Genetic diversity of Tibetan goats of Plateau type using microsatellite markers

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Abstract

The 10 microsatellite markers (XBM7, XBM11, XBM16, XBM19, XBM24, XBM31, XBM84, TGLA53, SRCRSP-10 and ILS005) were selected to investigate the genetic diversity of Tibetan goat of Plateau type (NM, n=108), and the other 5 goat populations, i.e., Tibetan goat of Valley type (TG, n=36), Baiyu black goat (BY, n=36), Jianchang black goat (JC, n=36), Meigu goat (MG, n=36) and Xinjiang goat (XJ, n=32) were served as control. The mean polymorphism information content, heterozygosity and effective allele number of these 6 populations were 0.660/0.777/4.476, 0.716/0.797/4.9416, 0.631/0.673/3.061, 0.649/0.680/3.125, 0.629/0.680/3.125 and 0.561/0.793/4.840 respectively. The allele frequencies of Tibetan goat of plateau type in 10 microsatellite loci were greatly different with other 5 goat populations. The 6 goat populations were grouped into three distinct clusters: the Tibetan goat cluster (NM goat and TG goat), Sichuan goat cluster (JC goat, MG goat and BY goat), and Xinjiang goat cluster (XJ goat). These 3 distinct clusters were finally clustered together. The genetic differences among populations were in accordance with their geographical and historical origins.

Keywords: Microsatellite DNA, genetic diversity, Tibetan goat

Zusammenfassung

Genetische Diversität von Tibetischen Hochplateau-Ziegen unter Verwendung von Mikrosatellitenmarkern

Die 10 Mikrosatellitenmarker (XBM7, XBM11, XBM16, XBM19, XBM24, XBM31, XBM84, TGLA53, SRCRSP-10 und ILS005) wurden ausgewählt, um die genetische Diversität von Tibetischen Hochplateau-Ziegen (NM, n=108) zu untersuchen, und die anderen 5 Ziegenpopulationen, d. h. Tibetische Tal-Ziege (TG, n=36) schwarze Baiyu-Ziege (BY, n=36), schwarze Jianchang-Ziege (JC, n=36), Meigu-Ziege (MG, n=36) und Xinjiang-Ziege (XJ, n=32) und Xinjian-Ziege (XJ, n=32) dienten als Kontrolle. Der durchschnittliche Polymorphismus-Informationsgehalt, die Heterozygosität und die effektive Allee-Anzahl dieser 6 Populationen betrugen 0,660/0,777/4,476,0,716/0,797/4,9416,0,631/0,673/3,061,0,649/0,680/3,125,0,629/0,680/3,125 bzw. 0,561/0,793/4,840. Die Allelhäufigkeiten von Tibischen Hochplateau-Ziegen an 10 Mikrosatelliten-Loci unterscheiden sich sehr stark von den anderen 5 Populationen. Die 6 Ziegenpopulationen wurden in 3 distinkte Cluster gruppiert: das Cluster Tibetische Ziege (NM- und TG-Ziege), das Cluster Sichuan-Ziege (JC-, MG- und BY-Ziege) und das Cluster

Xinjiang-Ziege (XJ-Ziege). Diese drei distinkten Cluster wurden schließlich miteinander verclustert. Die genetischen Unterschiede zwischen den Populationen standen mit ihrer geografischen und historischen Herkunft im Einklang.

