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RESEARCH ARTICLE

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Genetic differentiation and gene flow among six sheep breeds of Mongolian group in China

Abstract The level of genetic differentiation, gene flow and the relationship between geographical distance and genetic differentiation among six sheep populations of Mongolian group in China (Tong sheep, small-tailed Han sheep, Hu sheep, Tan sheep, Ujumuqin sheep and Bayinbuluk sheep) were analyzed using seven microsatellites. The trees were constructed from diversity coefficient (DC) distances among the six sheep populations. The overall heterozygote deficit across all the populations (F_{it}) was between 0.167 (OarAE101) and 0.044 (MAF33). The overall significant deficit of heterozygote, because of inbreeding within breeds, (F_{is}) was between 0.089 (OarFCB304) and 0.005 (MAF33). The coefficient of genetic differentiation (F_{st}) was between 0.100 (OarAE101) and 0.022 (OarFCB48). It indicated that 3.9% of the total genetic variation could be explained by breed differences and the remaining 96.1% by differences among individuals for each population. This illustrated that most variations existed within breeds and genetic differentiation level were very low among sheep breeds of the Mongolian Group in China. The average number of effective migrants exchanged per generation (*Nem*) ranged from 2.7369 (Tan sheep and Bayinbuluk sheep) to 44.3928 (Tong sheep and Hu sheep), and the mean value was 11.25213. Significantly positive relationships between the level of genetic differentiation and geographical distance and genetic distances were detected. It is concluded that genetic differentiation of sheep breeds of Mongolian group in China is mainly the result of natural selection (different living conditions).

Keywords sheep of Mongolian group, microsatellite, genetic differentiation, gene flow

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1 Introduction

Domesticated sheep originating from wild sheep is the earliest acclimated livestock following dogs. The history of domesticated sheep and goats has been long (Zou et al., 1994). With regards to sheep and goats, there are abundant resources in China. There are three systems of sheep in China including Mongolia sheep, Tibetan sheep and Kazakhstan sheep (Zou et al., 1994). Owing to long-term nature and artificial selection, transmutation of physical environment and hybridization, many illustrious local varieties have been formed and distributed in pastures and agro-areas extensively. Owing to extensive geographic distribution and adaptability, the differentiations have existed in traits of ecosystem, morphology and genetics (Yang et al., 2003; Lu et al., 2005; Yang et al., 2004b; Jia et al., 2003; Chu et al., 2002; Gen et al., 2002). In order to better understand their genetic heritage, we have carried out some work to preserve and develop the local sheep breeds (Yang et al., 2004a). Based on Yang et al. (2004b) and Gen et al. (2002), the present study investigated the level of genetic differentiation, gene flow and the relationship between geographical distance and genetic differentiation among six sheep populations of Mongolian group in China using 7 microsatellite loci.

2 Materials and methods

2.1 Sampling methods and experiment materials

The sampling process is of great importance since it will determine the kind of inferences which can be made. In order to reflect the current genetic composition and try to ensure that the animals chosen were unrelated, individuals were sampled using the method of ''Random sampling in typical colonies of central area''. Ujmuqin sheep ($n = 123$) were sampled from the Ujmuqin of Mongolia in China. Small-tailed Han ($n = 60$) and Tan sheep ($n = 73$) were from the Liangshan Town of Jining city in Shandong Province and the Yanchi countryside of Ningxia Hui

Autonomus Region of China, respectively. Hu ($n = 61$) and Tong sheep ($n = 65$) came from Suzhou city of Jiangsu Province and the Baishui countryside in Shanxi Province of China, respectively. Bayinbuluk sheep ($n = 71$) were from Bayinbuluk in the Hejing county of Inner Mongolia in China. Blood samples collected from the cervical vein were put into a centrifuge tube using heparin as an anticoagulant. The genomic DNA was extracted from whole blood using proteinase K digestion followed by a standard phenol-chloroform extraction procedure.

2.2 Microsatellite loci analysis

According to GenBank and the Meat Research Center of US Ministry of Agriculture, a panel of 7 sheep microsatellite loci was selected (the names of each primer were shown in Table 1), which have been researched in many sheep populations in our laboratory. All of the primers were synthesized by the Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. PCR was accomplished in a total volume of 20 μL of the following mixture: 100 ng template DNA, 1 μL 8 pmol·μL^{-1} each primer, 0.4 μL 10 mmol·μL^{-1} dNTP, 1.0–2.5 μL 25 mmol·L^{-1} $MgCl_2$, 0.3 μL 5 U Taq DNA polymerase, 2 μL 10 × buffer. PCR amplification was as follows: the first step was performed by initial denaturation for 5 min at 94°C, followed by 28–30 cycles at 94°C for 1 min, 54–66°C annealing for 1 min (according to different loci) and 72°C extension for 1 min. An extension step at 72°C for 10 min was added after the final cycle. Then, the product was conserved at 4°C (Crawford et al., 1995).

