51 | <0.01[3] (5) |

[1] p-value using Fisher's method implemented by GENEPOP v.3.1b.
[2] Number of loci showing departure from HWE
[3] p-value over all breeds and loci using Fisher's method

F-statistic estimates and their significances by locus across all three breeds are shown in table 2. Overall means for the F-statistics are significantly different from zero. $F_{IS}$ estimates over all breeds ranged between -0.049 (*CRSP26*) and 0.312 (*INRA005*), the global $F_{IS}$ being 0.053 ($p<0.01$). Given the multi-locus $F_{ST}$ value, levels of apparent breed subdivisions were considerable. Around 20.2% of the total genetic variation could be explained by breed differences, the remaining 79.8% corresponding to differences among individuals.

**Gene differentiation and genetic distances**

Based on pairwise $F_{ST}$ between each pair of three goat breeds, the gene differentiation values ranged from 7.3% for Chinese-Saanen goat pair to 26.3% for Korean-Saanen goat pair (table 3). A great genetic differentiation between Korean and the other two goat breeds was found. From

**Table 2.** F-statistic estimates and their significances by locus across three goat breeds

| Microsatellite | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ |
|---|---|---|---|
| INRA63 | -0.015 | 0.166** | 0.179** |
| ETH10 | 0.093 | 0.123 | 0.032** |
| ILSTS005 | 0.007 | 0.072 | 0.065** |
| TGLA53 | 0.178* | 0.476** | 0.362** |
| INRA005 | 0.312** | 0.596** | 0.413** |
| McMA47 | 0.062 | 0.195* | 0.142** |
| McMA49 | 0.040 | 0.085** | 0.048** |
| CRSP21 | -0.022 | 0.375** | 0.388** |
| CRSP26 | -0.049 | 0.036 | 0.081** |
| All loci | 0.053** | 0.245** | 0.202** |

* $p<0.05$, ** $p<0.01$.

indirect estimates (Nm) of gene flow, very low levels of gene flow between Korean goat and the other two goat breeds were also detected. Similarly, Nei's standard genetic distances based on allele frequencies showed a very high degree of genetic divergence between Korean and the other two goat breeds (table 3). The relationship tree based on the genetic distances showed that the Chinese goat and Saanen were grouped together and then Korean goat formed the basal branch (data not shown). An individual tree based on the proportion of shared alleles supported the relatively high heterozygosity of the Chinese goat, which formed three distinct clusters (figure 1). The Korean goat formed a clear level of clustering within the Chinese goat, whereas the Saanen revealed a fragmented pattern of clustering with the Chinese goat.

## DISCUSSION

Genotype data from nine microsatellites typed in 84 animals were used here to assess the genetic structures of three different goat breeds: Korean goat, Chinese goat and Saanen. The results show that HW disequilibrium is significantly high over all breeds and loci, showing a departure from HWE in the direction of heterozygote deficiency. On average, breed had a 5.3% deficit of heterozygotes, whereas the total population had a 24.5% deficit of heterozygotes (table 2). Considering the high genetic homogeneity in domestic goat, a certain degree of nonconformity to HWE is expected. In our study, allele non-amplification is unlikely to have contributed significantly to the deviation from HWE. The main reasons for the deviation from HWE are most likely the limited sample size, genetic drift or non-random mating.

Our estimate ($H_E$=0.67) of gene diversity in Chinese goat is lower than that of Chinese goat breeds (mean $H_E$=0.80) reported by Yang et al. (1999). Also, gene diversity ($H_E$=0.62) of Saanen in this study is slightly higher than that of Saanen ($H_E$=0.53) reported by Saitbekova et al. (1999). Yang et al. (1999) used hyper-variable microsatellites for their study, whereas Saitbekova et al. (1999) used microsatellites covering a wide range of variability. Thus, differences of gene diversity values obtained could be due to the choice of microsatellite loci as well as the choice of populations. Korean goat displays the lowest genetic diversity ($H_E$=0.38) from all analyses, showing a lower level of genetic diversity than those ($H_E$=0.43-0.60) of 11 indigenous south-east Asian goat populations (Barker et al., 2001).

It should be noted that the levels of apparent breed differentiation were considerable in domestic goat. The result shows that 20.2% of the total genetic variation is due to breed differentiation. The value is higher than those found in the south-east Asian goat populations (14.3%) (Barker et al., 2001) and other domestic animals, e.g. 10% in European cattle breeds (MacHugh et al., 1998), 10% in dogs (Jordana et al., 1992), 8% in horses (Canon et al., 2000), 13% in Iberian pig breeds (Martinez et al., 2000). It could be interpreted either as population subdivisions due to genetic drift and/or as the effect of a bottleneck through the reproductive isolation of rare populations (Takezaki and Nei, 1996).

In conclusion, the present study shows that the Korean goat exhibits a low level of genetic diversity and has been genetically subdivided from other goat breeds. It may be that Korean goat has been bred without any crossbreeding with foreign breeds after introgression from North East Asia to Korea. In addition, it is likely that Korean goat has experienced the loss of variation owing to non-random mating and/or genetic drift.

