Genetic Structure of Mongolian Goat Populations Using Microsatellite Loci Analysis*

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ABSTRACT: We studied genetic diversity and relationships among Mongolian goat populations on the basis of microsatellite DNA polymorphisms. DNA samples from eight populations (Bayandelger, Ulgii Red, Zavkhan Buural, Sumber, Zalaajinst White, Erchim Black, Dorgon, and Gobi Gurvan Saikhan) from geographically distinct areas of Mongolia were analyzed by using 10 microsatellite DNA markers. Since the 10 markers were highly polymorphic, the genetic characteristics of these native goat populations could be estimated. Genetic diversity within populations, as estimated by the expected heterozygosities, was high, ranging from 0.719 to 0.746, but genetic differentiation between populations was low, representing only 1.7% of the total genetic variation. The results suggest that Mongolian native goat populations still have a semi-wild genetic structure reflecting traditional Mongolian nomadism and the short history of artificial selection. The genetic relationships among the populations were not clear in the neighbor-joining tree generated from the modified Cavalli-Sforza chord genetic distances. By using principal components analysis, the five core populations of Mongolian native goats (Bayandelger, Ulgii Red, Zavkhan Buural, Sumber, and Dorgon) and the populations crossed with Russian breeds (Zalaajinst White, Erchim Black, and Gobi Gurvan Saikhan) were distinguished. There was no correlation between genetic relationships among the populations and the geographical distribution of the populations. (Key Words: Goat, Mongolia, Population Genetics, Microsatellite, Cashmere)

INTRODUCTION

Goats are important livestock in Mongolia and produce one of the major export products, cashmere. Mongolia produces about 30% of the world’s cashmere. A number of native cashmere goat populations, which have local names, are recognized in Mongolia. Of these populations, that of the Bayandelger goat is famous for the high quality of its cashmere (Mandakh and Zagdsuren, 1996). To increase cashmere productivity, Russian breeds such as Pri Don and Gorno Altai were introduced and crossed with indigenous goats in the Gobi and Altai mountain region in the 1960s. However, the quality of fiber from the original native goats was higher than in these crossbreeds (Zagdsuren et al., 2000). Research programs to improve Mongolian native goats have been conducted by Mongolian scientists, and resource populations have been developed by phenotypic selection in some districts to improve the cashmere fiber quality in the general goat population.

Populations of Mongolian native goats can be distinguished by their external characteristics, but genetic information on each population is limited. Genetic structure and relationships among Mongolian native goat populations have been studied on the basis of blood protein polymorphisms (Nozawa et al., 1998; Nyamsamba et al., 2003). These reports detected a limited number of polymorphic loci and alleles per locus, suggesting low
genetic diversity; the genetic distances among Mongolian goat populations were very close and they were clustered very tightly. These data suggested that: 1) resolution of blood protein polymorphisms is not sufficient to assess genetic diversity and establish the relationships among Mongolian goat populations; and 2) Mongolian goat populations are genetically very close and have not differentiated. To check these hypotheses and gain a meaningful assessment of genetic structure, sensitive genetic markers are needed. Microsatellite repeat sequences (for example, \((CA)_n\) repeats) are well dispersed in the genome. They are highly polymorphic and have been used to study the population genetics of goats (Saitbekova et al., 1999; Yang et al., 1999; Massohou et al., 2006). The use of microsatellites in population genetic analysis has the advantage of allowing accurate genetic assessment of population differentiation. Our purpose here was to examine the genetic structure of Mongolian goat populations by documenting microsatellite DNA polymorphisms.

