African Journal of Biotechnology Vol. 11(54), pp. 11671-11677, 5 July, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.539
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Genetic diversity of five goat breeds in China based on microsatellite markers

Liying Zhang, Qingfang Yang, Xianglong Li*, Rongyan Zhou and Lanhui Li

College of Animal Science and Technology, Agricultural University of Hebei, Baoding, 071001, China.

Accepted 12 April, 2012

The genetic diversity of five goat breeds in China was surveyed using 15 microsatellites. The five goat breeds included Tangshan dairy goat (TSD), Liaoning cashmere goat (LNC), Nanjiang yellow goat (NJY), Chengde polled goat (CDP) and Leizhou black goat (LZB). The mean polymorphism information content value (PIC) of the populations ranged from 0.6606 to 0.8405. The mean heterozygosity (H) of the populations ranged from 0.7936 to 0.8202. The mean number of effective allele (Ne) of the populations ranged from 5.3373 to 5.8812 and the coefficient of genetic differentiation between breeds was 0.0620. It was suggested that the five goat populations have abundant genetic diversity and extensive genetic basis, with limited inbreeding, especially in Leizhou black goat. The unweighted pair-group method with arithmetic averages (UPGMA) dendrogram based on the Nei's standard genetic distance indicated that Tangshan dairy goat, Chengde polled goat and Liaoning cashmere goat breeds / populations clustered together. The Nanjiang yellow goat and Leizhou black goat populations clustered together, consistent with the geographical distribution of goat breeds.

Key words: Goat, microsatellite, genetic diversity, clustering.

INTRODUCTION

Microsatellite markers have been proven useful in assessing genetic diversity of populations in different species. For goats, microsatellites have been used for parentage testing, estimation of genetic diversity, differentiation, and relationship between and within populations (Luikart et al., 1999; Saitbekova et al., 1999; Barker et al., 2001; Ganai and Yadav, 2001).

In China, there are approximately 24 indigenous goat breeds, distributed in different environmental areas including the north pastoral region, the Qinghai-Tibet plateau region, the mixed pastoral–agricultural region, and the north and south agricultural region (Compiling Group of Sheep and Goat Breeds in China, 1989). These different goat breeds represent important genetic resources because of their special economic and ecological characteristics. Some documents on the genetic diversity of goat breeds using microsatellites have been reported (Yang et al., 1999; Arranz et al., 2001; Li and Valentini, 2004 and Wang et al., 2006). The Liaoning cashmere goat, Chengde polled goat and Leizhou black goat breeds are important indigenous breeds distributed in north and south of China. Tangshan dairy goat and Nanjiang yellow goat are cultivated breeds for milk and meat production, respectively in north and south of China. However, the research on their genetic diversity and differentiation are limited at present. Therefore 15 microsatellites were used in this paper to evaluate the genetic diversity and differentiation of the previous five goat breeds.

*Corresponding author. Tel: E-mail: lixianglongcn@yahoo.com. Tel: +86 312 7528451. Fax: +86 312 7528451.

Abbreviations: TSD, Tangshan dairy goat; **LNC,** Liaoning cashmere goat; **NJY,** Nanjiang yellow goat; **CDP,** Chengde polled goat; **LZB,** Leizhou black goat; **PIC,** polymorphism information content value.

MATERIALS AND METHODS

Population samples and microsatellite DNA primers

The blood samples which were from five goat breeds (total of 319) which respectively include Tangshan dairy goat, Liaoning cashmere goat, Nanjiang yellow goat, Chengde polled goat and Leizhou black goat (Table 1). They were obtained with the typical population random sampling methods in center region (the mainly goat breeding area). The samples were stored at -20°C.

Table 1. The sources, names and sample numbers of five goat breeds.

Breed	Number	Material	Locality
TSD	140	Blood	Luanan, Hebei Province
LNC	50	Blood	Dashiqiao, Liaoning Province
NJY	50	Blood	Najiang, Sichuan Province
CDP	43	Blood	Longhua, Hebei Province
LZB	36	Blood	Xuwen, Guangdong Province

TSD, Tangshan dairy goat; LNC, Liaoning cashmere goat; NJY, Nanjiang yellow goat; CDP, Chengde polled goat; LZB, Leizhou black goat.