This study is the first report using microsatellite analysis to understand the genetic diversity of the Korean goat. Only little information is currently available to compare different goat breeds from North East Asia, but this information is very important for meeting the demands of future breeding programs as well as for formulating effective conservation strategies for genetic diversity within breeds. Although we have used only three representative goat breeds to understand the genetic backgrounds of domestic goats, the present study contributes to the knowledge and genetic characterization of local goat breeds.

**Table 3.** Nei's (1978) standard genetic distance, genetic differentiation ($F_{ST}$), migrants per generation (Nm; Wright, 1969) values between each pair of three goat breeds.

| Breed | Korean goat | Chinese goat | Saanen |
|---|---|---|---|
| Korean goat | - | 0.259** (0.71) | 0.263** (0.70) |
| Chinese goat | 0.422 | - | 0.073** (3.18) |
| Saanen | 0.432 | 0.160 | - |

Above the diagonal $F_{ST}$ values and Nm (between brackets) values, and below the diagonal genetic distance values are given. ($Nm=(1-F_{ST})/4F_{ST}$) ** $p<0.01$.

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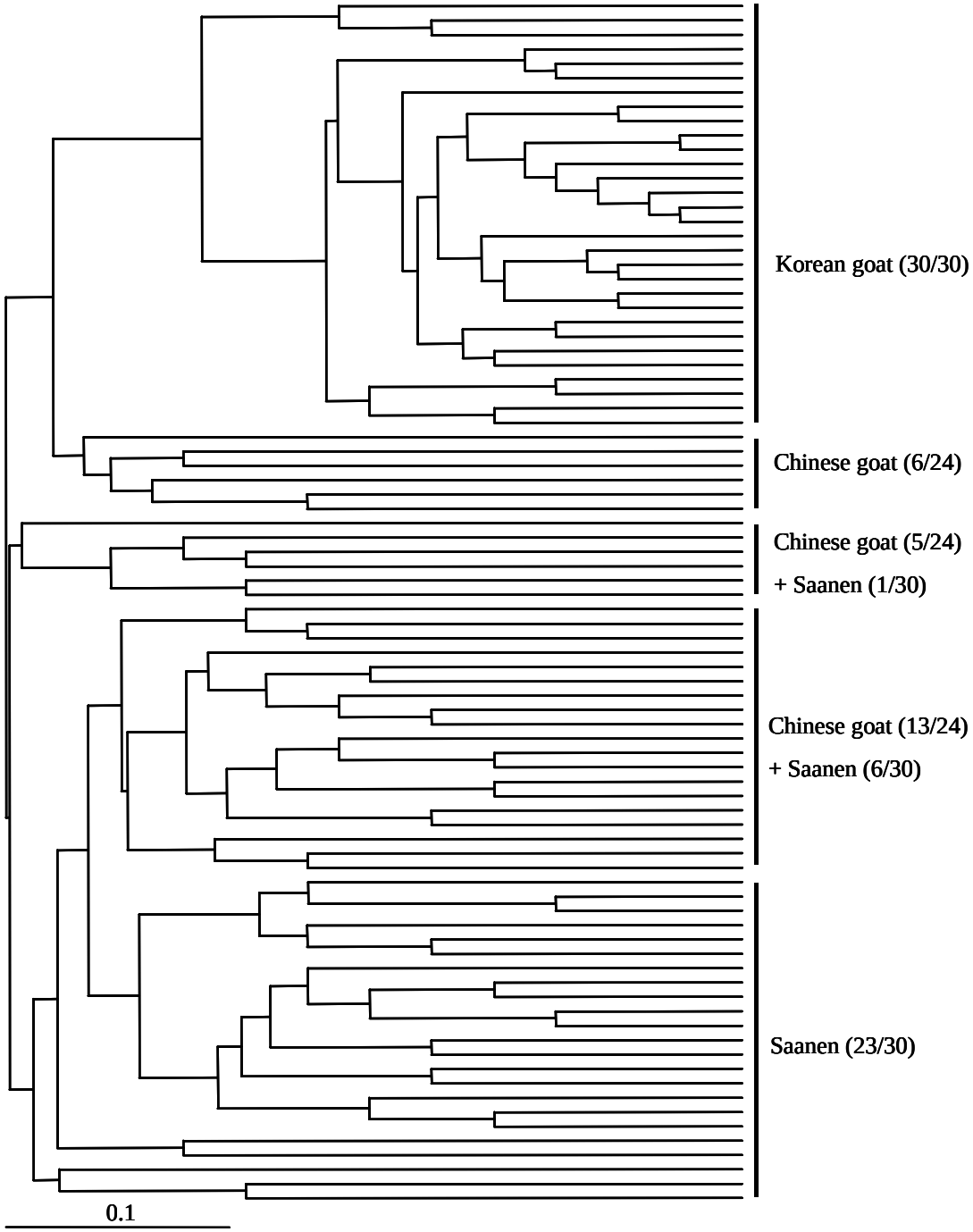


**Figure 1.** UPGMA dendrogram constructed from the pairwise distances inferred from microsatellite data between 84 individuals from three goat breeds. Numbers to the right indicate the fraction of individuals from the breed found in a cluster.

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