Schlüsselwörter: Mikrosatelliten DNA, genetische Vielfalt, Tibetische Ziege

Introduction

The Tibetan goat is mainly distributed in Qinghai-Tibet plateau of China. It is regarded as one of the world's most remarkable domestic animals as it thrives in conditions of extreme harshness and deprivation while providing meat and down for people. The archaeological evidence from Kaluo ruin in Changdu of Tibet suggests that the history of China's Tibetan goat industry is at least 4000 years old (Wang et al. 1993). At the present time, the total Tibetan goat population is estimated to number around 18 million in China. They were classified as plateau type and valley type (Ouyang et al. 1995). Tibetan goats are famous for their down production and good quality. In fact, what was called Cashmere goat by British businessmen in international cashmere trade during 1770s is Tibetan goat (Wang et al. 1994). There is, however, little study have been undertaken to investigate the genetic characteristics of Tibetan goat using molecular biological techniques. Microsatellites have been commonly utilized for the assessment of genetic diversity, construction of genetic maps, quantitative trait loci mapping and parentage testing etc. (Batendse et al. 1997, Buchanan et al. 1994, Li et al. 2004, Wang et al. 2004, Jin et al. 2005, Agha et al. 2008, Manatrinon et al. 2008, Ślaska et al. 2008, Kusza et al. 2010). Li et al. (2002) analyzed genetic relationships among twelve Chinese indigenous goat populations based on 26 microsatellite markers. Wang et al. (2006) studied microsatellite DNA polymorphism of 9 breeds (populations) of black goats in Sichuan province using 10 microsatellite markers, and the result showed these 9 breeds (populations) have a high genetic diversity. This observation is consistent with population's bodily form, economical purpose and geographical distributions. There is, however, no information is available for genetic diversity of Tibetan goat of plateau type by using microsatellites markers.

Therefore, the objective of this study was to analyze genetic diversity of Tibetan goat by using 10 microsatellite loci. The results would be useful for the protection and utilization of Tibetan goat genetic resources in China.

Materials and methods

Sampling and DNA extraction

Genomic DNAs were prepared from whole blood, which were collected from female goats of six populations: plateau-type Tibetan goats in Nima county of Tibetan Autonomous Region (NM, n=108), valley-type Tibetan goats in Mao county of Sichuan province (TG, n=36), Baiyu goats in Baiyu county of Sichuan (BY, n=36), Jianchang black goats in Huili county of Sichuan (JC, n=36), Meigu goats in Meigu county of Sichuan (MG, n=36), and Xinjiang goats in Changji city of Xinjiang Region (XJ, n=32). All breeds were kept at their own origin area. Individuals from each breed were sampled with the proportion of male:female equaling 1:4, according to Barker's (1994) guidelines for sample requirements of genetic diversity evaluation. Owners

were questioned in detail to minimize the sampling of closely related individuals. The original and distribution of these six populations were shown in Figure 1. Genomic DNAs from samples were extracted according to procedures described by Wang *et al.* (2006).

PCR and microsatellite analysis

Ten pairs of microsatellite primers used were synthesized (TakaRa, Dalian, China) and their sequences were shown in Table 1. PCR was accomplished in a total 15 μ l of the following mixture: 25 ng/ μ l of genomic DNA, 20 pmol/l of each primers, 2 U ExTaq DNA polymerase, 100 μ mol/l of each dNTPs, standard PCR buffer, 2 mmol/l MgCl₂ and ddH₂O. PCR amplification was as follows: first step was performed by initial denaturation for 4 min at 94 °C, followed by 33 cycles at 94 °C for 40 s, 54-59 °C for 40 s and 72 °C for 1 min. An extension at 72 °C for 5 min. PCR products were stored at 4 °C. Amplified fragments were analyzed on 9 % polyacrylamide denaturing sequencing gel which was stained in 0.1 % AgNO₃ solution. The PCR product size was calculated according to the pBR322 DNA/mspl marker on the computer.





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Loci	Forward 5`-3`	Reverse 5`-3`
XBM7	CTG TAT TAG AGT TCC CTG GAG AAA	GCC AAC ATG CCC TGT AGA AT
XBM11	TAC TGA TGG GAG GTT TCT GAG A	CCC AGA GTC TTT GTG TCA AGG
XBM16	AGG ATA ATT TGC TCT GTG CCC	ATG GCA ATA TGA GGA GTT GC
XBM19	AAG AAT CGG ACA TGA CTG AAT G	CCT CCT TCA TAA TCC ATT AAG CT
XBM24	TTA CCA CTG AGC CAC CTG G	ATG ATG CTT CTG TCA AGA GGT T
XBM31	GAT CCA ACG GAT GTT AGC AA	GCC ACA CAG TCA AAT GAA TCA
XBM84	TCA GGT GAA TAC TTT CCC ACG	TCC TTG TGT CCC TTT AGT TTT G
TGLA53	CAG CAG ACA GCT GCA AGA GTT AGC	CTT TCA GAA ATA GTT TGC ATT CAT GCA G
SRCRSP-10	ACC AGT TTG AGT ATC TTG CTT GGG	AGG AAG TTT ATT GGA CAG TGC TGG
ILS005	GGA AGC AAT GAA ATC TAT AGC C	TGT TCT GTG AGT TTG TAA GC

