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Study on Population Genetic Structure of Liangshan Semi-Wool Sheep Usimg Microsatellite Markers

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Abstract: To evaluate the genetic polymorphisms and to search for available molecular markers for Liangshan semi-wool sheep, 15 microsatellite markers of 187 samples were amplified by multiplex PCR. A total of 133 alleles were detected, with the number of alleles ranging from 6 (BM2830) to 15 (McM130), giving a mean No. of 8.87 alleles per locus. The total and mean effective allele No. were 64.29 and 4.29, respectively. The observed heterozygosity and expected heterozygosity were from 0.4486 (McM130) to 0.8877 (BMS1678) and 0.5704 (BMS0887) to 0.8373 (McM130), respectively. Mean observed heterozygosity and mean expected heterozygosity were 0.672 and 0.7536. Polymorphism information content values were from 0.5202 (BMS0887) to 0.8183 (McM130) and mean polymorphism information content of the 15 microsatellite loci was 0.7184. All 15 microsatellite loci were highly polymorphic, which showed that there were rich genetic polymorphisms at these detected microsatellite loci in Liangshan semi-wool sheep. Comparison of allele distributions among loci did not reveal consistent shapes. Distributions were centralized in some cases, whereas in others some kind of skewness was evident. Complex evolution at these loci is an important factor in the irregularity of microsatellite distributions.

Key words: Improved tibetan sheep, microsatellite, genetic variability, polymorphism, diversity

INTRODUCTION

In the past years, sheep breed registrations have led to genetic isolation of many sheep breeds. The selection of a few highly productive breeds has caused the decline of numerous other diverse breeds. This is the reason why the evaluation and the preservation of cattle genetic resources have already become a major and common problem that has attracted global concern. The genetic polymorphism and diversity found in the domestic breeds allow farmers to develop new characteristics in response to changes in environment, or market conditions. So, the importance of increasing, maintaining and conserving the genetic diversity in these animals for the future has been recognized. Gradually attention has been turned in this direction, with every tool including phenotypic parameters and biochemical and molecular genetic techniques having been used to assess the genetic diversity of the animal. DNA microsatellite sequences are valuable genetic markers due to their dense distribution in the genome, great variation, co-dominant inheritance and easy genotyping. In recent years, they have been extensively used in parentage testing, linkage analysis, population genetics and other genetic studies (Goldstein and Pollock, 1997; Calvo *et al*., 2006). Although, they show some limitations in the analysis of phylogenetically distant organisms, due to their irregular mutation processes involving range constraints and asymmetries, they have proved very useful in sheep population studies (Sun *et al*., 2007; Álvarez *et al*., 2008). This breed originated from Tibetan sheep in China, where intensive cross-breeding programme started in 1958. To improve wool production the crossing with wide range of breeds (Xinjiang fine-wool sheep Lincoln and Boioder Leicestler sheep) was attempted. The new breed, with name Liangshan Semi-wool Sheep, was announced in 1987. The number of animals is relatively high with 350,000 animals in China. Up to the last decade, microsatellite have been used in parentage testing, linkage analysis, association analysis and QTL detection of Liangshan Semi-wool Sheep (Gao and Wu, 2005; Zhang and Wu, 2005; Wang and Wu, 2006; Zhou *et al*., 2007). The purpose of this study was to reveal the genetic polymorphisms of Liangshan Semi-wool Sheep by examining the microsatellite DNA and to provide some theoretical basic for conservation and improvement of this sheep.

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MATERIALS AND METHODS

A total of 187 blood samples were randomly collected from genetically unrelated sheep across their breeding tract of Liangshan Semi-wool Sheep (Liangshan, of Sichuan, China) to make them representative of population. Blood samples (8-10 mL) were obtained from the jugular vein using vacuitaners treated with 15% Ethylene Diamine Tetra Acetic (EDTA) acid as an anticoagulant. This study has been done in China in Autumn 2004.

Fifteen microsatellite markers were selected in respect of polymorphisms and a non-linkage criterion for syntenic markers, based on the recommendations of either of the following organizations: United Staes Department of Agriculture (USDA); Australian Gene Mapping Web Site; Food and Agricultural Organization (FAO); International Society for Animal Genetics (ISAG).

