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Polymorphism analysis of three Chinese indigenous sheep breeds by microsatellite markers

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ABSTRACT

In this study, six microsatellite markers were adopted to detect the genetic diversity and analyze the genetic distance of three Chinese indigenous sheep breeds. The results showed that 161 alleles were detected in the three breeds of sheep populations, and the average effective number of alleles, the average polymorphism information content (PIC) of six microsatellite markers in fat-tailed sheep, small tailed han-sheep, Yuxi fat-tailed sheep were 5.8844, 6.3103, 4.8017 and 0.7463, 0.7790, 0.7140 respectively. Five markers were highly polymorphic except marker ILSTS011 which gave moderate polymorphic. Except markers OarFCB48, OarFCB304 and BL1038, the other three microsatellite markers deviated significantly from the Hardy-Weinberg Equilibrium ($P<0.01$) in the three breeds of sheep populations. The genetic differentiation coefficient of each locus ranged from 0.0059 to 0.1159, with an average of 0.0482, indicating a 4.82% gene variation among different populations. So the degree of differentiation among populations was low. The small tailed han-sheep and fat-tailed sheep with minimum genetic distance (0.2163) were first clustered as a group, then they clustered with Yuxi fat-tailed sheep.

Key words: Genetic distance, Genetic diversity, Microsatellite, Sheep.

INTRODUCTION

In mammalian genomes, a microsatellite occurs per 10kb. With the advantages of high PIC, uniform distribution in genome, and easy location, the microsatellite markers have been widely used in genetic map construction, gene location, relative relationship identification, population genetic structure analysis and marker assisted selection, etc. The polymorphic analysis of microsatellite markers played an important role in assessment of sheep genetic diversity and classification, conservation and utilization of breeds, and has been widely applied to study the genetic variation of different sheep breeds (Gizaw *et al*., 2007; Ozerov *et al*., 2008; Glowatzki-Mullis *et al*., 2009; Maria *et al.*, 2010; Arora *et al.*, 2011; Zhao *et al*., 2011; Sun *et al*., 2011; Al-Barzinji et al., 2011; Tolonea *et al.*, 2012; Ghazy *et al*., 2013). Recently, Soma *et al*. (2012) analyzed population genetic structure of the South African sheep breeds by microsatellite markers. The results showed that average unbiased heterozygosity (Hz) of microsatellite markers studied in fat-rumped breeds, composite breeds, indigenous fat-tailed breeds, Karakul breeds and the wool breeds was 0.466, 0.555, 0.598, 0.659 and 0.662 respectively. Crispim *et al* (2014) reported that the population of Pantaneiro sheep from the UFGD exhibited a high average number of alleles (11.13) and allelic richness (10.66). The polymorphic information content was highly informative in the locus studied, resulting in a mean value of 0.71. Observed heterozygosity was lower than expected for all molecular markers assessed. The analysis of molecular variance showed a differentiation rate of 5.2% between populations.

Fat-tailed sheep, small tailed han-sheep, and Yuxi fat-tailed sheep are three mainly indigenous sheep breeds in Henan, China. Small tailed han-sheep was a famous fur and meat-type breed in China and even all over the world, featured by its early-maturing, multiplets, multi-lamb, fast growth, big physique, large meat production, superior fur quality, genetic stability and strong adaptability, etc. Fat-tailed sheep was an excellent local sheep breed in China, mainly distributed in Jia county and Baofeng along Beiruhe river of Pingdingshan, Henan Province. It was featured by perennial oestrus, multiplets, fecundity, early-maturing, fast growth, high feed conversion, superior meat quality, high roughage and disease resistance, *etc*., and was thereby deeply loved by the masses (Zhao *et al*., 2008). Yuxi fat-tailed sheep was an ancient local skin and meat-purpose breed, which was collected in "The Fine Local Livestock and Poultry Breeds Record of Henan

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Province" in 1986, and was featured by its high roughage and disease resistance, easy fatting, sweet meat, etc (Tian *et al*.,2009).