The 15 pairs of primers for microsatellites published on NCBI UniSTS web database were from Bos Taurus and ovine. They were synthesized by Sango Biotech (Shanghai) Co., Ltd.

DNA extraction, PCR and polyacrylamide gel electrophoresis (PAGE)

DNA was extracted using the phenol-chloroform extraction kit (J Sambrook et al., 2002) according to manufacturer's instructions. PCR amplification was carried out in a PTC-100TM PCR instrument (MJ Research, MA, USA) with a total volume of 20 µL reaction containing 100 ng DNA, 2 µL 10× PCR standard reaction buffer (with Mg^{2+}), 1.5 µL 2.5 mmol/L dNTP, 0.8 µL each forward and reverse primer, 0.4 µL Taq DNA polymerase (2.5 U/µL) and add ddH_2O to 20 µL. Following an initial denaturation at 94°C for 5 min, 32 cycles were performed with 94°C for 30 s, annealing temperature at 54 to 62°C for 30 s, 72°C for 30 s. The final cycle was followed by an extension step at 72°C for 7 min and saved at 4°C. Amplification products were run on polyacrylamide gel electrophoresis (the concentration of 8%) sequencer. Compared with Marker pBR322DNA/MspI, the allele segment size was detected using the argentation. After the PAGE electrophoresis, we took photos with the X-PRESS gel imaging system and the image data were analyzed to obtain alleles classification.

Statistical analysis

The observed and expected heterozygosity for each population were estimated with GenePOP (Version 1.32; http:// www.ualberta.ca/fyeh/download.htm). For each locus, FSTAT software (Version 2.9.3; http://www.unil.ch/izea/softwares/fstat.html) was used to calculate Fit, Fst and Fis according to Weir and Cockerham (1984) and gene diversity for each locus in each population. The GenePOP software was used to calculate the polymorphism information content. With PopGene32 software, we calculated allele frequency of each point, heterozygosity, effective numbers of alleles and standard genetic distance between varieties. The polymorphic information content (PIC) for each locus and the standard genetic distance were from the web site http://jay.au. poznan.pl/tomjan/ds.htm. MVSP software was used to analyze the principal component and the unweighted pair-group method with arithmetic averages (UPGMA) of MEGA4.0 software was used to construct the phylogenetic tree.

RESULTS

Genetic diversity of each locus and population

The PIC, the numbers of effective allele (Ne) and the average heterozygosity (H) could assess the genetic diversity and reflect the level and degree of genetic variation within population. The Fis was used to estimate the parameters of the inbreeding degree within breeds. The range of the inbreeding degree was from -1 to 1. The inbreeding degree was high when the Fis was a positive number; on the other hand, there was outbreeding within breeds when the Fis was a negative number (Tang, 2006). The values of PIC, Ne, H and Fis for each locus and breed are listed in Table 2.

For all breeds, the averaged PIC, Ne, H and Fis were 0.7809, 5.5493, 0.8061 and 0.3839, respectively. The highest value of PIC (0.7973), Ne (5.8812) and and H (0.8202) were in Tangshan dairy goat, the ranges were from 0.7093 (BMS2508) to 0.8773 (BMS1004), 3.9894 (BMS2508) to 8.8889 (BMS1004), and 0.7493 (BM2508) to 0.8875 (BMS1004) respectively. Leizhou black goat has the lowest value of PIC (0.7675) and H (0.7936), ranging from 0.6496 (BMS2508) to 0.8635 (BMS1724) and 0.6566 (BM203) to 0.8472 (BMS1724), respectively. Ne was lowest in Nanjiang yellow goat (5.3773) ranging from 2.5839 (BMS2058) to 7.8370 (BMS1248). From these values, we could conclude that each microsatellite locus of different goat breeds and all microsatellites are considered as high polymorphic loci, abundant genetic diversity and large genetic variation degree.

The Fis value exceeded zero in the five goat breeds (Table 2), indicating some degree of inbreeding within the breeds.

