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Study on polymorphisms of microsatellite DNA of six Chinese indigenous sheep and goat breeds

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Abstract The genomes of six populations were screened using microsatellites as molecular markers, including Ujmuqin sheep, small-tailed Han sheep, Tan sheep, Hu sheep, Tong sheep and Yangtse River Delta (YRD) white goat. A total of seven microsatellite markers were used and genetic diversity and genetic distance were also determined. The results showed that there were 224 alleles in six populations, all seven loci showed polymorphism in all populations. The average heterozygosity of all populations was 0.949 9, and the mean polymorphism information content (*PIC*) of all six populations was 0.842 5–0.929 4. The six sheep (goat) populations were lowly differentiated with all loci, and the coefficient of phenotypic differentiation (*F_{st}*) was 2.6%, which was consistent with the coefficient of gene differentiation (*G_{st}*). The global heterozygote deficit across of all populations (*F_{it}*) amounted to 0.5%. The overall significant deficit of heterozygotes because of inbreeding within breeds (*F_{is}*) amounted to –2.2%. Two Unweighted Pair-group Method using Arithmetic Averages (UPGMA) dendrograms were constructed on the basis of Nei's standard genetic distance (*DS*) and Nei's genetic distance (*DA*) respectively. Hu sheep and Tong sheep were grouped at first, Ujmuqin sheep and small-tailed Han sheep clustered and then clustered with Tan sheep. Finally, Yangtse River Delta white goat joined in with all above. From this study, Ujmuqin sheep belongs to "Mongolia sheep" group, which corresponds with the historical records exactly. Ujmuqin sheep and small-tailed Han sheep, Tan sheep, Hu sheep and Tong sheep all vest in the "Mongolia sheep" group.

Keywords sheep, goat, microsatellite DNA, genetic diversity, phylogenesis

1 Introduction

Among the currently used molecular marker systems for genetic characterization, microsatellites, also known as simple sequence repeats (SSRs), are the most preferred because of their extremely informative polymorphic nature, their abundance in the genome, and the ease of amplification and typing by polymerase chain reaction (PCR). They are widely used for DNA fingerprinting, paternity testing, construction of linkage maps, population genetic studies (Li et al. 2005; Osmá et al., 2005), individual identification, heterosis analysis, genetic bottleneck analysis, estimation of cultivative power of discrimination (Olowofeso et al., 2005; Pandey et al., 2005; Zhang et al., 2005) and marker-assisted selection (Casacuberta et al., 2000).

The conservation of genetically unique breeds/populations is of a top priority to prevent loss of genetic diversity within each domestic species. In view of the massive costs involved, it is impossible to implement a conservation and improvement program for each livestock breed, and clustering derived from molecular characterization of general variability will provide valuable evidence to prioritize the breeds in terms of phylogenetic distinctness. This will help breeders to implement rational decisions for the conservation and improvement of valuable germplasm. With regards to sheep and goat, there are abounding resources in China. There are three systems of sheep in China including Mongolia sheep, Tibetan sheep and Kazakhstan sheep (Editorial Group of Sheep and Goat Breeds in China, 1989a). Owing to long-term nature and artificial selection, transmutation of physical environment and hybridization, many illustrious local varieties have been formed. More recently, with the generalization of modern livestock, numerous sheep and goat populations have been extinct. To be faced with the serious situation, measures must be taken to protect our resources. Ujmuqin sheep distributed in Ujmuqin County, Inner Mongolia, China, is a dual-purpose breed

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valued for its meat as well as wool utility. Because of high quality grass in the area, the breed grows fast with large body and rich fat content in body conformation (Editorial Group of Sheep and Goat Breeds in China, 1989b). But as exotic breeds importing and crossing, the number of pure breed Ujmuqin sheep decreased rapidly. The present study wants to describe the constitution diversity of the six native sheep and goat populations and phylogenesis of Ujmuqin sheep, so as to provide a basis for sheep and goat husbandry and the genetic resources protection.