In this research, six microsatellite markers were adopted to detect the genetic diversity and analyze the genetic distance of three breeds of local sheep (fat-tailed sheep, small tailed han-sheep, and Yuxi fat-tailed sheep) in Henan, China, in order to provide theoretical basis for protection and utilization of the local sheep breed resources.

MATERIALS AND METHODS

DNA extraction: 10 mL jugular venous blood taken from each of the 120 sheep (40 were small tailed han-sheep from Puyang, Henan province, 40 were fat-tailed sheep from Pingdingshan, Henan province and the other 40 were Yuxi fat-tailed sheep from Luoyang, Henan province) was preserved at -20°C with ACD (1:6) anticoagulation, and the genomic DNA extraction was completed by whole-blood DNA kit (Shanghai Sangon Biotech Co., Ltd, code: SK1262). The genomic DNA was preserved at - 20°C for later use.

Selection of microsatellite markers: Six pairs of microsatellite primers (Table 1) recommended by FAO were synthesized by Shanghai Sangon Biotech Co., Ltd.

PCR reaction condition: PCR amplification program included an initial denaturation step at 94°C for 4 min, followed by 30 cycles of 40 s at 94°C, 40 s at annealing temperature (56~60°C) and 30s at 72°C. Finally, an extension of 72°C for 7 min was followed. The total volume of PCR amplification reaction system was 15μL: 1.5μL 10×buffer, 1.2μL dNTPs (4×2.5mol/L), 0.3μL positive and reverse primerÿ10pmol/μL ÿrespectively, 1.0μL template DNA (100ng/μL), 0.3μL Taq DNA polymerase (5U/μL) and 10.4ìL distilled water.

8 μL of the PCR product was subjected to electrophoresis on 10% non-denaturing polyacrylamide gels for 6 ~ 8h under 150 ~ 180V. After electrophoresis, gels were stained with silver nitrate.

Statistical analysis: The molecular biology software POPGENE (Version1.32) was used to analyze Observed Heterozygosity (Obs.Het.), Expected Heterozygosity (Exp. Het.), Observed Homozygosity (Obs. Hom.), Expected Homozygosity (Exp. Hom.), Observed number of alleles(Na), Effective number of alleles(Ne), Shannon's Information index (I)

Genetic differentiation coefficient: Genetic differentiation coefficient (G_{st}) is an indicator evaluating the genetic differentiation in many loci among populations and the function of whole population's average heterozygosity (H_t) and average sub-population heterozygosity (H_s). G_{st}=1-H_s/H_t. Where H_t is total population average heterozygosity, H_s is average heterozygosity of different sheep populations, and G_{st} is coefficient of gene differentiation.

RESULTS AND DISCUSSION

Genetic variation within population: Effective number of alleles was the reciprocal of gene homozygosity and an indicator of genetic variation within population. The more uniform of alleles distribution in the population, the more approximate between the effective number of alleles and the absolute number of actually observed alleles. A total of 161 alleles were detected in the three sheep populations by the six microsatellite markers (Figure1), and the observed number of alleles in small tailed han-sheep, fat-tailed sheep and Yuxi fat-tailed sheep was 60, 53 and 48 respectively. In the small tailed han-sheep population, markers OarFCB48 and BL1038 gave the maximum observed number of alleles (13), while marker ILSTS011gave the minimum number (3). In the fat-tailed sheep population, marker OarFCB48 gave the maximum observed number of alleles (14), marker ILSTS011 gave the minimum number (3). In the Yuxi fat-tailed sheep population, marker OarFCB48 gave the maximum observed number of alleles (12), and marker ILSTS011 gave the minimum number (3). Marker ILSTS011 gave the minimum effective number of alleles in the three

TABLE 1: Primer information of microsatellite markers

Locus	Primer sequence(5'→3')	T_A(°C)	Size (bp)
BM757	F: TGGAAACAATGTAAACCTGGG R: TTGAGCCACCAAGGAACC	58	186-220
BL1038	F: GGCAAGCTAGAGTCAGACACG R: GCAAAAGTCTAGGTGAAATGCC	60	97-113
OarFCB304	F: CCCTAGGAGCTTTCAATAAAGAATCGG R: CGCTGCTGTCAACTGGGTCAGGG	56	150-188
BM4621	F: CAAATTGACTTATCCTTGGCTG R: TGTAACATCTGGGCTGCATC	58	135-157
OarFCB48	F: GACTCTAGAGGATCGCAAAGAACCAG R: GAGTTAGTACAAGGATGACAAGAGGCAC	58	143-167
ILSTS011	F:GCTTGCTACATGGAAAGTGC R:CTAAAATGCAGAGCCCTACC	52	260-282

