Diversity in Six Goat Populations in the Middle and Lower Yangtze River Valley

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ABSTRACT : Amplified fragment length polymorphism (AFLPs) markers were used to investigate the genetic variation in six autochthonous goat populations distributed in the middle and lower Yangtze River valley. The goat populations were Chengdu Grey Goat (CGG), Chuandong White Goat (CWG), Banjiao Goat (BG), Matou Goat (MG), Hui Goat (HG) and Yangtze River Delta White Goat (YRDWG). A total of 180 individuals (30 per population) were analysed using ten selected AFLP primer combinations that produced 78 clear polymorphism loci. The variability at AFLP loci was largely maintained within populations, as indicated by the average genetic similarity, and they were ranged from 0.745 to 0.758 within populations and 0.951 to 0.970 between populations. No breed specific markers were identified. Cluster analysis based on Nei's genetic distance between populations indicated that Chengdu Grey Goat is the most distant population, while CWG and YROWG were the closest populations, followed by BG, HG and MG. Genetic diversity of the goat populations didn't confirm what was expected on the basis of their geographical location, which may reflect undocumented migrations and gene flows and identify an original genetic resource. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 2 : 277-281)***

Key Words : Genetic Diversity, Indigenous Goat Population, Yangtze River Valley, AFLP

INTRODUCTION

The autochthonous goats of the middle and lower Yangtze River valley are not classified into breeds. Most of the colonies are similar in phenotype and their names correspond to the names of their distributed sites. Smallholder peasants, primarily for meat production purpose, keep the majority of the goats. Most of the goat mating is uncontrolled and indiscriminate. These goat populations are reared for most of the year on pastures in the valleys. Goat production may contribute to farm income in many poor farmers; the breeding and genetic improvement of indigenous goats had been received more attention in these regions in the past five years (Jiang et al., 2001). Boer goat is used to crossbreed with most of the indigenous goat populations (Li et al., 2000; Ding et al., 2001). Although the heterosis was remarkable, there is a growing recognition of the need for conservation of goat diversity in this area. For this purpose, study on the characteristics of breeds and populations, including their genetic biodiversity and relationship, was conducted in China (Sun et al., 1997; Chang et al., 2000). Unfortunately, there was no systematic research as to populations across this region. Previous studies had investigated the phenotypic variability and protein polymorphisms of goat populations in this area (Jiang et al., 1988; Cheng et al., 1995).

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This paper here presented a study of genetic structure and relationships of 6 colonies, using amplified fragment length polymorphisms (AFLPs). AFLP are neutral and biallelic markers that are randomly distributed over the goat genome as restriction sites and are not likely to be affected by selection or/and environmental pressure, and they are used to investigated goat populations in many countries in recent years (Crepaldi et al., 2001; Ajmone-Marsan et al., 2001).

MATERIALS AND METHODS

Animals

Sampling method was simple random sampling in typical colony. In all cases, owners were questioned in detail to minimize the sampling of genetical closely related individuals. Every sample consisted of 30 individuals. The sampling site and sample size were listed in Table 1. The average body weight and body size of the six populations were listed in Table 2. The means were calculated from data cited by Jiang (1988). Chengdu Grey Goat (CGG) population has the highest body weight and body size, this is the only grey goat population in the six populations. The other five populations are all white type. Figure 1 marked the geographical distribution of the sampling locations.

Chengdu Grey Goat (CGG) has a grey coat of the brown type which are based on an eumelanic coat of the brown type with different pigmentation patterns and eventual dilution.with a significant black strip in the middle of the back from neck to tail, with short hairs and horns. This population is mainly distributed around Chengdu city, including Shuangliu county, jingtang County, Peng county, Guan county, Dayi county and Wengchuan county.