**MATERIALS AND METHODS**

**Populations studied**

Three hundred and eighty-four individuals belonging to eight Mongolian goat populations were studied. Seven populations, i.e., Zavkhan Buural (ZB, 50 individuals), Ulgii Red (UR, 41), Bayandelger (BD, 73), Zalaajinst White (ZW, 51), an unnamed population from the town of Sumber in the Dormod district (SU, 60), Erchim Black (EB, 49), and Dorgon (DO, 30), were sampled as representative of native goats. Gobi Gurvan Saikhan (GGS, 30), which is a newly selected breed from a cross between local goats in the Gobi area and the Russian Pri Don breed at the Research Institute of Animal Husbandry, Mongolia, was also studied. Table 1 summarizes the status of each population. Goat genomic DNA for polymerase chain reaction (PCR) amplification was extracted from the buffy coat of blood by using a DNA extraction kit (Sepagene, Sanko-Junyaku, Tokyo, Japan).

**Microsatellite DNA markers**

Fourteen microsatellite loci were tested (Table 2) and four were subsequently eliminated from the analysis (OarFCB304, ILSTS30, BM2934, and CSSM43) owing to problems relating to PCR amplification or typing difficulties of well-amplified products. Consequenly, 10 markers (HUJ625, ILSTS0005, INRA127, INRABERN192, MAF50, MAF65, OarVH34, SRCRSP08, SRCRSP26, and TGLA53) were selected and used to analyze Mongolian goat populations.

**Detection of microsatellite DNA polymorphisms**

To detect microsatellite polymorphisms, amplification was carried out in a 15 μl reaction mixture that included 5 pmol of each primer (the forward primer in each pair was

<table>
<thead>
<tr>
<th>Table 1. Summary of goat populations in Mongolia examined</th>
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<tbody>
<tr>
<td>Population</td>
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<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Zavkhan Buural (ZB)</td>
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<tr>
<td>Zalaajinst White (ZW)</td>
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<tr>
<td>Erchim Black (EB)</td>
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<tr>
<td>Ulgii Red (UR)</td>
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<tr>
<td>Bayandelger (BD)</td>
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<tr>
<td>Dorgon (DO)</td>
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<tr>
<td>Sumber (SU)</td>
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<tr>
<td>Gobi Gurvan Saikhan (GGS)</td>
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</table>

GGS was developed by crossing local cashmere goats in the Gobi area with Don breed bucks up to the F2, followed by pure and selective breeding.
5'-end-labeled with FAM, HEX or NED), 200 μM of each dNTP, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 0.2 units of Platinum GenoTYPE Tsp DNA Polymerase (Gibco BRL, Life Technologies, France) and 50 ng of template DNA. PCR amplification was carried out in a 9700 thermal cycler (PE Applied Biosystems, Foster City, CA, USA), and the cycles were as follows: 1 min at 94°C, 15 cycles of 1 min at 94°C, 30 s at 55°C, 30 s at 72°C; then 25 cycles of 30 s at 89°C, 30 s at 55°C, and 1 min at 72°C; and then final elongation for 9 min at 72°C. PCR products were run with the internal size standard GenoTYPE ROX 60-500 DNA ladder (Gibco BRL) on an ABI 310 DNA sequencer (PE Applied Biosystems). The size of fragments was analyzed with Genotyper Version 2.0 (PE Applied Biosystems) software.

Data analysis

Alleles were designated according to PCR product size, and allelic frequencies and observed heterozygosity (H₀) were calculated directly from the observed genotypes. The expected heterozygosity (Hₑ) in each population at each locus was calculated with the GENETIX Version 4.01 (Belkhir et al., 2000) software package. The effective number of alleles (nₑ) and FIS (deficiency of heterozygotes relative to Hardy-Weinberg expectations) were calculated in each population at each locus with an FSTAT Version 2.9.3 (Goudet, 2001) package. Observed genotype frequencies in each population at each locus were tested for conformity to Hardy-Weinberg equilibrium by using the FSTAT package with 1,000 randomizations. Genetic differentiation among populations was estimated by using Dₑ, calculated in FSTAT Version 2.9.3 (Goudet, 2001) and by calculation from the modified Cavalli-Sforza chord distance (Dₑ; Nei et al., 1983). From the Dₑ genetic distance matrix, a tree was constructed according to the neighbor-joining method (Saitou and Nei, 1987), with 1,000 bootstrap replicates, by using the NJBAFD program (Takezaki and Nei, 1996). Principal components analysis (PCA) was performed by using the gene frequencies of all variable loci (Kidd et al., 1980).