Genetic differentiation among breeds

The total population and subpopulation heterozygosity were high in the five goat breeds (Table 3). The total gene diversity (Ht) of all loci exceeded 0.8 except for BM203 and BMS2508. The average heterozygosity within each population (Hs) was lower than Ht, which corresponds with the analysis result of PIC and H. We also could see that the genetic differentiation coefficient (Gst) were lower in all breeds, which indicated that between breeds the genetic differentiation was small so the genetic differentiation was mainly within breeds.

Genetic distance and dendrogram between goat breeds

The standard genetic distance and the UPGMA

Table 2. Polymorphism information content, the numbers of effective allele and the average heterozygosity at each loci of the five goat breeds.

Locus	Genetic index	Breed					Mean
		TSD	LNC	NJY	CDP	LZB	
INRA063	PIC	0.7958	0.7907	0.7821	0.7835	0.7711	0.7846
	Ne	5.5690	5.4289	5.1760	4.9906	5.2790	5.2887
	H	0.8204	0.8158	0.8068	0.8106	0.7996	0.8106
	Fis	0.6650	0.7340	0.7070	0.8280	0.7010	0.7270
BM203	PIC	0.7161	0.5982	0.6707	0.6569	0.6581	0.6606
	Ne	4.0719	2.9121	3.5689	3.3196	3.4195	3.4584
	H	0.7544	0.6988	0.7198	0.7076	0.6566	0.7074
	Fis	0.2890	0.2760	0.3060	0.2900	0.4110	0.3144
BM1818	PIC	0.7947	0.7812	0.8628	0.8128	0.7767	0.8056
	Ne	5.3935	5.1125	8.0515	5.0727	5.8909	5.9042
	H	0.8146	0.8044	0.8758	0.8302	0.8029	0.8256
	Fis	0.3550	0.3560	0.4380	0.3850	0.5110	0.4090
BMS1248	PIC	0.7250	0.8411	0.8591	0.7621	0.7250	0.7825
	Ne	4.1724	7.0028	7.8370	5.0952	4.8089	5.7833
	H	0.7603	0.8572	0.8724	0.7921	0.7558	0.8076
	Fis	0.6080	0.3560	0.3210	0.5770	0.3810	0.4486
BM1329	PIC	0.8208	0.8427	0.8045	0.8351	0.8041	0.8214
	Ne	6.2301	6.9348	5.8207	5.7691	6.7676	6.3045
	H	0.8395	0.8558	0.8282	0.8522	0.8267	0.8405
	Fis	0.5940	0.3780	0.3330	0.363	0.3280	0.3992
BM6526	PIC	0.7864	0.8289	0.7690	0.7371	0.7213	0.7685
	Ne	5.3002	6.5703	4.8876	4.0152	4.2843	5.0115
	H	0.8113	0.8478	0.7954	0.7666	0.7509	0.7944
	Fis	0.4220	0.3950	0.1300	0.3600	0.3240	0.3262
GC101	PIC	0.8479	0.7822	0.7703	0.7060	0.8272	0.7867
	Ne	7.3025	5.1106	4.9603	6.4538	3.9512	5.5677
	H	0.8631	0.8066	0.7984	0.7469	0.8451	0.8120
	Fis	0.4240	0.3890	0.2580	0.2950	0.3430	0.3418
BMS1678	PIC	0.8438	0.8038	0.7667	0.8423	0.7700	0.8053
	Ne	7.1182	5.6054	4.7125	4.9704	7.0244	5.8862
	H	0.8595	0.8216	0.7878	0.8576	0.7988	0.8251
	Fis	0.4460	0.4000	0.1720	0.3990	0.4290	0.3692
BM3413	PIC	0.8531	0.8264	0.8307	0.8290	0.8635	0.8405
	Ne	7.4909	6.3613	6.5963	8.0217	6.5455	7.0031
	H	0.8685	0.8548	0.8584	0.8563	0.8472	0.8561
	Fis	0.3620	0.4700	0.6280	0.3520	0.5220	0.4668
BMS1724	PIC	0.8531	0.8264	0.8307	0.8290	0.8635	0.8405
	Ne	7.4909	6.3613	6.5963	8.0217	6.5455	7.0031
	H	0.8685	0.8548	0.8584	0.8563	0.8472	0.8561
	Fis	0.3620	0.4700	0.6280	0.3520	0.5220	0.4668

PIC, polymorphism information content value; Ne, numbers of effective allele; H, heterozygosity.