Genomic DNA was isolated as per the method described by Sambrook *et al.* (1999) with minor modifications. Polymerase Chain Reactions (PCRs) were carried out to amplify microsatellite loci using fluorescent-labelled primers. The amplified products were separated using POP-4 polymer (Applied Biosystem). Genetic variants were visualized using ABI PRISM 310 DNA sequencer (Applied Biosystem). Results of the capillary electrophoresis were read directly with the Genescan software and interpreted with Genotyper.

Breed genetic variation of the microsatellites and breed structure was quantified using the allele frequencies, observed No. of alleles, effective No. of alleles (Kimura and Crow, 1964) observed heterozygosity and expected (Levene, 1949) heterozygosity. Within breed heterozygosity deficit (Wrights, 1978) was estimated using Wrights fixation index. All the estimates were derived using POPGENE, Version1.31 program (Yeh *et al.*, 1999). Polymorphism information content was calculated as per Botstein *et al.* (1980).

RESULTS AND DISCUSSION

The study of genetic diversity is the basis for any animal breeding program. The first step in a effective breeding or conservation program is accurate evaluation of available genetic resources and microsatellite analysis is a well tool for measuring the genetic polymorphisms in a population. Genotypic data from 15 microsatellites was used to assess the genetic structure of Liangshan Semi-wool Sheep population. All 15 microsatellite loci were polymorphic in the Liangshan Semi-wool Sheep population (Table 1). Total 133 alleles were detected from the 15 microsatellite loci examined. The No. of alleles per locus ranged from 6 (BM2830) to 15 (McM130) with an average of 8.8667. The total effective allele number and mean effective allele No. per locus were 64.2905 and 4.286. High mean number of observed alleles per locus showed high genetic variation in the sheep. At every microsatellite locus, allele size range was distinctive. And at every locus, there was a most frequent allele. At BMS0887 and BM1577, the most frequent allele was 149 and 184, which had an allele frequency of 0.6043.

Observed heterozygosity, expected heterozygosity, observed homozygosity, expected homozygosity, mean heterozygosity, Polymorphism Information Content (PIC) and fixation index (F) in Liangshan Semi-wool Sheep population were shown in Table 2.

Table 1: Allele No. allele size ranges, effective allele No. and most frequent alleles and their frequencies of each markers

Loci	Allele No.	No. of effective alleles	Minimum of allele size (bp)	Maximum of allele size (bp)	Alleles of the most frequent alleles	Frequencies of the most frequent alleles
BM2830	6	3.8215	116	126	120	0.3663
BM4513	9	3.7394	141	163	161	0.3476
BMC1009	8	3.8074	286	308	288	0.4465
BMS0887	7	2.3195	141	157	149	0.6043
BMS1126	9	4.9584	155	171	161	0.3603
BMS1636	7	5.0820	124	136	128	0.2754
BMS1678	10	5.7833	206	226	222	0.2834
BMS2263	9	3.7394	141	163	161	0.3476
BMS835	11	2.7624	182	204	190	0.5561
ILSTS004	9	4.4194	99	119	103	0.3094
ILSTS0049	7	3.8413	198	216	206	0.3396
McM130	15	6.0629	146	174	150	0.3027
OarCP0034	7	4.1250	76	88	84	0.3844
OarCP0079	10	5.1979	95	119	109	0.2781
OarFCB11	9	4.6307	118	140	134	0.2727
Total	133	64.2905				
Mean	8.8667	4.2860				

Table 2: Observed heterozygosity, expected heterozygosity and observed homozygosity, expected homozygosity, Polymorphic Information Content (PIC) and fixation indices (F) in Liangshan Semi-wool Sheep population

Loci	Observed heterozygosity	Expected heterozygosity	Observed homozygosity	Expected homozygosity	PIC	Fixation indices
BM2830	0.6364	0.7403	0.3636	0.2597	0.6968	0.1381
BM4513	0.6952	0.7345	0.3048	0.2655	0.6889	0.0510
BMC1009	0.7647	0.7393	0.2353	0.2607	0.7093	-0.0371
BMS0887	0.5936	0.5704	0.4064	0.4296	0.5202	-0.0434
BMS1126	0.6648	0.8006	0.3352	0.1994	0.7767	0.1672
BMS1636	0.7166	0.8054	0.2834	0.1946	0.7754	0.1079
BMS1678	0.8877	0.8293	0.1123	0.1707	0.8061	-0.0733
BMS2263	0.6952	0.7345	0.3048	0.2655	0.6883	0.0510
BMS835	0.6738	0.6397	0.3262	0.3603	0.6042	-0.0561
ILSTS004	0.6796	0.7759	0.3204	0.2241	0.7391	0.1217
ILSTS0049	0.5829	0.7417	0.4171	0.2583	0.6963	0.2120
McM130	0.4486	0.8373	0.5514	0.1627	0.8183	0.4627
OarCP0034	0.7581	0.7596	0.2419	0.2404	0.7236	-0.0006
OarCP0079	0.5936	0.8098	0.4064	0.1902	0.7813	0.2650
OarFCB11	0.6898	0.7862	0.3102	0.2138	0.7516	0.1202
Mean	0.6720	0.7536	0.3280	0.2464	0.7184	0.0991