RESULTS

All 10 microsatellite loci examined were polymorphic in all populations. A total of 126 alleles were observed in the eight populations, ranging from 2 to 21 alleles according to the microsatellite under scrutiny. Table 3 shows the genetic variability in each population at each locus. The number of alleles per locus in the eight populations ranged from 7.9 to 9.5, whereas the effective number of alleles ranged from 3.8 to 4.6. The average observed and expected heterozygosities ranged from 0.669 to 0.730 and 0.719 to 0.746, respectively. The observed genotype frequencies for all populations were in agreement with the Hardy-Weinberg expectations. The average FIS value in each population ranged from 0.032 to 0.082.

Table 4 shows the θ values (upper right) and Dₑ distances (lower left) among the eight populations. Pairwise θ was significantly different (0.01<p<0.05) for all pairwise comparisons except the ZW-UR pair (p>0.05). The Dₑ genetic distance between ZW and UR was closest among all population pairs. The neighbor-joining tree generated from Dₑ values is shown in Figure 1. Mongolian goat populations formed one big group, because the bootstrap values (9% to 38%) were not considered significant.

Figure 2 shows the relative positions of the eight populations, as defined by the principal components. The first, second, and third principal components represented 21.5%, 16.6%, and 15.1% of the total variation, respectively. Five populations (BD, DO, SU, UR, and ZB) were grouped. GGS was distant from the other populations. EB was distinct from the five populations in the scatter diagram of first and second principal components. EB was distinct from the five populations in the scatter diagram of first and third principal components.
DISCUSSION

In Mongolia, herders have kept goats among their nomadic pastoral livestock since ancient times for meat, milk, hide, and fiber production. Goats are traditionally kept with sheep because the goats can find water and grass earlier than sheep and can lead the flock of sheep. Herders were able to move freely across the Mongolian steppes with
livestock for water and grass until 1924, when the border with Russia and China was fixed. Since 1949, when the district boundaries were fixed, the movement of livestock between districts has been restricted. In the 1960s, a Russian-style agricultural collective system was introduced to nomadic animal husbandry and several goat populations were crossed with Russian Pri Don, Gorno Altai, and other Russian goat breeds. A mass crossing of native goats with Pri Don and Gorno Altai breeds, aimed at increasing cashmere production, took place in the Gobi and Altai mountain area. This resulted in the establishment of the Gobi Gurvan Saikhan (GGS) analyzed here and of the Mountain Brown breed (Zagdsuren et al., 2000). On the other hand, the development of distinct native Mongolian goat populations started in the late 1970s, focusing on external characteristics such as coat color and adaptation to the local environment. As the Mongolian cashmere industry developed in the 1970s, the concept of fiber quality (i.e., thickness) was re-evaluated. Since fiber diameter in indigenous goats is thinner than in crossbreeds, the fiber of the crossbreeds is now distinguished as “cashgora” from the cashmere of native goats. Thus the economic value of native Mongolian goats is greater than that of crossbreeds. Consequently, the emphasis of current breeding programs of Mongolian native goats has been to improve fiber quality.

In a previous report (Nyamsamba et al., 2003), the relationships among Mongolian goat populations were estimated from few polymorphic loci and a limited number of alleles per locus. Consequently, microsatellites were used to obtain more meaningful genetic information about Mongolian goats. The fact that \( \theta \) values among Mongolian goat populations ranged from 0.004 to 0.027, with a mean value of 0.017, suggests that there is a high level of gene flow among the populations. The \( \theta \) values among Mongolian goat populations are lower than in other domestic animal breeds, e.g., horse (0.041 to 0.153; average 0.078; Canon et al., 2000), European cattle (0.050 to 0.174, 0.112; MacHugh et al., 1998), and European pig (0.116 to 0.737, 0.270; Laval et al., 2000). These data suggest that Mongolian goat populations still have semi-wild or feral genetic structures and have not reached the level of breeds yet. These data might reflect a long history of nomadism and the short history of goat breeding in Mongolia.