Table 1 The sequence structure of the 10 microsatellite primer pairs

Statistical analysis

Allele frequencies, polymorphism information content (PIC) (May *et al.* 1995), heterozygosity (H) (Heam *et al.* 1992), effective allele number (Ne) (Kimura & Crow 1974, Hines *et al.* 1981). The dendrogram was constructed by using UPGMA method from the calculated Nei's standard genetic distance (Da) (Nei 1978).

Results

Number of alleles and allele frequencies

Genotypes were observed according to the size of amplified fragments (a, b, c.....): a single band indicated homozygote; two bands indicated heterozygote (Figure 2). If SSR primers gave no amplified product were treated as missing values and they were discarded in the following study. The allele number and frequencies of 10 microsatellite loci in the 6 goat populations were shown in Table 2. The number of alleles per locus ranged from 3 (XBM31) to 8 (XBM16) with an average value of 5.7 in NM plateau type Tibetan goat. According to standard selection of microsatellite loci (Barker 1994), it has been suggested that microsatellite ought to have at least 4 alleles to be useful for the evaluation of genetic diversity, however, 3 alleles per locus were also used to evaluate genetic diversity in some studies (Li *et al.* 2010), therefore, all 10 microsatellites were used in this study.





There was a great difference in allele frequencies and genotype frequencies among different populations. For example, allele h (XBM16) was only observed in NM goat and TG goat with frequency of 0.017 where not observed in other breeds analyzed (BY goat, JC goat, MG goat and XJ goat). Allele a and b (ILS005 and XBM24) were not observed in XJ goat, but allele g (ILS005 and XBM24) was only observed in this population with frequency of 0.317 and 0.383, respectively. Allele f (XBM7) was only detected in NM goat, TG goat and XJ goat with frequency of 0.250, 0.250 and 0.333, respectively.