2 Materials and methods

2.1 Sampling methods and experimental materials

One hundred and twenty-three Ujmuqin sheep were sampled from Ujmuqin of Mongolia in China; 60 small-tailed Han and 73 Tan sheep were from Liangshan Town of Jining City in Shandong Province and Yanchi countryside of Ningxia Province of China, respectively; 61 Hu and 65 Tong sheep came from Suzhou City of Jiangsu Province and Baishui countryside in Shanxi Province of China, respectively; 29 Yangtse River Delta (YRD) white goats were from the suburb of Yangzhou City of Jiangsu Province of China. The method of "Random sampling in typical colonies of central area" (Chang et al., 1995) was applied and it was tried to avoid sampling two (or more) individuals that have traceable genetic relationship. Their blood samples were collected from the cervical vein of each animal. Each blood sample was put into a centrifuge tube using heparin as an anticoagulant. The genomic DNA was extracted from whole blood using proteinase K digestion followed by the standard phenol-chloroform extraction procedure.

2.2 Microsatellite loci analysis

According to genebank and the Meat Research Center of US Ministry of Agriculture and previous studies, a panel of seven

sheep microsatellite markers was selected (Yang et al., 2004). Their characteristics are shown in Table 1. PCR amplification was performed on a HBPX220 (Hybaid Ltd., UK). Each 20 μ L PCR reaction contained 100 ng template DNA, 2 μ L 8 pmol/ μ L each primer, 0.4 μ L 10 mmol/ μ L dNTP, 1.0–2.5 μ L 25 mmol MgCl₂, 0.2–0.4 μ L 5 U Taq DNA polymerase, 2 μ L 10 \times buffer. An initial denaturizing at 94°C for 5 min was followed by 30 cycles of 1 min at 94°C denaturizing, 1 min of 53–63.5°C annealing and 1 min of 72°C extension. The final cycle was followed by an extension at 72°C for 10 min. Then the product was conserved at 4°C.

The amplified fragments were electrophoresed on a 10%–14% polyacrylamide gels in 1 \times TBE with 150–180 v of running voltage. Then the gels were detected by ethidium bromide staining. The fragment sizes were calculated by using the Kodak Digital Science ID Image Analysis Software. The genotype of each individual animal at seven different loci was recorded by direct counting.

2.3 Statistical analysis

Allele frequencies (P) on seven microsatellite loci were determined by direct counting. Genetic variability measures such as average heterozygosity (H) (Nei, 1978), information content of polymorphism (PIC) (Bostein et al., 1982), number of effective allele (N_e) (Kimura and Crow, 1974) were calculated for each population. The genetic distance (Nei, 1972) and the dendrogram between populations using UPGMA method were estimated with Population (1.2.28) and Treeview. For each locus, FSTAT software (V2. 9.3) was used to calculate the global heterozygote deficit across of all populations (F_{it}), the coefficient of phenotype differentiation (F_{st}) and the overall significant deficit of heterozygotes because of inbreeding within breeds (F_{is}) according to Weir and Cockerham (1984). Island model (Slatkin, 1993) was used to analyze gene flow between populations. The average number of effective migrants exchanged per generation (N_{em}) was calculated with the flowing formula: $N_{em} = (1 - F_{st}) / (4F_{st})$.

Table 1 Primer sequence, chromosome assignment, annealing temperature and MgCl₂ volume

