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Short Communication

POLYMORPHISM ANALYSIS OF *HENAN FAT-TAILED SHEEP* USING MICROSATELLITE MARKERS

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ABSTRACT

Five microsatellite markers were selected for studying the DNA polymorphism of fat-tailed sheep, aiming to understand the genetic diversity of this species. The genetic diversity of five microsatellite markers (BL1038, BM757, BM4621, OarFCB304 and OarFCB48) in fat-tailed sheep population was detected through the native polyacrylamide gel electrophoresis. The results indicated that fat-tailed sheep population has 68 alleles in these five microsatellite markers and its effective number of alleles was 7.7352-13.0962. The average effective number of alleles (Ne) of these five microsatellite markers was 8.9896, among which OarFCB48 has the highest Ne (13.0962). The average heterozygosity of these five microsatellite markers valued 0.8840, among which OarFCB48 achieved the highest heterozygosity (0.9236), which has also the highest PIC content (0.9187), while BM757 achieved the lowest PIC content (0.8571).The average PIC valued 0.8749 in this study. This indicated that the studied fat-tailed sheep population belongs to the genetic population with higher polymorphism information content. Furthermore, the fat-tailed sheep can be bred purely and selectively further to increase the uniformity of the population.

Key words: Henan Fat-tailed sheep, Microsatellite marker, Genetic diversity.

INTRODUCTION

In China, fat-tailed sheep is mainly distributed in Jiaxian and Baofeng County, Pingdingshan City, Henan Province, this breed has various advantages, such as perennial estrus, multiple birth, early-maturing, quick growing development, high feed reward, good meat quality, resistance to coarse feeding, strong premonition, which received wide appreciation in the production area (Tian *et al.*,2010). The genetic relationship of six Chinese sheep species including fat-tailed sheep was analyzed by microsatellite marker ILSTS011 (Lei *et al.*,2006 and Bai *et al.*,2009). Yuan *et al.,* (2006) studied the microsatellite genetic polymorphism of four local sheep species in Shandong Province. Sequence difference as well as system of cytochrome b gene of mitochondria of four sheep breeds was analyzed by Zhang *et al.*,(2005). Recently, the genetic diversity of fat-tailed sheep confronts serious threat because of the development of stock farming and the introduction of foreign species with high productivity as well as the wide popularization of breeding techniques like artificial insemination and embryo transplantation. Therefore, resource protection becomes one of important issues currently. In order to have a relative deeper comprehensive understanding on the germplasm, hereditary character and other aspects of this species, we selected five microsatellite markers to study the DNA polymorphism of fat-tailed sheep, aiming to understand its genetic diversity and provide some beneficial references for further increasing the conservation breeding.

MATERIALS AND METHODS

40 fat-tailed sheep in Pingdingshan, Henan Province were selected to collect 10ml blood from jugular vein, which was processed by ACD anti-freezing (1:6) and stored under -20^{o}C. The genomic DNA was extracted through whole blood DNA kit (SK1262) of Sangon and the extracted DNA was stored under -20℃ for standby application. The primers of five microsatellite markers were detected by GENEBANK（Table 1）, all of which were synthesized by Shanghai Sangon Biology Engineering Technology Service Co., Ltd.

PCR amplification program: pre-degenerated under 94^{o}C for 5 min; degenerated under 94℃ for 30s; annealing under 56～60℃ for 30s and extend under 72℃ for 30s, repeat for 30 times; extend under 72℃ for 7 min; stored under 4℃. The total volume of PCR amplification reaction system was 15μL, among which there were 1.5μL 10×buffer, 1.2μL dNTP（4×2.5mol/Ll）, 0.3μL positive and reverse primer respectively, 1.0μL template DNA (100ng/μL), 0.3Ml Taq polymerase (5μ/μL) and 10.4μL double distilled water. Process the PCR products with 10% native polyacrylamide gel electrophoresis for

6~8h under stable voltage 150~180V, and then fixing them for silver nitrate dye, development and other process. Finally, gel and imaging for systematic photographing.

Finally the frequency and size range of alleles were calculated through Excel Microsatellite Toolkit. The polymorphic information content (PIC), heterozygosity (He) and effective amount of allele (Ne) were calculated by Dispan Software.

Table 1. Relational information for microsatellite locus

Locus	Primer sequence (5'→ 3')		T_A(℃)
BM757	F: TGGAAACAATGTAAACCTGGG	R: TTGAGCCACCAAGGAACC	58
BL1038	F: GGCAAGCTAGAGTCAGACACG	R: GCAAAAGTCTAGGTGAAATGCC	60
OarFCB304	F: CCCTAGGAGCTTTCAATAAAGAATCGG	R: CGCTGCTGTCAACTGGGTCAGGG	56
BM4621	F: CAAATTGACTTATCCTTGGCTG	R: TGTAACATCTGGGCTGCATC	58
OarFCB48	F: GACTCTAGAGGATCGCAAAGAACCAG	R: GAGTTAGTACAAGGATGACAAGAGGCAC	58

RESUITS AND DISCUSSION

It can be seen from Table 2 that there were 68 alleles observed with an average allele of 13.5, among which OarFCB48 marker achieved the maximum alleles (16), while BM757 and BM4621 market achieved the least alleles (12). BL1038, BM757, BM4621, OarFCB304 and OarFCB48 witnessed polymorphism. The segment sizes of microsatellite markers OarFCB48, BL1038, BM757, BM4621 and OarFCB304 were 126-225bp, 101-172bp, 125-239bp, 93-134bp and 120-228bp respectively. Based on the Gene Frequency Table, alleles were distributed unevenly in each marker. Furthermore, different alleles as well as frequencies were observed in same microsatellite site of same population.