Locus allele	NM	TG	BY	JC	MG	ΧJ	Locus allele	NM	TG	BY	JC	MG	ΥJ
ILS005							XBM24						
а	0.167	0.083	0.150	0.217	0.133	0.000	а	0.200	0.183	0.267	0.217	0.250	0.000
b	0.000	0.167	0.067	0.050	0.050	0.000	b	0.117	0.150	0.350	0.350	0.350	0.000
с	0.150	0.117	0.217	0.250	0.250	0.133	с	0.150	0.150	0.083	0.067	0.067	0.000
d	0.200	0.167	0.217	0.200	0.283	0.233	d	0.300	0.250	0.300	0.233	0.183	0.000
e	0.283	0.267	0.167	0.233	0.250	0.000	e	0.233	0.150	0.000	0.133	0.150	0.317
f	0.200	0.200	0.183	0.050	0.033	0.317	f	0.000	0.117	0.000	0.000	0.000	0.300
g	0.000	0.000	0.000	0.000	0.000	0.317	g	0.000	0.000	0.000	0.000	0.000	0.383
XBM7							XBM84						
а	0.000	0.083	0.200	0.217	0.217	0.000	а	0.1	0.117	0.233	0.233	0.233	0.000
b	0.033	0.000	0.217	0.217	0.217	0.000	b	0.483	0.417	0.317	0.283	0.333	0.317
с	0.283	0.267	0.367	0.367	0.417	0.200	С	0.417	0.25	0.133	0.133	0.133	0.300
d	0.233	0.200	0.217	0.033	0.150	0.233	d	0.000	0.117	0.2	0.183	0.283	0.383
e	0.200	0.200	0.000	0.167	0.000	0.233	e	0.000	0.100	0.117	0.167	0.017	0.000
f	0.250	0.250	0.000	0.000	0.000	0.333							
SRCR-10							XBM19						
а	0.183	0.167	0.267	0.233	0.383	0.367	а	0.033	0.100	0.467	0.450	0.350	0.000
b	0.200	0.183	0.267	0.300	0.050	0.000	b	0.217	0.200	0.483	0.400	0.500	0.000
С	0.300	0.300	0.233	0.317	0.450	0.633	С	0.400	0.367	0.050	0.150	0.150	0.500
d	0.317	0.217	0.067	0.083	0.050	0.000	d	0.350	0.333	0.000	0.000	0.000	0.500
e	0.000	0.133	0.167	0.067	0.067	0.000							
XBM11							XBM31						
а	0.083	0.183	0.450	0.350	0.300	0.317	а	0.233	0.167	0.000	0.000	0.000	0.467
b	0.200	0.217	0.000	0.000	0.000	0.217	b	0.317	0.417	0.583	0.536	0.550	0.533
C	0.250	0.183	0.433	0.533	0.600	0.467	C	0.450	0.417	0.417	0.467	0.450	0.000
d	0.467	0.417	0.117	0.117	0.100	0.000							
XBM16							TGLA53						
a	0.000	0.133	0.150	0.117	0.183	0.167	a	0.150	0.100	0.000	0.067	0.067	0.000
b	0.000	0.000	0.383	0.400	0.267	0.000	b	0.167	0.150	0.017	0.067	0.067	0.000
c	0.000	0.000	0.400	0.267	0.267	0.000	c	0.100	0.067	0.250	0.050	0.050	0.000
d	0.300	0.233	0.000	0.150	0.167	0.000	d	0.150	0.133	0.100	0.050	0.083	0.267
e	0.333	0.300	0.000	0.000	0.000	0.300	e	0.233	0.167	0.233	0.350	0.283	0.233
t	0.350	0.317	0.067	0.067	0.117	0.267	f	0.200	0.150	0.067	0.083	0.083	0.250
g	0.000	0.000	0.000	0.000	0.000	0.267	g	0.000	0.133	0.167	0.167	0.200	0.250
h	0.017	0.017	0.000	0.000	0.000	0.000	h	0.000	0.100	0.167	0.167	0.167	0.000

Table 2
Allele frequencies of 10 microsatellite loci in the 6 goat breeds (populations)

Polymorphism information content (PIC), heterozygosity (H) and effective allele number (Ne)

The PIC, H and Ne of 10 microsatellite loci in six goat populations were shown in Table 3. The PIC ranged from 0.832 (TGLA53) to 0.526 (XBM31). The PIC in 10 microsatellite loci was higher than 0.5, indicated that the genetic diversity was abundant. The PIC was higher in

NM goat (0.660) and TG goat (0.716) than BY goat (0.631), GC goat (0.649), MG goat (0.629) and XJ goat (0.561).

The mean of heterozygosity of the six goat populations in 10 microsatellite loci was 0.733 ranged from 0.822 (XBM24 and TGLA53) to 0.617 (XBM11), revealed abundant polymorphism in these 10 microsatellite loci. Among populations, the mean of heterozygosity was low in MG goat (0.680), JC goat (0.680) and BY goat (0.673). However, NM goat and TG goat had a high heterozygosity, 0.777 and 0.797 respectively, which is greater than the expected heterozygosity (0.726 and 0.770, respectively). These observations indicated NM goat and TG goat were greatly different from MG goat, JC goat and BY goat in the genetic diversity.

Table 3

Polymorphism	information	content	(PIC),	heterozygosity	(H)	and	effective	allele	number	(Ne)	of	10
microsatellite lo	oci in 6 goat b	reeds (po	pulati	ons)								