Table 2. Contd.

Locus	Genetic index	Breed					Mean
		TSD	LNC	NJY	CDP	LZB	
BMS2508	PIC	0.7093	0.8073	0.5417	0.7520	0.6496	0.6920
	Ne	3.9894	5.8617	2.5893	4.5610	4.5714	4.3146
	H	0.7493	0.8294	0.6138	0.7812	0.6933	0.7334
	Fis	0.2600	0.1900	0.0330	-0.0950	0.3370	0.1450
BMS574	PIC	0.7664	0.8349	0.7550	0.7816	0.8014	0.7879
	Ne	4.9197	6.7568	4.5872	6.5194	5.1840	5.5934
	H	0.7967	0.8520	0.7820	0.8071	0.8188	0.8113
	Fis	0.2500	0.0480	0.1400	0.3290	0.3240	0.2182
MAF065	PIC	0.8480	0.7298	0.7552	0.8327	0.7803	0.7892
	Ne	7.2485	4.1667	4.6685	5.2011	6.6632	5.5896
	H	0.8620	0.7600	0.7858	0.8499	0.8077	0.8131
	Fis	0.2990	0.3250	0.5240	0.5190	0.5840	0.4502
ILSTS087	PIC	0.8006	0.8357	0.7836	0.7654	0.7743	0.7919
	Ne	5.6845	6.7935	5.2192	5.0245	4.8722	5.5188
	H	0.8241	0.8528	0.8084	0.7948	0.8010	0.8162
	Fis	0.3440	0.3520	0.5130	0.5150	0.2790	0.4006
BMS1004	PIC	0.8773	0.7694	0.8546	0.7753	0.8071	0.8167
	Ne	8.8889	4.8733	7.5988	7.7156	5.0135	6.8180
	H	0.8875	0.7948	0.8684	0.8005	0.8250	0.8353
	Fis	0.4720	0.4790	0.5690	0.4100	0.4560	0.4772
Mean	PIC	0.7973	0.7890	0.7699	0.7807	0.7675	0.7809
	Ne	5.8812	5.6149	5.3773	5.4568	5.4162	5.5493
	H	0.8202	0.8122	0.7976	0.8067	0.7936	0.8061
	Fis	0.4004	0.3663	0.3536	0.3858	0.4131	0.3839

Table 3. Total gene diversity (Ht), average heterozygosity within each population (Hs) and coefficient of gene differentiation (Gst) of fifteen microsatellite loci.

Locus	Ht	Hs	Gst	Locus	Ht	Hs	Gst
INRA063	0.8311	0.8106	0.0246	BM3413	0.8630	0.8025	0.0701
BM203	0.7968	0.7074	0.1121	BMS1724	0.8871	0.8561	0.0350
BM1818	0.8889	0.8256	0.0712	BMS2508	0.7876	0.7334	0.0687
BMS1248	0.8671	0.8076	0.0687	BMS574	0.8688	0.8113	0.0662
BM1329	0.8830	0.8405	0.0477	MAF065	0.8562	0.8131	0.0503
BM6526	0.8322	0.7944	0.0454	ILSTS087	0.8718	0.8162	0.0638
GC101	0.8839	0.8120	0.0813	BMS1004	0.8900	0.8353	0.0616
BMS1678	0.8799	0.8251	0.0623	All	0.8592	0.8061	0.0620

dendrogram of the populations are shown in Table 4 and in Figure 1, respectively. The smallest standard genetic distance (Ds) (0.3039) showed the closest relationship between Tangshan dairy goat and Liaoning cashmere goat. The largest Ds (0.5417) displayed the earliest differentiation between Nanjiang yellow goat and Chengde polled goat.

The allele frequency was obtained by GenPOP32 software. The allele frequency is used to estimate genetic variation between the breeds and genetic diversity.

Table 4. The standard genetic distance of the populations.