Marker	Chromosome assignment	Primer sequence	Annealing temperature/°C	25 mmol·L ⁻¹ MgCl ₂ /μL
MAF70	4	(CA strand)GCAGGACTCTACGGGGCCTTTGC (GT strand)CACGGAGTCACAAAGAGTCAGACC	58–65.5	1.3–1.9
OarFCB11	2	(CA strand)GGCCTGAACTCACAAGTTGATATATCTATCAC (GT strand)GCAAGCAGGTTCTTTACCACTAGCACC	63–65	1.3–2.5
OarAE101	6	(CA strand)TTCTTATAGATGCACTCAAGCTAGG (GT strand)TAAGAAAATATATTTGAAAAAACTGTATCTCCC	58.3–59	2.2–3.0
OarFCB48	17	(CA strand)GAGTTATGTACAAGGATGACAAGAGGCAC (GT strand)GACTCTAGAGGATCGCAAAGAACCAG	53	1.8–2.6
MAF33	9	(CA strand)GATCATCTGAGTGTGAGTATATACAG (GT strand)GACTTTGTTTCAATCTATTCCAATTTTC	58–63.2	1.8–2.2
OarFCB128	2	(CA strand)CAGCTGAGCAACTAAGACATACATGGCG (GT strand)ATTAAGCATCTTCTCTTTATTTCCTCGC	59.8–60.2	1.8–2.6
OarFCB304	19	(CA strand)CCCTAGGAGCTTTCAATAAAGAATCGG (GT strand)CGCTGCTGTCAACTGGGTCAGGG	61–66	1.8–2.5

3 Results

3.1 Structural and microsatellite loci characterization

The number of alleles observed at every single microsatellite locus ranged from 26 (MAF70) to 46 (OarFCB304), with an average number of alleles per locus of 32. For each population, there were at least seven alleles per locus. This showed that microsatellite markers could give a lot of genetic information. The numbers of alleles determined on seven microsatellite loci in six sheep (goat) populations were showed in Table 2.

From Table 2, it was found that there were great differences in the number of alleles among populations, which concluded that different breeds would show a great genetic diversity, owing to geographical isolations and different evolutionary processes. When comparing the number of alleles and effective alleles in the six populations, it was found that their values were different, which indicated that alleles in each population were distributed unevenly. The reason could be geographical isolation and artificial selection.

3.2 Diversity analysis

Diversity measures were calculated on the basis of allele frequencies. Tables 3 and 4 show the heterozygosity (H) and information content of polymorphism (PIC) for each population respectively. From the results, it could be seen that the mean heterozygosity of the six sheep (goat) populations ranged from 0.755 8 to 0.933 6. The highest was in the Han

sheep (0.933 6), and the YRD was the lowest (0.755 8). The results of the heterozygosity were closely consistent with those of PIC . The highest value of H was MAF70 (0.966 1), and the lowest was OarFCB11 (0.935 1). The gene heterozygosity of all populations at all loci was high (0.949 9). Meanwhile, the coefficient of gene differentiation was low (0.036 7).

The three fixation indexes (F_{it} , F_{st} and F_{is}) could reflect the extent of inbreeding in populations. The greater the value was, the more obvious the deviation of Hardy-Weinberg. From the result of FSTAT statistics (Table 5), it was concluded that the extent of inbreeding in the six populations was low, 2.6% of hereditary variation was caused among populations, while 97.4% of genetic variation was from individual variation within populations, which was consistent with the coefficient of gene differentiation (G_{st}). The results certified that large gene migration could exist among populations.

Pairwise estimates of F_{st} and N_{em} of the six sheep (goat) populations were shown in Table 6. It was found that the value of F_{st} was larger between five sheep populations and goat population than that within sheep populations, which induced that the number of effective migrants exchanged per generation (N_{em}) was lower. This conclusion exactly corresponded with the feature of population differentiation. Meanwhile, according to the conclusion, it was considered that this fact reflected the validity of this statistical method laterally. Among sheep populations, F_{st} was the largest between Tan and Hu sheep (0.040 0), while the lowest between Tong and Hu sheep.

Table 2 The number of alleles determined and effective number of alleles (N_e) on seven microsatellite loci in six sheep (goat) populations

