DNA Fingerprint Polymorphism of 3 Goat Populations from China Chaidamu Basin

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ABSTRACT : The DNA fingerprint polymorphism and the genetic relationship were studied by RAPD technology on Chaidamu goat (CG), Chaidamu Cashmere goat (CCG) and Liaoning Cashmere goat (LCG) from Chaidamu Basin of Qinghai province, China. The results showed that: The amplified bands were all 94 in 3 goat populations by using 8 random primers, and the DNA polymorphism frequencies of CG, CCG and LCG were 0.8404, 0.8617 and 0.8511, respectively, and the length of these DNA fragments were 176-2937 *bp*. The mean heterozygosities of the 3 goat populations were 0.5148, 0.5142 and 0.5075, respectively. The genetic relationship between CCG and CG or LCG were similar (Gst=4.37% and 3.79%; D_{ij}=0.0109 and 0.0106), and that between CG and LCG was further (Gst=13.14%; D_{ij} =0.0230). These results also showed that the genetic relationship between CCG and LCG was the closest, then CG and LCG, and CG and CCG was distant. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 8 : 1076-1079)***

Key Words : RAPD Marker, Native Goat Population, Genetic Relationship, Chaidamu Basin of China

INTRODUCTION

Chaidamu Basin is located in the north of Qinghai-Tibetan plateau, west China. Its elevation is between 2,600 and 3,200 meters above sea level, the annual mean rainfall and temperature are 25.1-179.1 mm and 2.3-4.4°C, respectively. In the basin, Chaidamu goat (CG) is an ancient native breed that can resist coldness and drought. Liaoning Cashmere goat (LCG) came from Liaoning province of China, Chaidamu Cashmere goat (CCG) is a new breed that has many blood lines. The DNA fingerprint polymorphism and the genetic relationship on the goat populations have been studied by Cargill et al. (1995), Vaiman et al. (1996), Geng et al. (2000), Li et al. (1999, 2000) and Qin et al. (2000), using RAPD technology. The aim of the paper is to examine the DNA fingerprint polymorphism and the genetic relationships of the 3 goat populations on the molecular level for the protection and utilization of goat genetic resources.

MATERIALS AND METHODS

Experimental materials

Applying the typical population random sampling, the blood samples of 95 goats (CG=32, CCG=31, LCG=32) were respectively from Delingha district, Yingdeer and Mehe goat farm in Qinghai province of China. The photos of the 3 goats see figure 1, 2 and 3. The blood samples were preserved at -4°C.

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DNA isolation and PCR amplified reaction

The DNA was isolated from venous blood white cells as described by Sambrook et al. (1989). The mixture solution of DNA amplified reaction was contained 1.5 μl DNA template (50 ng/μl), 2.5 μl 10×PCR Buffer, 2 μl dNTPs (2.5 mM), 1.5 μl $MgCl_2$ (25 mM), 2 μl primer (5 pmol), 1 U Taq DNA polymerase, 14.5 μl H_2O dein. The DNA amplified reaction was carried on the PTC^{1M}100 with the following cycle program: 5 min denaturation at 94°C, then 45 cycles at 94°C for 1 min, 36°C for 1 min, 72°C for 2 min, and the final extension step was at 72°C for 10 min. The PCR reaction product was analyzed by electrophoresing in a 1.4% agarose gel containing a final concentration of 1.5×10^{-6} M bisbenzimide for 4 h at 4 V/cm. After that, the electrophoresis gel was examined under *UV* light and taken photos (Sambrook et al., 1989).

Statistical analysis

Estimation of the DNA fragments length (Sambrook et al., 1989)

Gene heterozygosity and genetic differentiation coefficient

Gene heterozygosity (h_K)

$$h_K=1-j_K=1-\sum_{i=1}^{K} P_i^2$$

Mean loci heterozygosity (H)

$$H=1-J=1-\sum_{K=1}^{r} j_K/r$$

Genetic differentiation coefficient

$$G_{st}=1-Hs/Ht,$$

In the above formurlas, P_i stands for frequency of band i,

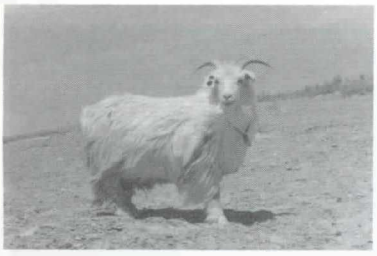


Figure 1. Chaidamu Cashmere Goat (♂).