Table 1. The population size and sampling sites

Population name (abbreviation)	Size of population (thousand)*	Sampling site and sample size
Chengdu Grey Goat (CGG)	120	Jingtang county 20, Peng county 10, Sichuan province
Chuandong White Goat (CWG)	1,000	Wang county 30, Chongqing city
Banjiao Goat (BG)	100	Wuxi county 30, Chongqing city
Matou Goat (MG)	210	Simeng county 30, Hunan province
Hui Goat (HG)	5,000	Fuyang county 30, Anhui province
Yangtze River Delta White Goat (YRDWG)	3,000	Haimeng county 20, Haian 10, Jiangsu province

* Cited by Jiang (1988).

Table 2. Means (sample number) of body weight and body size of the six populations (Jiang et al., 1988)

Population	Body weight (kg)			Body size in adult (cm)		
	Birth	Yearling	Adult	Body height	Body length	Chest width
CGG	1.86 (107)	22.33 (107)	35.54 (107)	57.51 (246)	63.31 (246)	72.37 (246)
CWG	1.74 (90)	16.54 (556)	23.67 (1479)	49.71 (1479)	55.38 (1479)	64.54 (1479)
BG	1.71 (103)	21.72 (396)	28.48 (648)	50.79 (648)	58.64 (648)	68.62 (648)
MG	1.72 (98)	23.39 (329)	33.15 (477)	53.78 (42)	62.17 (42)	68.60 (42)
HG	2.52 (125)	19.48 (12)^{A}	27.03 (345)	64.20 (420)	65.96 (420)	76.67 (420)
YRDWG	1.12 (62)	16.07 (129)^{B}	18.01 (108)	48.65 (108)	51.70 (108)	60.93 (108)

A, 9 months old; B, 1.5 years old.

Figure 1. Geographical distribution of the sampling locations.
● Capital of the provinces.
1.CGG; 2. CWG; 3. BG; 4. MG; 5. HG; 6.YRDWG.

Chuandong White Goat (CWG) has a great variability in body conformation, mainly classed into big type and small type. All are white, with short hair and horns. CWG is mainly ditributed in Wan, Fulin and Dachuan areas.

Banjiao Goat (BG) has a white coat, with coarse short hair and long horns. This population is mainly distributed in Wanyuan, Chengkou, Wuxi and Wulong County.

Matou Goat (MG) mainly natived in Hunan and Hubei province. Most of the individuals are white coat type, with hair long or short and without horns.

Hui Goat (HG) has a white coat, short hair, with or without horns. This population as a popular breed is mainly distributed in Henan, Anhui and part of Jiangsu province.

Yangtze River Delta White Goat (YRDWG) has a white coat, with short hair and horns. This population is mainly distributed in Yangtze River Delta, including Haimeng, Qidong and Chongming County.

DNA preparation

About 0.15 g ear tissue was collected from every goat into Eppendorf tubes and transported to laboratory as quickly as possible. The tissue samples were stored in a -80°C freezer until DNA extraction. DNA was extracted from frozen ear tissue samples by referrence method (Meng et al., 1993). Extracted DNA was precipitatedly by gentle addition of 2.5 volumes of ethanol. The resulting DNA were pooled out and washed twice with ice cold 70% ethanol to remove excess salt and diluted to 25 ng/μl respectively .

AFLP methods

The AFLP protocol used in this experiment was modified from Cao (2000). Enzyme cleavage comprised a total reaction volume of 40 μl: ddH_2O 33.6 μl, 10×ligase buffer 4.0 μl, 10 mg/ml BSA 0.4 μl, 10 U/μl MseI 0.5 μl, 10 U/μl EcoRI 0.5 μl and 25 ng/μl DNA 1 μl. Incubated at 37°C for 3 h and 70°C for 15 min, then 10 μl ligation reaction mixture was added in the reaction . incubated at 22°C overnight and 70°C for 15 min . The ligation mixture comprised as: 10×Ligase buffer 1.0 μl, EcoRI adaptor pair (5 pmol) 1.0 μl, MseI adaptor pair (50 pmol) 1.0 μl, T_4 DNA ligase (3 U/μl) 1 μl, ddH_2O 6 μl.