Breed	TSD	LNC	NJY	CDP	LZB
TSD	****	0.7384	0.6420	0.7240	0.7152
LNC	0.3039	****	0.6554	0.6542	0.6386
NJY	0.3970	0.3825	****	0.4759	0.7010
CDP	0.3058	0.3823	0.5417	****	0.6260
LZB	0.3127	0.4061	0.3293	0.4138	****

Nei's genetic identity (above diagonal) and standard genetic distance (below diagonal).

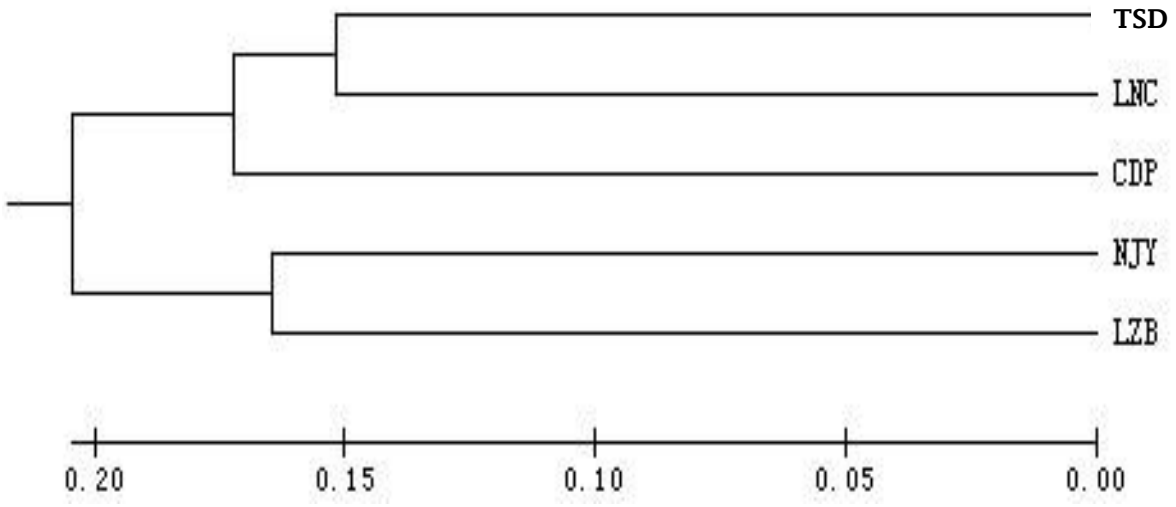

Figure 1. UPGMA dendrogram of five goat breeds based on Ds genetic distance. UPGMA, Unweighted pair-group method with arithmetic averages. TSD, Tangshan dairy goat; LNC, Liaoning cashmere goat; NJY, Nanjiang yellow goat; CDP, Chengde polled goat; LZB, Leizhou black goat.

Andrzej and Waldemar, (1993) has put forward the principal components analysis (PCA), which can translate a lot of mutation data to a small amount of mutation data and not lost too much information. It can make up for the shortage of the bottleneck effect on the groups and mixed degree of groups which cannot be found in dendrogram tree. Based on the allele frequency, the MVSP software was used to analyze the PCA of the five goat breeds. First four characteristics root of accumulative total contribution were 34.676, 61.012, 83.938 and 100%, respectively. From Figure 2, we can see that the first principal component separated the Leizhou black goat from others and the fourth principal component to the Nanjiang yellow goat. From Figure 3, Leizhou black goat and Nanjiang yellow goat were separated from the other three goat breeds obviously. The results are well proved in the Figure 1.

DISCUSSION

Genetic diversity and polymorphism among loci

The standard of microsatellite selection is that the numbers of alleles in each site, which is used to estimate the group genetic diversity must not be less than four (Barker, 1994). In this study we detected 169 alleles from 15 microsatellites in the five goat breeds. The average number of alleles over loci was 11.3. The highest number of alleles was 14 at BM3413 and BMS1724 and the least was eight at BM203. Based on the research of Botstein et al. (1980), the polymorphism information content could represent the degree of the variation of microsatellites, all of the 15 microsatellite loci in the five goat breeds have abundant polymorphism and the allele size was consistent with the original reference index results. Therefore, these loci can be used for the assessment of genetic diversity.