Locus	Parameters	NM	TG	BY	JC	MG	XJ	Mean
ILS005	PIC	0.756	0.786	0.791	0.758	0.737	0.676	0.807
	Н	0.700	0.733	0.733	0.633	0.633	0.767	0.700
	Ne	3.333	3.745	3.745	2.725	2.725	4.292	3.428
XBM7	PIC	0.720	0.743	0.684	0.700	0.661	0.692	0.777
	Н	0.733	0.733	0.600	0.667	0.567	0.767	0.678
	Ne	3.745	3.745	2.500	3.003	2.309	4.292	3.266
SRCRSP-1	0 PIC	0.687	0.750	0.733	0.699	0.575	0.357	0.701
	Н	0.700	0.733	0.667	0.700	0.700	0.667	0.694
	Ne	3.333	3.745	3.003	3.333	3.333	3.003	3.292
XBM11	PIC	0.619	0.664	0.509	0.499	0.466	0.561	0.647
	Н	0.700	0.733	0.467	0.500	0.533	0.767	0.617
	Ne	3.333	3.745	1.876	2.000	2.141	4.292	2.808
XBM16	PIC	0.607	0.690	0.602	0.686	0.748	0.691	0.824
	Н	0.733	0.767	0.633	0.767	0.833	0.833	0.761
	Ne	3.745	4.292	2.725	4.292	5.988	5.988	4.505
XBM19	PIC	0.603	0.649	0.442	0.534	0.527	0.375	0.696
	Н	0.767	0.800	0.667	0.667	0.667	0.800	0.728
	Ne	4.292	5.000	3.003	3.003	3.003	5.000	3.884
XBM24	PIC	0.744	0.798	0.653	0.714	0.715	0.589	0.807
	Н	0.833	0.867	0.833	0.833	0.800	0.767	0.822
	Ne	5.988	7.519	5.988	5.988	5.000	4.292	5.623
XBM31	PIC	0.569	0.545	0.368	0.374	0.372	0.374	0.526
	Н	0.833	0.800	0.700	0.667	0.767	0.800	0.761
	Ne	5.988	7.519	5.988	5.988	5.000	4.292	5.796
TGLA53	PIC	0.798	0.852	0.786	0.778	0.806	0.702	0.833
	Н	0.900	0.933	0.800	0.733	0.733	0.833	0.822
	Ne	10.000	14.925	5.000	3.745	3.745	5.988	7.234
XBM84	PIC	0.494	0.686	0.738	0.752	0.689	0.589	0.715
	Н	0.867	0.867	0.633	0.633	0.567	0.933	0.750
	Ne	7.519	7.519	2.725	2.725	2.309	14.925	6.287
Mean	PIC	0.660	0.716	0.631	0.649	0.629	0.561	0.733
	Н	0.777	0.797	0.673	0.680	0.680	0.793	0.733
	Ne	5.128	6.175	3.655	3.680	3.555	5.636	4.639

The mean effective number of alleles per locus was 4.476 in NM goat, ranged from 3.333 (ILS005, SRCRSP-10, XBM11 to 10.000 (TGLA53). Among the different populations, TG goat had the highest mean effective number of alleles (4.916) ranged from 3.745 (ILS005, XBM 7, SRCRSP-10, XBM11) to 14.925 (TGLA53). BY goat had the lowest mean effective number of alleles (3.061) ranged from 1.876 (XBM11) to 5.988 (XBM24, XBM31). The mean effective number of alleles in 10 microsatellite loci was 3.750 ranged from 3.061 (BY goat) to 4.916 (TG goat), and the mean effective number of alleles per locus ranged from 2.609 (XBM11) to 5.623 (XBM24). These results indicated that the 10 microsatellite loci of these 6 goat populations had abundant genetic diversity.

Genetic distances and population relationship

Estimates of the Da genetic distances among the 6 populations were shown in Table 4. The smallest Da distances were observed between JC goat and MG goat (0.063), and the largest Da distances between BY goat and XJ goat. Using UPGMA cluster method, the dendrogram of relationships among these six goat populations was obtained (Figure 3). The NM goat and TG goat populations were grouped together. JC goat, MG goat and BY goat populations were grouped together, but the XJ goat has separate branch. These three distinct groups were finally clustered together.

Table 4

Nei's standard genetic distances (Da) below the diagonal and standard errors above the diagonal between NM and other 5 goat populations

Populations	NM	TG	BY	JC	MG	XJ
NM		0.008	0.133	0.106	0.085	0.086
TG	0.063		0.112	0.866	0.068	0.074
BY	0.318	0.235		0.009	0.017	0.216
JC	0.261	0.194	0.043		0.013	0.172
MG	0.266	0.200	0.042	0.026		0.166
XJ	0.311	0.270	0.464	0.435	0.404	



Figure 3

The UPMG dendrogram showing the genetic relationship among 6 goat populations using Nei's standard genetic distance for 10 microsatellite loci.