MseI adaptor pair sequence is:
5'-GACGATGAGT CCTGAG-3'
3'-TACTCAGGACTCAT-5'
EcoRI adaptor pair sequence is:
5'-AATTGGTACGCAGTCTAC-3'
3'-CCATGCGTCAGATGCTC-5'
The pre-amplify primer nucleotides are Mse:
GATGAGTCCTGAGTAA
Eco:
GACTGCGTACCAATTC.

The pre-amplify PCR comprised a total reaction volume of 20 μl: 5.0 μl of template DNA, 0.2 μl of *Taq* polymerase (5 U/μl), 1.6 μl dNTPs (2.5 mmol/l), 1.0 μl of each pre

primer (75 ng/μl), 7.6 μl of ddH_2O, 1.6 μl of $MgCl_2$ (25 mmol/l) and 2.0 μl 10×Reaction buffer provided by the enzyme supplier. An Eppendorf Thermal Cycler was programmed for an initial incubation at 94°C for 3 min; 30 cycles each with denaturing at 94°C for 30s, annealing at 56°C for 30s and extention at 72°C for 1 min; and a final cycle at 72°C for 5 min.

Mse I/EcoRI AFLP markers were produced using the method as described by Cao (2000). Ten primer combinations were selected according to our previous experiment (Cao, 2000). The selection of these primer combinations was based on the reproducibility of the patterns, the level of polymorphism obtained and clarity of the bands for scoring. These selective nucleotides were MseACT/EcoAAC, MseCAC/EcoAAC, MseACC/EcoACC, MseCAG/EcoACG, MseACC/EcoACG, MseCAG/EcoACT, MseCAT/EcoACG, MseCAA/EcoACG, MseACT/EcoACG and MseCAA/EcoACT.

Statistical analysis

The AFLP polymorphisms were classified as dominant markers in the binary manner (1=band presence and 0=band absence). Allelic frequencies were calculated assuming AFLP loci in Hardy-Weinberg equilibrium. The method based on band sharing frequency and band frequency was tested to estimate genetic variation and relationship within and between populations. The band sharing frequency between goat populations of X and Y (BSF_{XY}) was calculated using the following formula:

$$BSF_{XY} = 2N_{XY}/(N_X + N_Y)$$

Where N_X and N_Y were the number of bands scored for goat population X and Y, respecteively; N_{XY} was the number of bands common to population X and Y.

The within population genetic similarity (WGS) was calculated as an average of BSF_{XY} across all comparisons between individuals within a population (Lynch, 1990).

The between population genetic similarity (BGS) corrected for WGS, was calculated according to Lynch (1990) as:

$$BGS_{ij} = 1 + s'_{ij}(S_i + S_j)/2,$$

Where S'_{ij} was the average of band sharing frequency estimates of the comparisons between population i and j, S_i and S_j were values of WGS for population i and j, respectively. S'_{ij} were used to determine genetic distance (D_{ij}) according to Lynch (1991) as follows:

$$D_{ij} = -\ln\left(S'_{ij}/\sqrt{S_i S_j}\right)$$

This formula was an analogue of Nei's (1978) estimator. Neighbour-joining tree based on the genetic distance was constructed by SGS software (Jiang et al., 2001).

RESULTS AND DISCUSSIONS

The selected primer combinations detected 971 bands and 78 of the bands have polymorphisms (as seen in Table 3). The average polymorphism percent was 8.03% (ranged approximately 4-16%). The MseCAG/EcoACG primer combination had the highest polymorphisms, about 16%.

Average genetic similarity

The ten AFLP primer combinations used to study the genetic variability of the six populations produced 78 polymorphic bands. No population specific markers were identified. The average genetic similarity within and between goat populations were listed in the diagonal and in the lower triangle respectively (see Table 4). The BGS values between every two populations were higher than the average WGS values of the same two populatons, which indicated that most of the diversity was within populations. From this standpoint, it could be said that the native populations contained abundance genetic variability. This was a good result for the genetic improvement of these goat populations.

