Genetic diversity of five Chinese goat breeds assessed by microsatellite markers

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ABSTRACT: The genetic diversity was studied using microsatellite DNA markers in Laoshan dairy goat (LS), Xinong Saanen dairy goat (SN), Guanzhong dairy goat (GZ), Banjiao goat (BJ) and Guizhou white goat (GW). Within the nine polymorphic loci, allele frequencies, number of effective alleles (Ne), heterozygosity (He), polymorphism information content (PIC), genetic identity (I) and Nei's standard genetic distance (D) were calculated, and UPGMA phylogenetic tree was constructed based on allele frequencies. The average number of alleles was 9.4, ranging from four to eleven at the nine assessed loci. The average values of Ne, He, PIC of all loci were 4.716, 0.765, 0.732, respectively. The GZ population showed the highest variability (PIC = 0.78, He = 0.80). There was a relatively high level of genetic diversity in these goat breeds. A UPGMA diagram, based on Nei's standard genetic distances, yielded relationships between populations that agreed with what is known about their origin, history and geographical distribution.

Keywords: goat; microsatellites; biodiversity

Microsatellite markers, also known as simple sequence repeats (SSRs) or short tandem repeats (STRs), are regions of DNA that exhibit short repetitive sequence motifs. Because of their high degree of polymorphism, random distribution across the genome and possibility of automated scoring of genotypes, microsatellite markers have been proved to be one of the most powerful tools for evaluating genetic diversity and estimating genetic distances among closely related populations of ruminant species (Moore et al., 1991; Buchanan et al., 1994; Ellegren et al., 1997).

There are close similarities between cattle, sheep and goat chromosomes (Crawford et al., 1994; Kemp et al., 1995; Vaiman et al., 1996). Microsatellite markers present in all three species could be amplified with the same primer pairs, so microsatellite markers developed in cattle and sheep also work in goats (Vaiman et al., 1996) and they can be used for the analysis of genetic diversity (Saitbekova et al., 1999).

The existence of a large gene pool is important for the potential future breeding preservation and for the development of a sustainable animal production system. Comprehensive knowledge of the existing genetic variability is the first step for the conservation and exploitation of domestic animal biodiversity.

With the development of China booming economy, some superior goat breeds were introduced to

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Table 1. Number of animals, mean number of alleles per locus, Ne, He, PIC and related SE (standard error) in five goat breeds

Breed	No. of animals	Mean number of alleles	Ne	He	PIC
LS	80	8.2 (1.72)	5.191 (1.40)	0.794 (0.11)	0.768 (0.07)
SN	69	7.1 (2.20)	4.421 (2.17)	0.731 (0.06)	0.693 (0.12)
GZ	62	8.1 (1.76)	5.280 (1.33)	0.800 (0.05)	0.775 (0.05)
BJ	25	6.3 (1.66)	4.328 (1.20)	0.751 (0.08)	0.714 (0.09)
GW	30	6.6 (1.81)	4.360 (1.49)	0.746 (0.09)	0.711 (0.11)

improve Chinese native goat production abilities. Some indigenous goat breeds, represented by small population sizes, have been threatened by extinction, and some have already disappeared.

The Xinong Saanen dairy goat is regarded as an introduced breed in China, which was imported from America during the 1930's and then it was selected to adapt to house-feeding in Northwestern Agricultural University; so it was called Xinong Saanen dairy goat. Laoshan dairy goat and Guanzhong dairy goat were developed breeds, which were crossbred with the Saanen dairy goat and native goat endemic to China with a complex genetic background. Banjiao goat and Guizhou white goat are native breeds used for meat, which have been kept in Sichuan province and Guizhou province.

In order to develop objective criteria for *in situ* and *ex situ* conservation and further usage of the above goat breeds, eight bovine microsatellite markers and three sheep microsatellite markers were employed to study the genetic diversity and genetic distance and to design a breeding strategy aimed at increasing the genetic diversity within and across breeds.

MATERIAL AND METHODS

Blood samples were collected on five different farms in China and the total number was 266 (Table 1). The five Chinese goat breeds were Xinong Saanen dairy goat (SN), Guanzhong dairy goat (GZ), Laoshan dairy goat (LS), Banjiao goat (BJ), Guizhou white goat (GW), which were reared in the province of Shaanxi, Shandong, Sichuan, Guizhou (China), respectively (Lan et al., 2007).