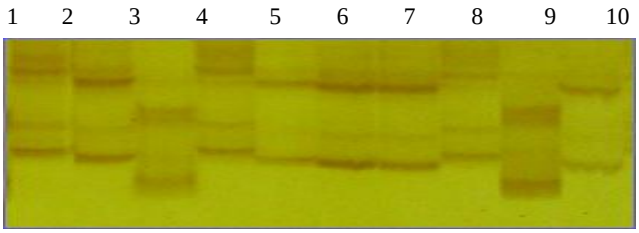

FIG 1: Results of microsatellite marker OarFCB304
Notes:1、4、8：CG，2、5、6、7、10：BE，3、9：AD

sheep populations (2.2200), while marker OarFCB48 gave the maximum effective number of alleles (10.5026) . The average effective number of alleles of the six microsatellite markers in small tailed han-sheep, fat-tailed sheep and Yuxi fat-tailed sheep was 5.8844, 6.3103 and 4.8017, respectively. On the whole, the effective number of alleles was lower than the actually observed number of alleles, which was caused by uneven distribution of alleles in populations, resulting in some alleles frequencies relatively high and some relatively low.

The polymorphism of molecular genetic markers could also be measured by heterozygosity of populations, and the average heterozygosity of populations was an optimal parameter to measure the variation. For the same molecular markers, the average heterozygosity reflected the genetic consistency. The lower the average heterozygosity was, the higher the consistency, the less the genovariation, and the lower the genetic diversity were. As was shown in Table 2, the average observed heterozygosity (Obs.Het.) of the six microsatellite markers in small tailed han-sheep, fat-tailed sheep and Yuxi fat-tailed sheep was 0.6406, 0.4872 and 0.5000 respectively, and the average expected heterozygosity (Exp. Het.) was 0.7903, 0.8216 and 0.7673 respectively, which showed that the observed and expected heterozygosities in the same sheep population varied to a certain extent, and genetic diversity of small tailed han-sheep population was more abundant and slightly higher than that of fat-tailed sheep and Yuxi fat-tailed sheep. Yang *et al.* (2004) analyzed three local sheep breeds of Hu sheep, Tong sheep and Yangtze River delta white goat, obtaining the average heterozygosity of 0.9092, 0.9177, and 0.8867, respectively. Chen *et al.*(2007) analyzed the genetic diversity of Yunnan local sheep breeds and showed that heterozygosities of Tengchong, Zhaotong, Diqing and Ninglang sheep were 0.8226, 0.8505, 0.8457, and 0.8505 respectively. Compared with the research above, expected heterozygosities of small tailed han-sheep, fat-tailed sheep and Yuxi fat-tailed sheep in our study were a little lower, therefore genetic consistency of the three breeds was better.

PIC was an indicator of gene richness and a function of allele frequency and number, which reflected the diversity