Using 5 μL amplified product to detect the fragments on 1%–3% agarose gels. Then the valid amplified fragments were electrophoresed on 10%–14% polyacrylamide gels in 1 × TBE with 90–150 V of running voltage and the gels were detected by silver staining. The fragment sizes were calculated by using the Kodak Digital Science ID Image Analysis Software. Genotype of each individual animal at 7 different loci was recorded by direct counting.

2.3 Statistical analysis

FSTAT software (V2. 9.3.2) was used to calculate the *F*-statistics (F_{it}, F_{st}, F_{is}) raised by Wright (1978) and run significance test. According to the flowing formula: $Nem = (1 - F_{st})/(4F_{st})$, we analysed gene flow between populations. The relationship between geographic distribution of each population and genetic differentiation was tested by the formula: $y = a + b\ln(D)$ (Satkin, 1993). In the two formulae above: *Nem* was the average number of effective migrants exchanged per generation; *D* was the geographic distance among populations. Phylogenetic trees were constructed from the DC genetic distance matrix according to the neighbor-joining (NJ) and the unweighted pair-group method using arithmetic averages (UPGMA) methods (Rousset, 1997; Saitou and Nei, 1987).

3 Results

3.1 Microsatellite polymorphism

The heterozygosity (*H*), polymorphic information content (*PIC*) and effective number of allele (*Ne*) in six populations are shown in Table 2, indicating that the six sheep populations (Tong sheep, small-tailed Han sheep, Hu sheep, Tan sheep, Ujmuqin sheep and Bayinbuluk sheep) at the seven microsatellite loci had a high *PIC* value. The average heterozygosity (*H*) was 0.9184, 0.9336, 0.9094, 0.9158, 0.9211 and 0.8208, and the average value of *PIC* was 0.9116, 0.9294, 0.9024, 0.9049, 0.8452 and 0.8091. In all the loci, the small-tailed Han sheep had the highest *PIC* and *H* values and the Bayinbuluk sheep the lowest.

3.2 Genetic differentiation among populations

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.

Table 1 Primer sequence of microsatellite markers and related information

locus	primer sequence	chromosome assignment	No. of alleles	size of alleles	anneal temperature
OarFCB11	GGCCTGAACTCACAAGTTGATATATCTATCACGCA-AGCAGGTTCTTTACCACTAGCACC	2	11	118–174	66°C
OarFCB128	CAGCTGAGCAACTAAGACATACATGGCGATTAAAG-CATCTTCTCTTTATTTCCTCGC	2	9	91–159	54°C
OarFCB304	CCCTAGGAGCTTTCAATAAAGAATCGGCGCTGCTG-TCAACTGGGTCAGGG	19	12	126–222	63°C
OarFCB48	GAGTTATGTACAAGGATGACAAGAGGCACGACTCT-AGAGGATCGCAAAGAACCAG	17	11	127–197	62°C
MAF70	GCAGGACTCTACGGGGCCTTTGCCACGGAGTCACA-AAGCGTCAGACC	4	11	129–179	64°C
MAF33	GATCATCTGAGTGTGAGTATATACAGGACTTTGTTT-CAATCTATTCCAATTTC	9	12	110–176	60°C
OarAE101	TAAGAAATATATTTGAAAAAACTGTATCTCCCTCCT-TATAGATGCACTCAAGCTAGG	6	7	75–139	59°C

Table 2 Heterozygosity, polymorphism information contents and effective number of alleles of seven microsatellite loci at six sheep populations