Table 2. Allele frequencies of microsatellite loci

Allele	BL1038	BM757	BM4621	OarFCB304	OarFCB48
A	0.011	0.014	0.098	0.025	0.014
B	0.020	0.030	0.160	0.030	0.010
C	0.111	0.027	0.043	0.035	0.025
D	0.078	0.167	0.065	0.035	0.095
E	0.178	0.167	0.022	0.035	0.125
F	0.089	0.042	0.152	0.125	0.016
G	0.111	0.042	0.043	0.165	0.016
H	0.022	0.125	0.043	0.125	0.098
I	0.144	0.167	0.065	0.038	0.014
J	0.101	0.139	0.132	0.238	0.065
K	0.022	0.069	0.087	0.025	0.095
L	0.111	0.014	0.011	0.014	0.095
M			0.076	0.025	0.095
N				0.065	0.025
O				0.025	0.065
P					0.065

Effective amount of allele (Ne) is the main indicators in measuring the genetic structure of animal population. Among five microsatellite markers, Ne valued between 7.3520 and 13.0962, with the average value of 8.9896, among which OarFCB48 achieved the highest effective amount of allele (Ne) (13.0962). Abundant literatures revealed that generally, the allele amount of microsatellite site of sheep was within range of 2-20. The allele amount of the selected microsatellite markers in this paper lies within this range.

In this study, the microsatellite marker OarFCB48 owns the highest PIC (0.9187), whereas BM757 owns the lowest PIC (0.8571), and the average PIC values 0.8749, which indicated that all of five microsatellite markers selected in this research are high polymorphic sites. In a study on 10 sheep breeds using 21 microsatellite markers, the results indicated that all of these microsatellite markers are high polymorphic sites except for SRCRSP9 (moderate polymorphic site). Among them, SRCRSP9 site owns the lowest PIC, reaching 0.489 (Zhong *et al*., 2008). The research on 8 Sinkiang sheep species by microsatellite markers

demonstrated that the average PIC was 0.5631 (Jia *et al*., 2003). The analysis on the microsatellite DNA polymorphism of Lanzhou big-tailed sheep by 15 microsatellite markers revealed the average PIC valued 0.7762 (Lang *et al*., 2011). In another study, average PIC of 8 microsatellite markers in Tan sheep valued 0.855 (Xu *et al*., 2009). All these findings confirmed that the fat-tailed sheep population belongs to the genetic population with high polymorphism information content.

Table 3. Number of effective alleles, polymorphism information content and heterozygosity of microsatelliate loci

Locus	Polymorphism information content (PIC)	Heterozygosity(H)	Number of effective alleles(Ne)
BL1038	0.8741	0.8845	8.6597
BM757	0.8571	0.8702	7.7028
BM4621	0.8654	0.8707	7.7352
OarFCB304	0.8595	0.8710	7.7544
OarFCB48	0.9187	0.9236	13.0962
Average	0.8749	0.8840	8.9896

Gene heterozygosity, or known as gene diversity, reflected the hereditary variation of population on various sites and was regarded as one of the most appropriate parameter for measuring the hereditary variation of population. In terms of same molecular marker, the average heterozygosity of population reflects the degree of genetic consistency of the population. The lower the population heterozygosity is, the higher the genetic consistency of the population will be; otherwise, the fewer the hereditary variation of the population is, the lower the genetic diversity of the population will be (Lei *et al*., 2003). Different studies indicated that the average heterozygosity of four microsatellite markers in Suffolk sheep population was 0.8653 (Ren *et al*., 2008). The microsatellite analysis on the genetic diversity of Yunnan local sheep species revealed that the heterozygosity of Tengchong Sheep, Zhaotong Sheep, Diqing Sheep and Ninglang Black Sheep valued 0.8226, 0.8505, 0.8457 and 0.8396, respectively (Chen *et al*.,2007). The heterozygosity of the five microsatellite markers in the present study was from 0.8702-0.9236, with an average heterozygosity of 0.8840, all of which have high heterozygosity, thus belonging to high heterozygosis sites. This indicated that the studied fat-tailed sheep population possesses high heterozygosity and richer genetic diversity. Furthermore, it is successful in selecting microsatellites, thus enabling to reflect the genetic diversity of fat-tailed sheep.

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