Figure 2. Chaidamu Goat (♂)

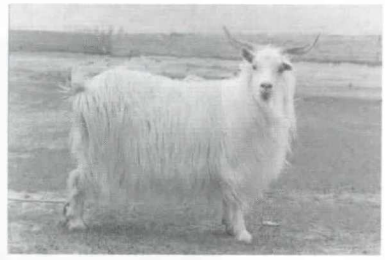


Figure 3. Liaoning Cashmere Goat (♂)

r stands for number of loci, Hs stands for mean loci heterozygosity of one population, Ht stands for mean loci heterozygosity of 3 populations.

Genetic similarity coefficient S_{ij} and standard genetic distance D_{ij} (Chang, 1998; Geng, 2001)

$$S_{ij}=\sum P_{ik}P_{jk}/(\sum P_{ik}^2 P_{jk}^2)^{1/2}$$

$$D_{ij}=-\ln(S_{ij})$$

Note: P_{ik} or P_{jk} stands for frequency of band k in population i or population j.

RESULTS AND DISCUSSIONS

Estimation of the length and polymorphic frequency on DNA fragments

8 polymorphism primers were selected from 100 random primers and used for the DNA fingerprint polymorphism and the genetic relationship on the 3 goat populations. The results were shown in table 1. Each goat population was all amplified 94 bands in the 8 random primers (The amplified results of CY18 and OPW19 were showed in figure 4). The amplified polymorphic bands were 79, 81 and 80, and its polymorphic frequencies were 0.8404, 0.8617 and 0.8511 respectively in CG, CCG and LCG. The results were similar to those of Qin et al. (2000) and Geng et al. (2000), who studied on Tibetan Yadong goat, Tibetan Plateau goat, Lvliang black goat of Shanxi province and White cashmere goat of north Shaanxi province. Those results showed that RAPD marker had rich polymorphism. They also showed that polymorphic primers in sheep could also be used in goats. The results were in accordance with Cargill et al. (1995) analysis on the difference between goat and sheep.

Taking bands of λDNA/EcoRI+HindIII as molecular marker, the estimation of the length of DNA fragments was shown in table 2, the length of the DNA fragments was 176-2937 bp on the 3 populations.

Genetic variation in the 3 goat populations

The variation of population genetics was analyzed by applying RAPD marker in table 3.

The mean heterozygosities were more than 0.5 in the 3 goat populations (CG was 0.5148, CCG was 0.5124, LCG was 0.5075), but the difference among populations was little. According to the research on blood protein marker, it was found that RAPD marker was more polymorphic than blood protein marker in the 3 populations (Shen et al., 2001). That showed that RAPD marker had wider selective range than blood protein marker.

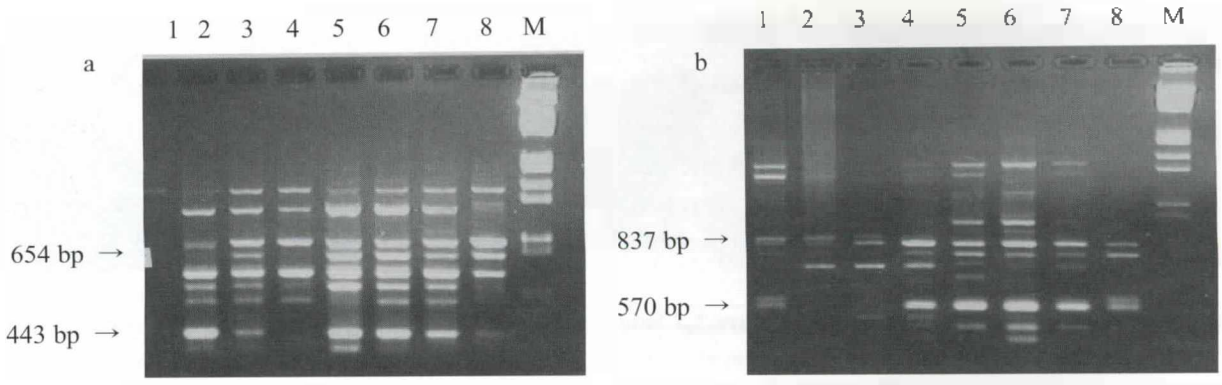
Genetic differentiation and similarity among the 3 goat populations

The relationships between the 3 goat populations were analyzed by RAPD marker (table 4).