locus	index	MAF70	OarFCB11	OarAE101	OarFCB48	MAF33	OarFCB128	OarFCB304	mean
Tong	*PIC*	0.8856	0.9198	0.8987	0.9252	0.8986	0.9242	0.9291	0.9116
	H	0.9008	0.9246	0.9061	0.9296	0.9059	0.9288	0.9331	0.9184
	Ne	9.4382	13.2683	10.6474	14.2043	10.6312	14.0351	14.9520	12.4528
Xiaow	*PIC*	0.9347	0.9359	0.8816	0.9114	0.8507	0.9351	0.9563	0.9294
	H	0.9382	0.9392	0.8914	0.9173	0.9528	0.9384	0.9580	0.9336
	Ne	16.1818	16.4584	9.2081	12.0948	21.1864	16.2338	23.8095	16.4533
Hu	*PIC*	0.8926	0.9409	0.8520	0.9240	0.8985	0.9055	0.9036	0.9024
	H	0.9000	0.9438	0.8664	0.9284	0.9056	0.9120	0.9101	0.9094
	Ne	10.0796	17.7772	7.4839	13.9738	10.5960	11.3637	11.1285	11.7723
Tan	*PIC*	0.9028	0.9178	0.8855	0.8898	0.8866	0.9334	0.9182	0.9049
	H	0.9096	0.9230	0.8945	0.8983	0.8953	0.9370	0.9233	0.9158
	Ne	11.0561	12.9903	9.4831	9.8370	9.5482	15.8653	13.0389	11.6884
Wu	*PIC*	0.9100	0.8894	0.8962	0.4274	0.9224	0.9233	0.9480	0.8452
	H	0.9160	0.8979	0.9038	0.9248	0.9270	0.9278	0.9502	0.9211
	Ne	11.9090	9.7935	10.3816	13.2948	13.6994	13.9431	20.0997	13.2890
Ba	*PIC*	0.8615	0.8257	0.4978	0.8674	0.8852	0.8431	0.8827	0.8091
	H	0.8744	0.8440	0.5297	0.8795	0.8666	0.8590	0.8924	0.8208
	Ne	7.9642	6.4120	2.1264	8.2976	7.4950	7.0923	9.2978	6.9550

The result of *F*-statistics was shown in Table 3, the F_{it} ranged 0.0440–0.1670 (the average value was 0.0950); the F_{st} ranged 0.0220–0.1000 (the average value was 0.0390); the F_{is} ranged 0.0050–0.0890 (the average value was 0.0580). 3.9 percent of hereditary variation was caused among populations, while 96.1% of genetic diversity in the total population could be attributed to differences among individuals, which indicated the genetic differentiation among breeds was very low.

Table 3 The result of *F*-statistics for each of 7 markers across 6 sheep populations of Mongolian group in China

locus	F_{is}	F_{st}	F_{it}
MAF70	0.0300	0.0270	0.0560
OarFCB11	0.0480	0.0320	0.0790
OarAE101	0.0740	0.1000	0.1670
OarFCB48	0.0730	0.0220	0.0940
MAF33	0.0050	0.0390	0.0440
OarFCB128	0.0880	0.0260	0.1120
OarFCB304	0.0890	0.0260	0.1130
all	0.0580	0.0390	0.0950

From Table 4, we observed that the F_{st} among all populations was significant ($P < 0.001$), but it was not between the Tong sheep and other populations. The F_{st} value was the largest between the Bayinbuluk sheep and the Tan sheep (0.0837), while the lowest was between the Tong and the Hu sheep (0.0056). According to the F_{st} value, the smallest *Nem* was 2.7369 between the Bayinbuluk sheep and the Tan sheep, and the largest was 44.3928 between the Tong and the Hu sheep. The average of *Nem* among 6 sheep populations was 11.2521.

3.3 Genetic distance and phylogenetic trees

Estimates of DC genetic distance among 6 populations based on the allele frequency data on 7 microsatellite loci are presented in Table 5. The formula of $F_{st}/(1 - F_{st}) = 0.0342 \ln(D) - 0.2254$ demonstrated the relationship of F_{st} and D. Through significance test, we concluded F_{st} and D ($r = 0.7490^{**}$), DC genetic distance and D ($r = 0.3536^{*}$) were all significantly related.

Table 4 Pairwise estimates of F_{st} (below the diagonal) and *Nem* (above the diagonal) of 6 sheep populations of Mongolian group in China

breed	Hu	Tong	Xiaow	Tan	Wu	Ba
Hu		44.3928	12.7708	5.9844	9.1485	3.9236
Tong	0.0056		13.7949	6.5435	10.5259	4.6907
Xiaow	0.0192	0.0178		12.250	23.3349	4.5950
Tan	0.0401	0.0368	0.0200		10.9107	2.7369
Wu	0.0266	0.0232	0.0106	0.0224		3.1794
Ba	0.0599	0.0506	0.0516	0.0837	0.0729	

Note: Xiaow: small-tailed Han; Ba: Bayinbuluk. Same as below.