DNA was isolated from blood using a standard phenol-chloroform extraction protocol according to Mullenbach et al. (1989).

The 11 microsatellite markers used in this study were *BM203*, *BM143*, *BM6425*, *BM302*, *BM315*, *BM415*, *BM1329*, *CSN3*, which originated from

Table 2. Information on 11 microsatellite loci

Loci	Primer sequences(5'–3')	Annealing temperature (°C)
BM203	F: GGGTGTGACATTTTGTTCCC R: CTGCTCGCCACTAGTCCTTC	63.0
BM143	F: ACCTGGGAAGCCTCCATATC R: CTGCAGGCAGATTCTTTATCG	56.9
BM6425	F: AGTTGAACCTGGGTCTCCTG R: TGCAATGGCAGTGAAAAAAG	64.0
BM302	F: GAATTCCCATCACTCTCTCAGC R: GTTCTCCATTGAACCAACTTCA	64.0
BM315	F: TGGTTTAGCAGAGAGCACATG R: GCTCCTAGCCCTGCACAC	58.0
BM415	F: GCTACAGCCCTTCTGGTTTG R: GAGCTAATCACCAACAGCAAG	63.0
BM1329	F: TTGTTTAGGCAAGTCCAAAGTC R: AACACCGCAGCTTCATCC	63.0
CSN3	F: ATGCACCCTTAACCTAATCCC R: GCACTTTATAAGCACCACAGC	54.0
TGLA73	F: GAGAATCACCTAGAGAGGCA R: CTTTCTCTTTAAATTCTATATGGT	55.6
GC101	F: ATCCTCACCCTTCAAACAG R: CTGGGGAGTTTTCTCTGAC	61.9
TGLA68	F: ATCTTACTTACCTTCTCAGAGCT R: GGGACAAAATTTTACATATACACTT	59.6

Table 3. Nei's (1978) genetic identity (I, above diagonal) and genetic distance (D, below diagonal) in five breeds

Breed	SN	LS	GZ	BJ	GW
SN		0.8492	0.8250	0.6079	0.5880
LS	0.1635		0.8975	0.7120	0.6924
GZ	0.1924	0.1081		0.6226	0.6611
BJ	0.4978	0.3397	0.4738		0.7945
GW	0.5311	0.3676	0.4139	0.2301	

cattle, and *TGLA0073*, *GC101*, *TGLA68*, derived from sheep (Table 2). The primer sequences were obtained from the website: http://www.ncbi.nlm.gov. All PCR amplifications were conducted in a reaction volume of 15 µl, containing 25–100 ng of DNA, 10 pmol of each primer, 1× PCR buffer (10mM Tris-HCl, pH 9.0; 50mM KCl), 1.67mM of $MgCl_2$, 200µM dNTP and 1 unit of *Taq* polymerase (MBI Fermentas, Vilnius, Lithuania). The cycling protocol was as follows: 2 min at 95°C, followed by 35 cycles of 30 s at 94°C, 30 s at annealing temperatures, 45 s at 72°C, with a final extension at 72°C for 10 min.

PCR products were separated on a 10% polyacrylamide gel and detected by silver staining and visualized under white light on a BIO-RAD Gel Doc XR system (Hercules, USA). Microsatellite alleles were identified by their sizes in base pairs using gel works software package (Quantity One) in the same system. Sizes of amplified fragments were determined by comparing with the standard base-pair ladder, pBR322/*Msp*I.

The observed number of alleles (A), effective number of alleles (Ne) and expected heterozygosity (He) were computed by the software POPGENE (Version 3.2). Polymorphism information content (PIC) was computed according to Botstein et al. (1980). Genetic diversities in subpopulations (*Hs*) and in the total population (*Ht*) were assessed using the computer program GENETIX (Belkhir et al., 2000). The extent of differentiation between subpopulations, called the coefficient of gene differentiation *Gst* (Nei, 1973), was calculated by the same program. Genetic identity and Nei's standard genetic distance were calculated by POPGENE (Version 3.2), a phylogenetic tree was constructed by UPGMA method based on pairwise Nei's standard distances using the same software by a bootstrapping method.