It is known that RAPD marker was a dominant marker,

Table 1. Polymorphism of genome DNA-RAPD markers on 3 goat populations

Primer	Base sequence	CG			CCG			LCG		
		N	Marker band	Polymorphism band	N	Marker band	Polymorphism band	N	Marker band	Polymorphism band
CY13	ACGCTGCGAC	32	10	7	31	10	7	32	10	8
CY14	TGGTGCACTC	32	11	8	31	11	8	32	11	8
CY16	AAGGCACGAG	32	16	15	31	16	16	32	16	16
CY17	CCTCACGTCC	32	10	9	31	10	9	32	10	7
CY18	TCGCGGAACC	32	11	9	31	11	9	32	11	9
F-09	AAGGCGGCAG	32	15	13	31	15	13	32	15	12
OPQ05	CCGCGTCTTG	32	11	10	31	11	10	32	11	10
OPW19	CAAAGCGCTC	32	10	8	31	10	9	32	10	10



* 1-8 are results of RAPD-PCR, M indicates λDNA/HindⅢ+EcoR I marker

Figure 4. RAPD patterns generated by primer OPW19 (a) and CY18 (b)

Table 2. Length of DNA-RAPD markers on goat

Primer	Length of DNA fragment															
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
CY13	1,792	1,501	1,284	927	819	695	634	521	345	236						
CY14	2,937	2,054	1,590	1,372	1,091	856	756	602	306	254	198					
CY16	1,889	1,721	1,476	1,245	1,102	1052	886	829	746	691	560	478	351	249	176	
CY17	1,478	1,352	1,231	1,107	1,035	952	854	827	753	682						
CY18	2,054	1,985	1,456	1,102	837	764	685	570	548	389						
F-09	2,020	1,459	1,301	1,096	1,002	845	827	796	705	653	611	484	432	396	301	276
OPQ05	1,405	1,354	1,102	1,086	886	819	649	560	451	346	267					
OPW19	1,592	1,390	1,102	887	826	654	560	531	482	443	296					

Table 3. Heterozygosity of RAPD marker gene on 3 goat populations

Population	Heterozygosity of RAPD marker								Mean heterozygosity
	CY13	CY14	CY16	CY17	CY18	OPW19	F09	OPQ05	
CG	0.4387	0.5217	0.8357	0.4824	0.4670	0.4419	0.5318	0.4085	0.5148
CCG	0.4294	0.4916	0.8597	0.4313	0.4639	0.3878	0.5644	0.4708	0.5124
LCG	0.4365	0.5231	0.8044	0.3903	0.4667	0.4008	0.5422	0.4961	0.5075

Table 4. Genetic differentiation and genetic similarity among the goat populations

Population	Genetic differentiation			Genetic similarity	
	Hs	Ht	Gst	S_{ij}	D_{ij}
CG and CCG	0.5136	0.5391	4.37%	0.9892	0.0109
LCG and CCG	0.5100	0.5301	3.79%	0.9895	0.0106
CG and LCG	0.5112	0.5885	13.14%	0.9773	0.0230

protein marker was a co-dominant marker and both of them had their marker characteristics. It is believed that Rogers' genetic distance was more effective than that of Nei's and Hedrizk's on the analysis of population genetic relationship by using RAPD (Lu et al., 1997). Qin et al. (2000) estimated the genetic similarity index of 3 goat populations (i.e. Yadong goat and Gaoyuan goat from Tibetan of China, and Lvliang goat from Shaanxi province of China) by RAPD markers. The genetic relationships of 3 goat populations were studied by 2 methods (i.e. genetic differentiation coefficient and genetic similarity coefficient). The result showed that genetic relationships between LCG and CCG was the closest (D_{ij}=0.0106, Gst=3.79%), then CG and CCG (D_{ij}=0.0109, Gst=4.37%). However, the genetic relationship between CG and LCG was distant (Gst=13.14%, D_{ij}=0.0230). It is shown that the different

analytical methods had similar results, and RAPD marker was more effective in analyzing the genetic relationship of populations.

CONCLUSIONS

8 random primers were all amplified 94 bands in 3 goat population. The polymorphic bands and frequencies were 79, 81 and 80, 0.8404, 0.8617 and 0.8511, respectively, in CG, CCG and LCG. The mean loci heterozygosity was over 0.5 in the 3 goat populations. The results were similar by using genetic differentiation coefficient and genetic similarity coefficient to analyze the genetic relationship among the 3 goat populations. The genetic relationship between CG and LCG was the closest, then CCG and CG, and that between CG and LCG was distant.

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