TABLE 2: Genetic polymorphism

Populations	Locus	Na	Ne	Obs. Hom.	Obs. Het	Exp. Hom.	Exp. Het.	I	PIC	Chi-square
Fat-tailed Sheep	ILSTS011	3	2.8643	0.3125	0.6875	0.3388	0.6612	1.0738	0.5759	0.8471
	BL1038	5	2.7527	0.2812	0.7188	0.3532	0.6468	1.1921	0.5810	21.9547*
	BM757	10	3.8138	0.4375	0.5625	0.2505	0.7495	1.7057	0.7121	128.3780**
	BM4621	9	7.0137	0.9375	0.0625	0.1290	0.8710	2.0786	0.8429	267.5764**
	OarFCB304	12	8.3592	0.1250	0.8750	0.1057	0.8943	2.2567	0.8687	70.2538
	OarFCB48	14	10.5026	0.0625	0.9375	0.0809	0.9191	2.4942	0.8974	115.7857*
	Mean	8.8	5.8844	0.3594	0.6406	0.2097	0.7903	1.8002	0.7463	
Small tailed han-sheep	ILSTS011	3	2.2200	0.3846	0.6154	0.4397	0.5603	0.8908	0.4612	0.7958
	BL1038	13	8.8947	0.3846	0.6154	0.0950	0.9050	2.3374	0.8773	204.7547**
	BM757	10	6.3178	0.5769	0.4231	0.1418	0.8582	2.0261	0.8234	123.8606**
	BM4621	10	6.6601	0.8077	0.1923	0.1335	0.8665	2.0363	0.8320	194.3928**
	OarFCB304	11	6.1735	0.6538	0.3462	0.1456	0.8544	2.0653	0.8217	159.1482**
	OarFCB48	13	7.5955	0.2692	0.7308	0.1146	0.8854	2.3138	0.8583	132.8972**
	Mean	10.0	6.3103	0.5128	0.4872	0.1784	0.8216	1.9450	0.7790	
Yuxi Fat-tailed sheep	ILSTS011	3	2.6205	0.2000	0.8000	0.3690	0.6310	1.0181	0.5391	6.0548
	BL1038	10	4.2230	0.4400	0.5600	0.2212	0.7788	1.6989	0.7274	79.9179**
	BM757	10	4.8450	0.5600	0.4400	0.1902	0.8098	1.8795	0.7711	95.3844**
	BM4621	9	5.8411	0.5200	0.4800	0.1543	0.8457	1.9797	0.8110	95.1387**
	OarFCB304	4	2.6596	1.0000	0.0000	0.3633	0.6367	1.1184	0.5625	103.8646**
	OarFCB48	12	8.6207	0.2800	0.7200	0.0980	0.9020	2.2828	0.8731	75.8583
	Mean	8.0	4.8017	0.5000	0.5000	0.2327	0.7673	1.6629	0.7140	

of genetic basis of the breeds. Botstein *et al.* (1980) first put forward the PIC indicator to measure the genovariation degree: loci with PIC≥0.50 were highly polymorphic, loci with PIC≤0.25 were low-grade polymorphic and loci with 0.25<PIC<0.50 were moderately polymorphic. According to Table 2, ILSTS011 had the lowest PIC (0.4612) and OarFCB48 had the highest PIC (0.8974) among the three sheep populations. The average PIC of the six microsatellite markers in small tailed han-sheep, fat-tailed Sheep and Yuxi fat-tailed sheep was 0.7463, 0.7790, and 0.7140 respectively. Zhong *et al*. (2008) studied 10 sheep breeds by 21 microsatellite markers and obtained the average PIC of 0.697, which was lower than that of small tailed han-sheep, fat-tailed Sheep and Yuxi fat-tailed sheep obtained in this study. In the six microsatellite markers, five markers were highly polymorphic except for marker ILSTS011which was moderately polymorphic.

In the fat-tailed sheep population, the maximum index of Shannon's Information in the six microsatellite markers was 2.4942, and the minimum was 1.0738, with the average of 1.8002. In small tailed han-sheep population, the maximum index was 2.3374, and the minimum was 0.8908, with the average of 1.9350. While in Yuxi fat-tailed sheep population, the maximum index was 2.2828, and the minimum was 1.0181, with the average of 1.6629. The results were consistent with that of Nei's Gene Diversity.

Before conducting genetic research on a population, Hardy-Weinberg equilibrium state test should be carried out to determine whether the population genetic equilibrium condition were met. Microsatellite marker ILSTS011 in the three sheep breeds accorded with Hardy-Weinberg equilibrium (P>0.05); OarFCB48 in Yuxi fat-tailed sheep populations accorded with the equilibrium (P>0.05); OarFCB304 in fat-tailed sheep populations accorded with the equilibrium (P>0.05); BL1038 and OarFCB48 in fat-tailed sheep populations significantly deviated from the equilibrium (P<0.05); and the other markers in three sheep populations very significantly deviated from the equilibrium (P<0.01). There were many factors causing the disequilibrium: firstly, constant introduction of alien breeds may change the original gene frequency. Secondly, it may be associated with the sample size, generally, the greater sample size gave the better result. In this study, 40 samples were used in each population, which was likely to cause incomplete population structure. Thirdly, it may be associated with the inbreeding within population, which may cause the loss of related genes.