RESULTS

All the bovine and ovine microsatellite markers were successfully amplified in all the breeds. Nine loci, except *BM203* and *BM6425*, were found to be polymorphic in all populations, and generated a total of 85 alleles from the 266 individuals analysed, with locus *GC101* and *CSN3* showing the highest number of alleles. The number of observed alleles for each locus ranged from 4 to 11, with the mean of 9.4. The population statistics generated by the nine microsatellite markers in five goat populations is presented in Table 1. The mean effective number of alleles, polymorphism information content and

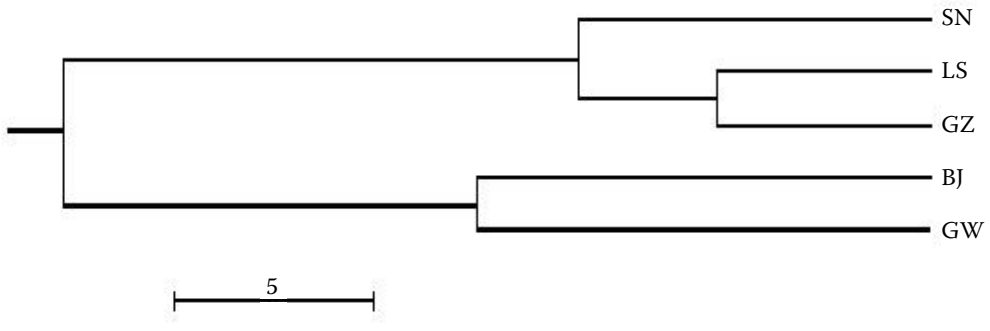

Figure 1. UPGMA of 5 native goat breeds by Nei's (1978) genetic distance

expected heterozygosity over all populations were 4.716, 0.765 and 0.732, respectively. No significant difference in the number of alleles, Ne, He and PIC was found between these goat populations. Among the five populations, GZ displayed the highest values for both mean He and PIC, while the SN breed showed lower variability levels.

The overall genetic diversity (*Ht*), the average gene diversity within a population (*Hs*) and the coefficient of gene differentiation (*Gst*) of the nine microsatellite markers were calculated. Overall *Gst* was 0.03, indicating that only 3.0% of the total genetic diversity was partitioned among breeds. In fact, one of the components of the total genetic variability (*Ht* = 0.35) based on the diversity among populations is only 0.21, showing that most of the variability is within populations (*Hs* = 0.34) with a percentage of 97.0%.

The genetic identity and standard genetic distances were calculated for these populations. The closest distance was observed between LS and GZ (D = 0.1081) and the largest between GW and SN (D = 0.5311). A cluster analysis based on Nei's standard distance matrix (Nei, 1978) (Table 3; Figure 1) showed two main groups: one consisting of the three dairy breeds, which exhibited the separation of SN breed, followed by LS and SN in one cluster, and the other consisting of BJ and GW.

DISCUSSION

This paper provides the description of genetic variability using molecular markers in five Chinese goat breeds. The results demonstrated that bovine and ovine microsatellite markers were effective for the detection of polymorphism in the five goat breeds. Apart from *BM6425* and *BM203*, nine of them were polymorphic and the mean number of alleles in this study was 9.4, which was relatively high and the results of microsatellite analysis may reflect the introduction of alleles from other breeds. In the last few years, Nanjiang yellow goat, a breed selected for meat production, was used to improve meat production of Banjiao goat and Guizhou white goat, which increased the genetic variability of the two breeds.

The results obtained in this study showed that the levels of genetic diversity were relatively high in these populations. The overall value of *Gst* (0.03) in this study was considerably lower than that found in an analysis of 20 microsatellite loci in Swiss goat breeds (0.17) (Saitbekova et al., 1999). This could lead to the conclusion that a high level of genetic variation has been maintained within the five goat breeds. Iamartino et al. (2005) found that the value of *Gst* also depended on the microsatellite markers used. Further study should be performed in future with a greater number of microsatellites in order to obtain more accurate results.

The result of UPGMA was consistent with the background of the origin, history and geographical location of these breeds. The UPGMA tree shows that three dairy goat breeds (SN, LS, GZ) are highly distinct from the other two goat breeds analyzed (BJ, GW). The close kinship between LS and GZ might suggest some past crossing between these two geographically close breeds. Likewise, the close genetic relationship between BJ and GW and their geographic proximity suggest the possibility of admixture between the two breeds. However, only a small number of microsatellite loci and breeds were analyzed. Additional markers and samples are required to increase the accuracy of the results.

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