Table 5 The geographical distance (above the diagonal) and the DC distances (below the diagonal) among 6 sheep populations of Mongolian group in China

breed	Hu	Tong	Xiaow	Tan	Wu	Ba
Hu		1548	917	2159	2706	5128
Tong	0.3654		1288	1006	2091	3050
Xiaow	0.4463	0.4410		1620	2031	4588
Tan	0.5545	0.5455	0.4193		1672	3671
Wu	0.4534	0.4465	0.3403	0.3927		5144
Ba	0.5334	0.4892	0.5221	0.6318	0.5989	

Phylogenetic trees were constructed based on the NJ and UPGMA methods according to DC genetic distance (Fig. 1). When the phylogenetic tree was constructed by the NJ method, the Tan and Ujmuqin sheep were grouped first, then clustered with the small-tailed Han sheep, the Tong sheep and the Hu sheep clustered. Finally, the Bayinbuluk sheep joined with all above. The bootstrap values of each node were all higher than 50% (the average

value was 0.7775). However, when phylogenetic tree was constructed by the UPGMA method, the result was different from the above. The small-tailed Han sheep and the Ujmuqin sheep were clustered first and the average of bootstrap value was 0.8580 and higher than that based on the NJ method.

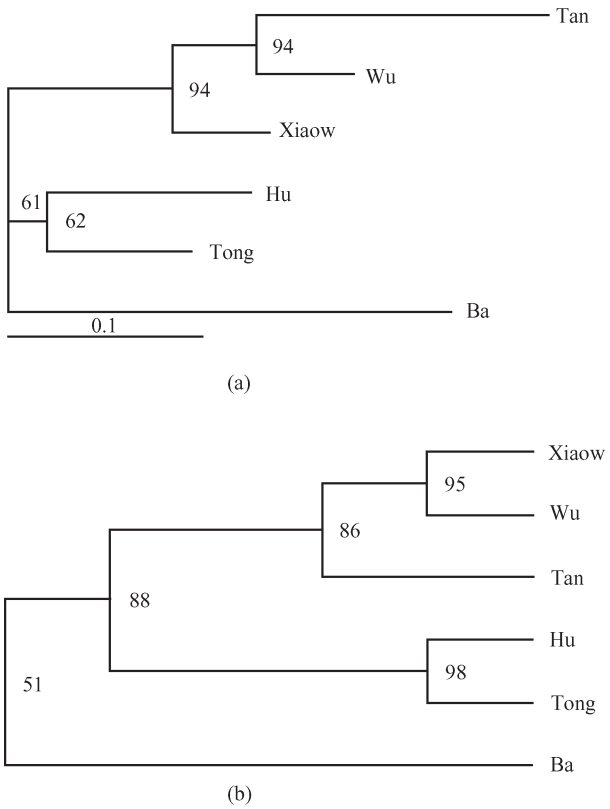

Fig. 1 The trees constructed from DC distances among 6 sheep populations of the Mongolian group in China
Note: (a): DC-NJ method; (b): DC-UPGMA method; the numbers on the node were the bootstrap percentage value from 1000 times.

4 Discussion

4.1 Polymorphism of seven microsatellite loci

Microsatellite loci chosen in this study were in different chromosomes (OarFCB11 and OarFCB128 were in the same chromosome). For each population, there were at least 7 alleles per locus and the average value of *PIC* was 0.9116, 0.9294, 0.9024, 0.9049, 0.8452 and 0.8091. According to Bolstein et al. (1980), these 7 microsatellite loci involved in this research were all highly polymorphic. Therefore, we considered the 7 microsatellite markers suited the study of genetic diversity in sheep populations generally.

4.2 Genetic differentiation among sheep populations

The estimation of coefficient of genetic differentiation could discriminate the genetic variances among or in populations (Nei, 1931). The average of genetic differentiation among breeds, measured as the F_{st} value was 3.9 percent, thus 96.1% of genetic diversity in the total population could be attributed to differences among individuals, which indicated that the variation of the "Mongolia sheep" was mainly caused in breeds. The genetic differentiation among breeds was very low.

4.3 Geographic isolation and gene flow

Geographic isolation, a nature barrier of gene exchange is an important factor to influence the genetic differentiation among populations (Tian et al., 2005; Qu et al., 2004). The genetic distances among the 6 "Mongolia sheep" were significantly related with geographic distance in this study, which indicated geographic distribution affected the genetic distance among populations and the genetic diversity was related with the geographic distance.

Gene flow is also an important factor influencing the genetic differentiation among populations and the high and steady gene flow may prevent genetic differentiation among populations. According to Wright (1931), gene flow could restrain genetic differentiation efficiently caused by genetic drift when it was larger than 1. In our study, gene flows among sheep populations were all greater than 1 (the average value was 11.2521). Thus, larger gene flow was a predominant reason of low genetic differentiation among "Mongolia sheep". The distribution areas of the 6 sheep breeds were different, which shows that there was a distinction in the ecosystem of each breed. The consequence of this study reflected low genetic differentiation among breeds, which indicated that this difference did not influence the genetic level. Therefore, it is concluded that genetic differentiation of sheep breeds of Mongolian group in China is mainly the result of natural selection (from different living conditions).

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