The PIC is a measure of the polymorphism fragments index. In a group, the genetic information is higher when the locus of heterozygous is bigger. According to the genetic variations level of PIC index, the locus is a high polymorphic locus when PIC>0.5. In this study, the average PIC at the 15 microsatellite loci ranged between 0.6920 and 0.8405. So the five goat breeds had high genetic diversity at the 15 polymorphic loci and a large potential of reserving seeds.

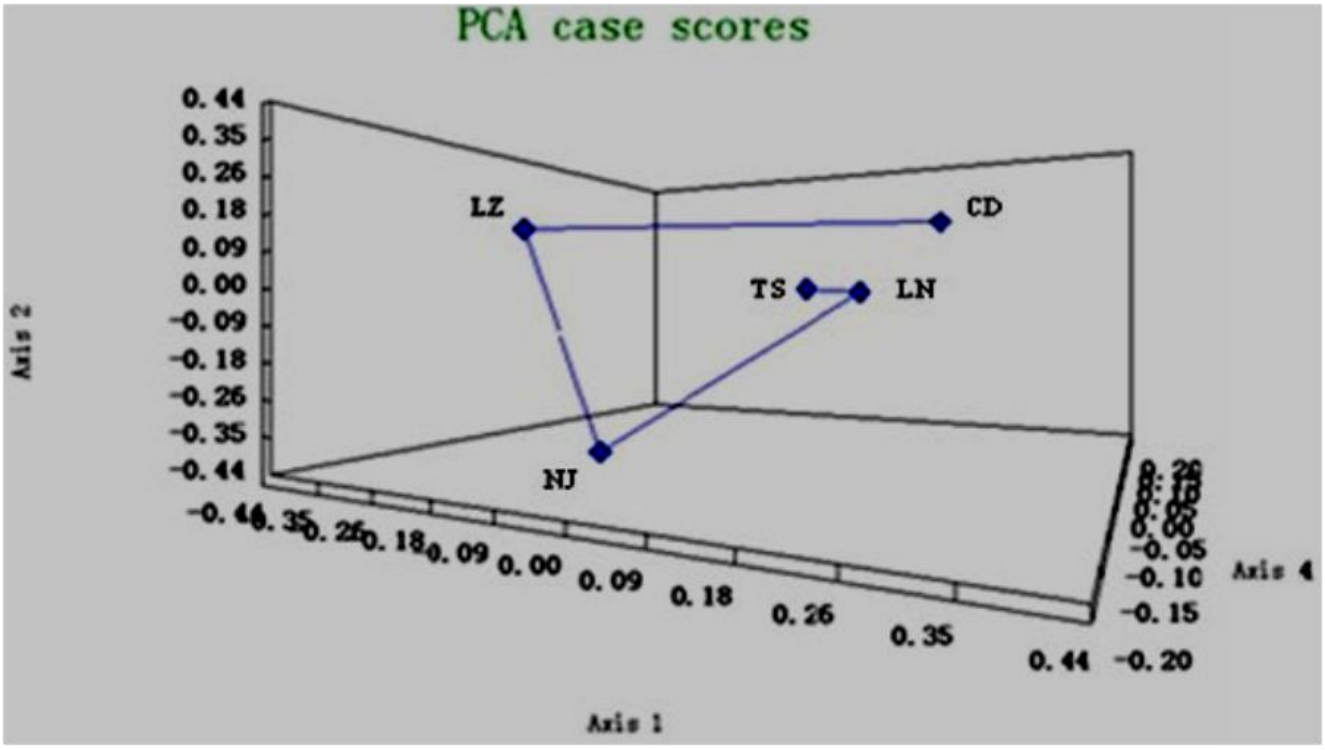

Figure 2. Three-dimensional scatter plot of the first, second and fourth principal component of the five goat breeds.