Genetic distance

The genetic distance values calculated from the frequency data of AFLP alleles by using Nei index was given in Table 5. The dendrogram constructed from the Nei distance shewd in Figure 2.

Among the six populations, CGG was the most distant population, while CWG and YROWG were the closest populations, followed by HG, MG and BG (Table 5 and Figure 2). Genetic distance of the goat populations did not confirm what was expected on the basis of their geographical location but mostly confirmed their somatic

Table 3. Ten selected primer combination sequences and amplification results

Primer combination	Total bands	Polymorphism bands	Polymorphism percent
MseACT/EcoAAC	126	8	6.35
MseCAC/EcoAAC	110	8	7.27
MseACC/EcoACC	90	6	6.67
MseCAG/EcoACG	70	11	15.71
MseACC/EcoACG	75	5	6.67
MseCAG/EcoACT	118	11	9.32
MseCAT/EcoACG	43	4	9.30
MseCAA/EcoACG	101	12	11.88
MseACT/EcoACG	89	7	7.87
MseCAA/EcoACT	149	6	4.03

Table 4. Estimates of average genetic similarity (±SE) within (in the diagonal) and between (in the lower triangle) goat populations at AFLP loci

Population	YRDWG	HG	CWG	CGG	MG	BG
YRDWG	0.756±0.087					
HG	0.964±0.075	0.745±0.082				
CWG	0.970±0.081	0.969±0.081	0.752±0.086			
CGG	0.957±0.084	0.956±0.066	0.956±0.041	0.755±0.081		
MG	0.967±0.079	0.965±0.078	0.961±0.058	0.951±0.092	0.758±0.071	
BG	0.968±0.080	0.967±0.084	0.962±0.063	0.956±0.065	0.966±0.058	0.749±0.084

Table 5. Indexes of genetic distance (D_A) at AFLP loci for the six goat populations

Population	YRDWG	HG	CWG	CGG	MG
HG	0.047				
CWG	0.037	0.038			
CGG	0.059	0.060	0.060		
MG	0.042	0.045	0.052	0.068	
BG	0.041	0.042	0.050	0.060	0.044

Figure 2. Dendrogram of relationships among 6 goat populations.

measurements. This may reflect undocumented migrations and gene flows and may also identify original genetic resources. The five white populations should be clustered as one native breed and the colored population CGG as another. There were high correspondence beween this result and views of some animal breeding experts. White goat colonies distributed in the valley of middle and lower Yangtze River, for example, could be looked as a same native breed. It was treasurable to classify our goat colonies in this area into two original breeds and to make respective preservation strategy. The colored CGG breed should be paid more attention to preserve for its relatively small population size and limited diversity. The white breed distributed in broad areas and had several colonies. Each colony had big population number and abundunce diversity, so the white breed was a safety breed concerning genetic resource. The preservation of native breed diversity should base both upon phenotypic information and genome characteristics. Most of the penotype traits selected by the environmental and artificial pressure, such as disease resistance, were certainly worth preservation. From population size and diversity, the CGG population should be paid more attention to preserve immediately.

Amplified fragment length polymorphism (AFLP) was employed in present study, and typical colony was carefully selected and simple random sampling method was used to minimize the sampling of genetical closely related individuals. These allowed a relatively small sample to deduce accurate genetic relationship among colonies (Zhang, 2000). This strategy was important for research in large native animals, because these animals dispersed in broad areas; there were some difficulties to collect large samples. Many researchs employed the same strategy to probe genetic relations within species such as cattle, goat, pig and other farm animals (Ajmone-Marsan et al., 2001, 2002; Ovilo et al., 2000). Information obtained from molecular data may identify populations with an original evolutionary history and harbour original alleles for traits that are so far not subjected to selection pressure, but potentially useful in the future.

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