Marker	Ujmuqin		Han		Tan		Hu		Tong		YRD	
	N	N_e	N	N_e	N	N_e	N	N_e	N	N_e	N	N_e
MAF70	23.00	11.91	22.00	16.18	20.00	11.06	16.00	10.08	17.00	9.438	16.00	10.67
OarFCB11	21.00	9.794	21.00	16.46	22.00	12.99	22.00	17.78	19.00	13.27	13.00	8.022
OarAE101	17.00	10.38	14.00	9.208	15.00	9.483	10.00	7.484	14.00	10.65	21.00	16.06
OarFCB48	28.00	13.3	21.00	12.09	16.00	9.837	23.00	13.97	21.00	14.2	17.00	12.42
MAF33	24.00	13.7	26.00	21.19	21.00	9.548	20.00	10.6	16.00	10.63	7.00	13.5
OarFCB128	23.00	13.84	25.00	16.23	22.00	15.87	16.00	11.37	17.00	14.04	14.00	11.54
OarFCB304	31.00	20.1	15.00	23.81	23.00	13.04	20.00	11.13	20.00	14.95	35.00	16.82
Average	23.86	13.29	20.57	16.45	19.86	11.69	18.14	11.77	17.71	12.45	17.57	12.72

Table 3 Gene heterozygosity and polymorphism information content (PIC) per locus and population

Marker	Ujmuqin		Han		Tan		Hu		Tong		YRD	
	H	PIC	H	PIC	H	PIC	H	PIC	H	PIC	H	PIC
MAF70	0.916 0	0.910 0	0.938 2	0.934 7	0.909 6	0.902 8	0.900 0	0.892 6	0.900 8	0.885 6	0.906 2	0.899 2
OarFCB11	0.897 9	0.889 4	0.939 2	0.935 9	0.923 0	0.917 8	0.943 8	0.940 9	0.924 6	0.919 8	0.875 3	0.863 8
OarAE101	0.903 8	0.896 2	0.891 4	0.881 6	0.894 5	0.885 5	0.866 4	0.852 0	0.906 1	0.898 7	0.937 6	0.934 3
OarFCB48	0.924 8	0.927 4	0.917 3	0.911 4	0.898 3	0.889 8	0.928 4	0.924 0	0.929 6	0.925 2	0.919 4	0.913 8
MAF33	0.927 0	0.922 4	0.952 8	0.950 7	0.895 3	0.886 6	0.905 6	0.898 5	0.905 9	0.898 6	0.714 4	0.686 9
OarFCB128	0.927 8	0.923 3	0.938 4	0.935 1	0.937 0	0.933 4	0.91 2	0.905 5	0.928 8	0.924 2	0.906 8	0.906 8
OarFCB304	0.950 2	0.948 0	0.958 0	0.956 3	0.923 3	0.918 2	0.910 1	0.903 6	0.933 1	0.929 1	0.937 4	0.937 4
Average	0.921 1	0.916 7	0.933 6	0.929 4	0.911 6	0.904 9	0.909 4	0.902 4	0.918 4	0.911 6	0.755 8	0.877 5

Table 4 Gene heterozygosity and coefficients of gene differentiation at each locus

Marker	<i>Hs</i>	<i>Ht</i>	<i>Gst</i>
MAF70	0.935 9	0.966 1	0.028 4
OarFCB11	0.901 4	0.935 1	0.030 1
OarAE101	0.922 1	0.944 9	0.036 1
OarFCB48	0.885 2	0.956 0	0.024 1
MAF33	0.913 4	0.940 1	0.074 0
OarFCB128	0.921 6	0.950 2	0.032 5
OarFCB304	0.926 0	0.957 1	0.031 2
Average	0.915 1	0.949 9	0.036 7

Note: *Hs* means heterozygosity for each group; *Ht* means heterozygosity for the whole group.

Table 5 Results of *F*-statistics for each of seven markers across six sheep (goat) populations

Loci	<i>Fis</i>	<i>Fst</i>	<i>Fit</i>
MAF70	-0.063 0	0.024 0	-0.037 0
OarFCB11	-0.04 0	0.019 0	-0.02 0
OarAE101	-0.038 0	0.031 0	-0.006 0
OarFCB48	-0.044 0	0.017 0	-0.026 0
MAF33	-0.006 0	0.054 0	0.048 0
OarFCB128	0.034 0	0.013 0	0.046 0
OarFCB304	-0.001 0	0.026 0	0.025 0
All loci	-0.022 0	0.026 0	0.005 0