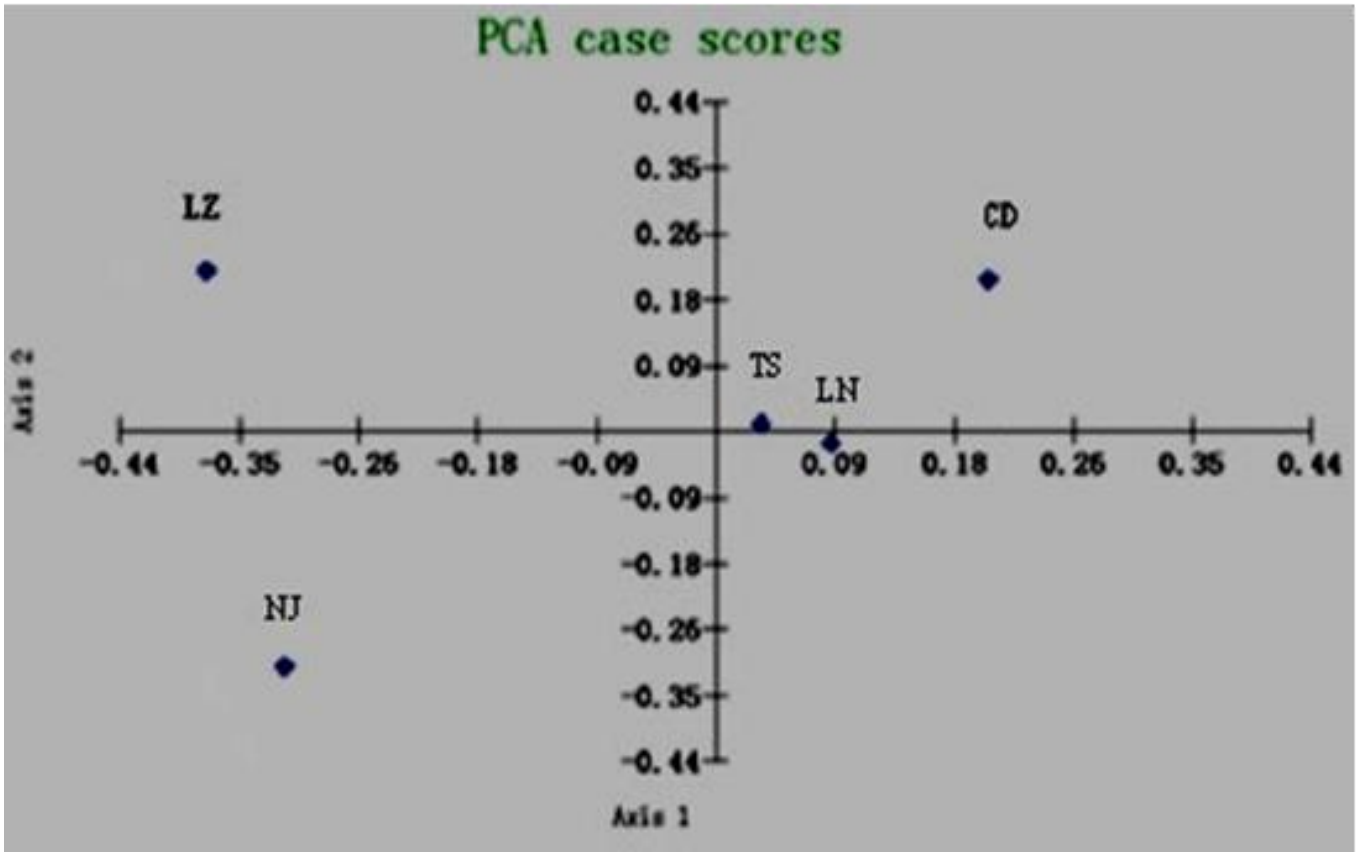

Figure 3. Two-dimensional scatter plot of the first and second principal component of the five goat breeds.

The average gene heterozygosity reflects the degree of genetic structure variation. In this study, the average gene heterozygosity of the 15 microsatellite loci in the five goat breeds varied from 0.7074 to 0.8561, which indicated that within the five goat breeds there was a larger genetic variation. Based on the average gene heterozygosity, the five goat breeds had a stronger adaptability to environment.