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<tr>
<th></th>
<th>ZB</th>
<th>ZW</th>
<th>EB</th>
<th>UR</th>
<th>BD</th>
<th>DO</th>
<th>SU</th>
<th>GGS</th>
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<tbody>
<tr>
<td>ZB</td>
<td>0.069</td>
<td>0.018*</td>
<td>0.010*</td>
<td>0.012*</td>
<td>0.013*</td>
<td>0.010*</td>
<td>0.016*</td>
<td>0.021*</td>
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<tr>
<td>ZW</td>
<td>0.069</td>
<td>0.072</td>
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<tr>
<td>EB</td>
<td>0.064</td>
<td>0.056</td>
<td>0.074</td>
<td>0.065</td>
<td>0.061</td>
<td>0.088</td>
<td>0.079</td>
<td>0.082</td>
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<tr>
<td>UR</td>
<td>0.068</td>
<td>0.067</td>
<td>0.065</td>
<td>0.061</td>
<td>0.088</td>
<td>0.089</td>
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<tr>
<td>BD</td>
<td>0.067</td>
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<td>0.088</td>
<td>0.089</td>
<td>0.083</td>
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<tr>
<td>DO</td>
<td>0.069</td>
<td>0.066</td>
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<td>0.083</td>
<td>0.086</td>
<td>0.086</td>
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<tr>
<td>SU</td>
<td>0.069</td>
<td>0.066</td>
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<td>0.074</td>
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<tr>
<td>GGS</td>
<td>0.076</td>
<td>0.082</td>
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<td>0.093</td>
<td>0.100</td>
<td>0.086</td>
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NS = Not significant, * p<0.05.

**Table 4.** \( \theta \) (above the diagonal) and \( D_a \) (below the diagonal) between pairs of populations

**Figure 1.** Dendrogram drawn by the NJ method from a genetic similarity matrix from \( D_a \) values, showing the genetic relationships between eight Mongolian goat populations from different regional areas. Numbers on the nodes show bootstrap values from 1,000 replications of resampled loci.

**Figure 2.** Three-dimensional scatter diagrams based on the first three principal components.
Although GGS was used as a population of outlier groups, Mongolian goat populations formed one big group in the neighbor-joining tree generated from $D_\theta$ values. Inside the group, they might fall into three clusters: a cluster including UR, BD, ZW, and SU, a cluster of EB and DO, and another cluster of the GGS population. We do not persist in saying that the clustering is correct, since the $\theta$ values are low and very similar to each other, and it is surprising that there is poor resolution of the NJ tree. Contrastingly, the data on PCA suggests that the influence of the Russian Pri Don breed is expressed in GGS. SU is believed to have characteristics similar to those of Mongolian goats of the past, since the area where SU is found is geographically isolated from the areas where the other seven populations are found. No special selection has been carried out, and SU has more varied coat colors than do other populations. Thus, the five populations (BD, DO, SU, UR, and ZB) identified by PCA are suggested to be the core populations of Mongolian native goats. The data showing that EB and ZW are distant from the other five populations do not contradict the undocumented information that limited introgression of Russian breeds has occurred in the districts where the EB and ZW populations are found. In addition, our data suggest that there is no correlation between genetic relationships among populations and the geographic distribution of the populations.

In conclusion, genetic diversity within Mongolian goat populations is high, but the genetic relationships among the populations are surprisingly close. The populations have not differentiated, even though one of the types analyzed here has been designated a breed (GGS). Therefore, we can say that the genetic structure of Mongolian goats is homogenous. Within the populations, the core of goat populations native to Mongolia was identified by using PCA. Our results allow for the future management and breeding of Mongolian native goats to be based on greater knowledge of the genetic structuring and relationships among populations.

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