Table 6 Pairwise estimates of *Fst* (below diagonal) and *Nem* (above diagonal) of six sheep (goat) populations

Populations	Ujumuqin	Han	Tan	Hu	Tong	YRD
Ujumuqin		22.686 0	10.911 0	9.044 0	10.434 0	5.862 0
Han	0.010 9		12.313 0	12.771 0	13.795 0	5.445 0
Tan	0.022 4	0.019 9		6.000 0	6.543 0	3.835 0
Hu	0.026 9	0.019 2	0.040 0		44.393 0	4.852 0
Tong	0.023 4	0.017 8	0.036 8	0.005 6		5.306 0
YRD	0.040 9	0.043 9	0.061 2	0.049 0	0.045 0	

Table 7 showed that Nei's standard genetic distance (DS) and Nei's genetic distance (DA) between Hu and Tong were the smallest (0.062 5 and 0.171 7). Comparing with Ujumuqin and other sheep populations, Ds and DA genetic distances between Ujumuqin and Han sheep were the smallest (0.180 2 and 0.143 9). The two genetic distances between sheep and goat were rather greater than those in sheep.

Generally, the shorter time the populations differentiate, the smaller the genetic distance is. Two UPGMA dendrograms were constructed on the basis of Nei's standard genetic

Table 7 Standard genetic distance (DS) (below diagonal) and genetic distances (DA) (above diagonal) of six populations

Population	Ujumuqin	Han	Tan	Hu	Tong	YRD
Ujumuqin		0.143 9	0.194 8	0.256 1	0.248 3	0.445 6
Han	0.180 2		0.227 1	0.247 0	0.240 9	0.515 5
Tan	0.331 8	0.300 3		0.397 7	0.376 5	0.587 1
Hu	0.432 4	0.304 8	0.644 2		0.171 7	0.49 8
Tong	0.388 5	0.302 5	0.591 6	0.062 5		0.527 7
YRD	0.712 8	0.880 9	1.174 2	0.907 9	0.845 7	

distance and Nei's genetic distance respectively. Hu sheep and Tong sheep were grouped first, Ujumuqin sheep and small-tailed Han sheep clustered and then clustered with Tan sheep. Finally, YRD white goat joined with all above (Figs. 1 and 2).

4 Discussion

Gene heterozygosity is gene variability. The average of heterozygosity could reflect the level of genetic variation. Information content of polymorphism (*PIC*) was a diversity index. The higher the *PIC* value was, the greater the rate of heterozygote. Arranz et al. (1998) analyzed genetic diversity of six Spain sheep populations, and results showed the mean heterozygosity for each population was from 0.713–0.771; Wang et al. (2006) studied genetic diversity among seven goat populations in the Middle and Lower Yangtse River Valley and East and South Mainland near the sea, and the mean heterozygosity was from 0.677 to 0.737. Our study has demonstrated far greater heterozygosity than all the above studies (0.755 8–0.933 6). This high value could be concerned with the selection of microsatellites. This observation indicated that the six sheep (goat) populations adapted their living environments individually through chronic acclimatization. Our observations with sheep and goats also showed that the conservation of microsatellite markers between these two related species existed in all microsatellite loci detected in this study. According to Bolstein et al. (1980), these seven microsatellite loci involved in this research were all highly polymorphic. Therefore, it was considered that the seven microsatellite markers suited the study of genetic diversity in sheep and goat populations generally.

Fis, *Fst* and *Fit* are parameters to estimate inbreeding coefficient. *Fis* represents the heterozygote deficit within populations. The results showed that all of the populations had a lower inbreeding coefficient, which was consistent with the high heterozygosity. The six sheep (goat) populations were lowly differentiated with all loci, which was consistent with *Gst*. The coefficients of genetic differentiation at the structural loci were all below 7.34% (Lu et al., 2005). Using microsatellites to study the determination of evolutionary relationships among sheep breeds, the genetic differentiation at all loci was extremely low (Buchanan et al., 1994). According to the genetic diversity analysis among twelve Chinese indigenous goat populations based on microsatellite, the low genetic differentiation at all loci was also found (Li et al., 2002). The results of this study corresponded with the historical records exactly.