Genetic differentiation between the breeds

The Gst is used to measure the index of genetic differentiation over multiple groups of microsatellites. It is the index of average heterozygosity in the total population and subpopulation. When all the alleles in a group are almost the same, the value of the Gst is close to 0, and there is almost no differentiation between the groups. When the genetic differentiation between groups is increased, Gst value is close to 1, which indicated that between groups, there exist genetic diversity. In this study, the gene differentiation coefficient at total loci was 0.0620, so the genetic variation level between breeds was low.

From the dendrogram (Figure 1) we can see that Tangshan dairy goat, Liaoning cashmere goat, and Chengde polled goat were all consistent with their geographical position. In this study, the genetic variation distribution of the five goat breeds was consistent with the geographical distribution, which reflected that the formation and evolution of species were effected by the geographical environment. In addition, we also found that the Nanjiang yellow goat and Leizhou black goat were together. Whether the microsatellites used in this paper have linked with color genes needs to be investigated furthermore.

ACKNOWLEDGEMENT

This work was supported by National Key Scientific and Technological Project (2006BAD04A10-6).

REFERENCES

Andrzej M, Waldemar R (1993). Principalcomponentsanalysis (PCA) [M]. Comput. Geosci., 19 (3): 303-342.

Arranz JJ, Bayon Y, Primitivo FS (2001). Genetic variation at microsatellite loci in Spanish sheep [J]. Small Rumin. Res., 39 (1): 3-10.

Barker JSF (1994). A global protocol for determining genetic distances among domestic livestock breed. In: Proceedings of the 5th World Congress on Genet. Appl. Livestock Prod. Guelph, Ontario, Canada. 21: 501-508.

Barker JSF, Tan SG, Moore SS, Mukherjee TK, Matheson JL, Selvaraj OS (2001). Genetic variation within and relationship among populations of Asian goats (*Capria hircus*). Anim. J. Breed. Genet., 118: 213-233.

Botstein D, White R, Skolnick M, and Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms [J]. Am. J. Hum. Genet., 32: 314-331.

Compiling Group of Sheep and Goat Breeds in China (1989). Sheep and Goat Breeds in China. Shanghai Science and Technology Publisher, Shanghai, China.

Ganai NA, Yadav BR (2001). Genetic variation within and among three Indian breeds of goat using heterologous microsatellite markers. Anim. Biotechnol., 12: 121-136.

Hines HC, Zikakis JP, Haenlein GF, Kiddy CG, Trowbridge CL (1981). Linkage relationships among loci of polymorphisms in blood and milk of cattle [J]. J. Dairy Sci., 64(1): 71-76.

Sambrook J, Fritsch EF, Maniatis T (2002). Molecular Cloning [M]. Beijing: Sci. Technol. Publishing House.

Li XL, Valentini A (2004). Genetic diversity of Chinese indigenous goat breeds based on microsatellite Markers [J]. J. Anim. Breed. Genet., 121: 350-355.

Luikart G, Biju-Duval MP, Maudet C (1999). Power of 22 microsatellite markers in fluorescent multiplexes for parentage testing in goats (*Capra hircus*). Anim. Genet., 30: 431-438.

Saitbekova N, Gaillard C, Obexer-Ruff G, Dolf G (1999). Genetic diversity in Swiss goat breeds based on microsatellite analysis. Anim. Genet., 30: 36-41.

Tang C (2006). Analysis of Genetic Diversity of microsatellite among 13 Sheep Population in Xinjiang[D]. Master's Degree Thesis of Xinjiang Agric. Univ.

WANG Zhi-guo, YANG Zhang-ping, MA Yue-hui ,WANG Qing-hua, MAO Yong-jiang (2006). Analysis of Genetic Diversity among Seven Goat Population in the Middle and Lower Yangtse River Valley and East and South Mainland near the sea [J]. Acta Veterinaria Zootechnica Sinica. 37 (1): 1-6.

Weir BS, Cockerham CC (1984). Estimating F-statistics for the analysis of population structure. Evolution, 38: 1358-1370.

Yang L, Zhao S H, Li K, Peng ZZ, Montgomery GW (1999). Determination of genetic relationships among five indigenous Chinese goat breeds with six microsatellite markers [J]. Anim. Genet., 30(6): 452-455.