Discussion

Genetic diversity within populations

The level of mean population heterozygosity reflects the degree of population genetic consistency. The lower is the population heterozygosity, the higher is the population genetic consistency and *vice versa* (Barker 1994). The present work showed that plateau type Tibetan goat and the other five goat population had a high heterozygosity in 10 microsatellite loci within population with an average of 0.733 ranged from 0.673 (BY goat) to 0.797 (TG goat) (Table 3). These results showed abundant polymorphism of these populations, i.e., a small effort for breeding has been performed in these populations. The effective number of alleles is an estimate of the number of alleles with equal frequencies corresponding to a particular PIC value. It is an inverse function of the theoretical homozygosity and it allows comparison of populations with different distributions of allele frequencies, reducing the effect of infrequent alleles (Barker 1994, Jin *et al.* 2005). The mean effective allele numbers were highest in XJ goat (4.840) and lowest in NM Tibetan goat (4.476) in this study.

Morin *et al.* (1994) reported that the polymorphism of microsatellite loci can reflect the evolution history of populations. The alleles with the highest frequency within a population are the most original and conservative, and the other alleles are originated from them during evolution by mutation. Accordingly, allele c and e (ILS005), d (XBM7), c (SRCRSP-10, XBM11), d (XBM24), b (XBM31, XBM84) and e (TGLA53) were the original alleles of these microsatellite loci in these goat populations. Some alleles observed or not observed in other microsatellite loci within goat populations should be as a result of evolution or mutation. These loci can be used as candidate microsatellite markers for studying population characteristics.

Genetic relationship analysis between populations

The allele frequencies were greatly different in different populations. In addition, some alleles were only detected in some populations, for example, allele h (XBM16) was only observed in Tibetan goats (both NM goat and TG goat) with frequency of 0.017; a and b (ILS005 and XBM24) were not observed in XJ goat, but g was only observed in this population with the frequency of 0.317 and 0.383. These variations might be related to the genetics and variation, evolution, selection and ecological conditions (Chen & Ma 2001, Li *et al.* 2002).

The mean polymorphism information was an ideal index to measure the polymorphism of allele fragments. PIC>0.5, indicated the locus of high-polymorphism; 0.25<PIC<0.5, indicated the locus of medium- polymorphism; PIC<0.25, indicated the locus of low-polymorphism (Cho 2006, Wang *et al.* 2006). In the present study, PIC of Tibetan goat of plateau type (0.660) and valley type (0.716) were higher than the other four goat populations, but the PIC of other 4 populations were also higher than 0.5 ranged from 0.561 to 0.649. These results indicated that genetic information was also abundant in these populations.

The 6 goat populations are grouped into three distinct clusters: the Tibetan goat cluster (NM goat and TG goat), Sichuan goat cluster (JC goat, MG goat and BY goat) and Xinjiang goat cluster (XJ goat). These three distinct groups are finally clustered together. These clustering results were in accordance with their geographical and historical origins of these 6

populations (Zheng 1989, Sun 1997). TG goat is distributed in Sichuan, but its historical origin is Tibetan goat. In contrast, BY goat is distributed in Baiyu county, Qinghai-Tibet Plateau, but its historical origin is not Tibetan goat (Figure 1 and 3). Although genetic diversity was not studied in these goat populations by molecular biological methods, evidences in chicken and pigs obtained by microsatellite markers (Chen *et al.* 1991, Wang *et al.* 2004) are consistent with those by blood protein markers and mtDNA diversity (Chen *et al.* 1991, Huang *et al.* 1998, Mo *et al.* 2003).

In conclusion, Tibetan goat of plateau type has abundant genetic diversity. It is a unique gene pool, developed for a long history by natural selection and artificial selection, with a good adaptation to the extremely cold and low oxygen conditions. The results would be useful for the protection and utilization of Tibetan goat genetic resources in China.

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