It could also be noticed that breeds with close relationships had the similar genetic diversity. Compared with goat breeds, sheep breeds had similar genetic diversity. Using microsatellite markers, it was discovered that the two kinds of genetic distances between sheep and goat were rather greater than those in sheep, which reflected the objectivity of microsatellite markers indirectly. Genetic distances could reflect phylogenetic evolution of the studied populations. Kinship

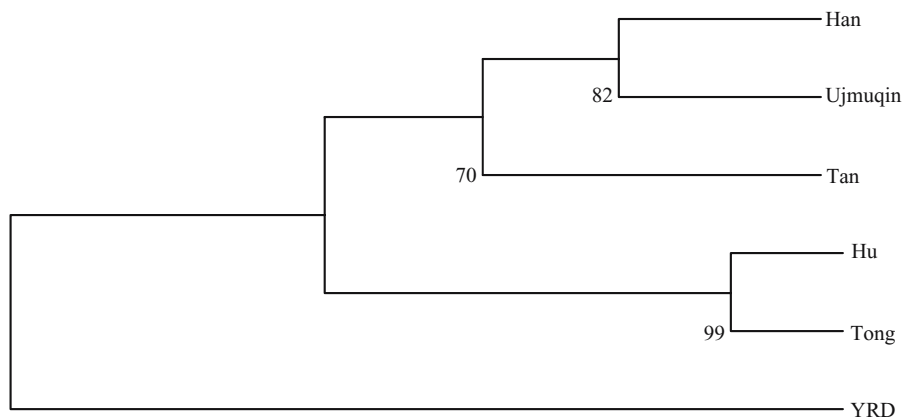


Fig. 1 UPGMA cluster figure based on standard genetic distances

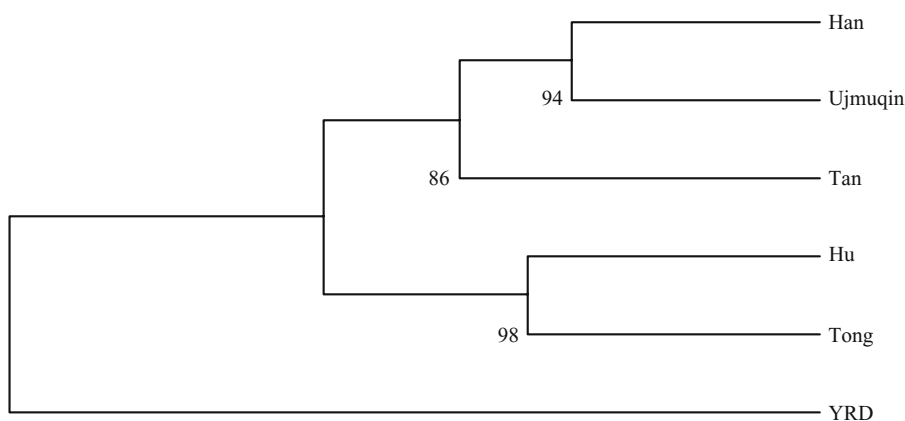


Fig. 2 UPGMA cluster figure based on DA distances

between Ujmuqin and Han sheep was close (Wang et al., 2004). This study using random amplified polymorphic DNA (RAPD) showed that Ujmuqin sheep, Han sheep, Tan sheep and Hu sheep were recognized belonging to the Mongolian group (Gong et al., 2002). According to the traceable origin and breeding history (Lei, 1999) and Yang et al. (2004), there were close genetic relationships among Ujmuqin sheep, Han sheep, Tan sheep, Hu sheep and Mongolian sheep populations. From this study, it could be concluded that Ujmuqin sheep belongs to the “Mongolia sheep” group; the results correspond with the historical records exactly. Ujmuqin sheep and small-tailed Han sheep, Tan sheep, Hu sheep, Tong sheep are also part of the “Mongolia sheep” group.

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