Genovariation among populations: Heterozygosity of the total population (Ht), heterozygosity of the subpopulation (Hs) and coefficient of genetic differentiation (Gst) were the three indicators used to measure the genovariation among populations. The total population meaned that all populations in the research were regarded as a population. Hs was the average heterozygosity of all populations, and Gst refered to the degree of genetic differentiation, and was the function of the average Ht and Hs. It can be seen from Table 3 that Ht of each loci was between 0.6215 and 0.9076, with an average of 0.8352, showing very high heterozygosity; Hs was between 0.6175 and 0.9022, with an average of 0.7931, lower than

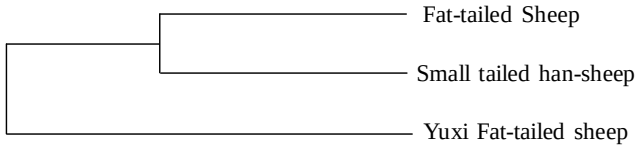

FIG 2: Dendrogram of sheep populations

TABLE 3: Total gene diversity(Ht)ÿAverage heterozygosity within each population(Hs)and Coefficient of gene differentiation(Gst) of 6 microsatellite loci

Locus	Total gene diversity(Ht)	Average heterozygosity within each population(Hs)	Coefficient of gene differentiation(Gst)
ILSTS011	0.6215	0.6175	0.0064
BL1038	0.8697	0.7769	0.1067
BM757	0.8367	0.8058	0.0369
BM4621	0.8763	0.8611	0.0173
OarFCB304	0.8993	0.7951	0.1159
OarFCB48	0.9076	0.9022	0.0059
Mean	0.8352	0.7931	0.0482

TABLE 4: Genetic distance of three sheep populations

Populations	Fat-tailed Sheep	Small tailed han-sheep	Yuxi Fat-tailed sheep
Fat-tailed Sheep		0.8055	0.5375
Small tailed han-sheep	0.2163		0.6972
Yuxi Fat-tailed sheep	0.6208	0.3607	

Note :Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Ht. Ht and Hs were larger, which reflected large genovariation in the population, which was consistent with PIC and heterozygosity analysis results. Gst of each loci were between 0.0059 and 0.1159, with an average of 0.0482, indicating the genovariation among different populations was 4.82%, a low differentiation among populations. Gst values in most loci were not high, which showed that the genovariation occurred mainly in each breed and little among breeds. Despite there were differences among breeds, there was no obvious geographical separation among them, which was consistent with the geographical location and the ecological environment of Henan where these sheep breeds were located.

Genetic distance reflected the phyletic evolution of the population studied, and was used to describe the genetic structure of populations and the differences among breeds. Currently, there were many types of genetic distance available, of which Nei's standard genetic distance was traditionally used. Generally, the shorter the time of differentiation was, the smaller the genetic distance became. Rich polymorphism has provided the safeguard for clustering analysis. It can be seen from Table 4 that the minimum genetic distance between the small tailed han-sheep and fat-tailed sheep was 0.2163, while genetic distances between Yuxi fat-tailed sheep and small tailed han-sheep, fat-tailed sheep were 0.3607 and 0.6208 respectively. According to the clustering diagram of Figure 2, the small tailed han-sheep and fat-tailed sheep were first clustered as a class, and then clustered with Yuxi fat-tailed sheep.

CONCLUSION

Research shows that,indicating the closer phylogenetic relationship between small tailed han-sheep and fat-tailed sheep than that between small tailed han-sheep, fat-tailed sheep and Yuxi fat-tailed sheep. The results above provides reference basis for germplasm resources protection and utilization of Chinese sheep.

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