Observed heterozygosity and expected heterozygosity at the 15 microsatellites in Liangshan Semi-wool Sheep population were from 0.4486 (McM130) to 0.8877 (BMS1678) and from 0.5704 (BMS0887) to 0.8373 (McM130), respectively. Mean observed heterozygosity and mean expected heterozygosity were 0.672 and 0.7536, respectively. The average genetic variation (0.672) observed in this study was higher than that of Muzzafarnagri 0.652 (Arora and Bhatia, 2004), Nali 0.651 and Chokla 0.657 (Sodhi *et al*., 2006) as well as this is higher than Swiss sheep breed Mouflon 0.450 (Stahlberger-Saitbekova *et al*., 2001), Northern Spanish sheep-Latxa 0.661, Rubin del Molar 0.600, Churra 0.661, Xalda 0.572 (Alvarez *et al*., 2004). The high mean heterozygosity values could be attributed to low selection pressure and large number of alleles present in a population. The present estimates of gene diversity (Ho) were, however, lower than that of Spanish sheep breeds (Mean Ho = 0.713-0.771) and Awassi sheep from Turkey (Ho = 0.750) as reported by Arranz *et al*. (1998). Thus, differences in gene diversity (Ho) values observed might be ascribed to choice of microsatellite loci typed as well as existing population structure.

Another parameter also indicative of the genetic variation is Polymorphism Information Content (PIC). PIC was from 0.5202 (BMS0887) to 0.8183 (McM130) and mean PIC was 0.7184. PIC is a parameter indicative of the degree of informativeness of a marker. Following the criteria of Botstein *et al*. (1980), in this study, all 15 microsatellite loci appeared to be highly informative (PIC>0.5), According to the selective standard of the microsatellite loci, microsatellite loci ought to have at least four alleles to be considered useful for the evaluation of genetic diversity. Based on this criterion, the 15 microsatellite loci used in this study were useful for the evaluation of genetic diversity in Liangshan Semi-wool Sheep. These results imply that abundant genetic polymorphisms exist in the Liangshan Semi-wool Sheep. These high estimates of PIC substantiated the suitability of used set of markers to applications such as parentage control, linkage-mapping programs in addition, to genetic polymorphism studies in the sheep too. Of the 15 microsatellite loci, the fixation indices of BMS835, BMS0887, OarCP34, BMC1009, BMS1678 microsatellite loci were negative and others were positive. The mean fixation indices was 0.0991, reflecting that the degree of heterozygote surplus at these loci was not high and deviations from Hardy-Weinberg equilibrium were not significant.

Figure 1a-o reveal that for a few markers (e.g., BM2830, ILSTS0049) a narrow range of variation was found in the number of observed alleles. When this situation is found the most frequent alleles are likely to be the oldest, the others being the result of mutation process through insertion-deletion mechanisms. We can point out loci such as McM130 with a large number of alleles with low frequencies leading to flat distributions. The allele frequency distributions of 15 microsatellite loci show asymmetry. In several cases, the most frequent alleles showed an intermediate size (e.g., BMS0887, BMS1126), producing a centralised distribution. In other markers (e.g., OarFCB11, BMC1009) the variants with the greatest frequencies showed an extreme size. However, in most cases a common shape was not found. Even when some kind of skewness was evident it did not follow a consistent pattern, either to the right or to the left, in the populations. The distributions of allele frequency are in accordance with data previously reported for microsatellites. As an example, Forbes *et al*. (1995) analysed different domestic and bighorn sheep and found that distributions at some loci were highly centralised, while others were skewed, flat. Asymmetries of microsatellite mutation distributions have been found in

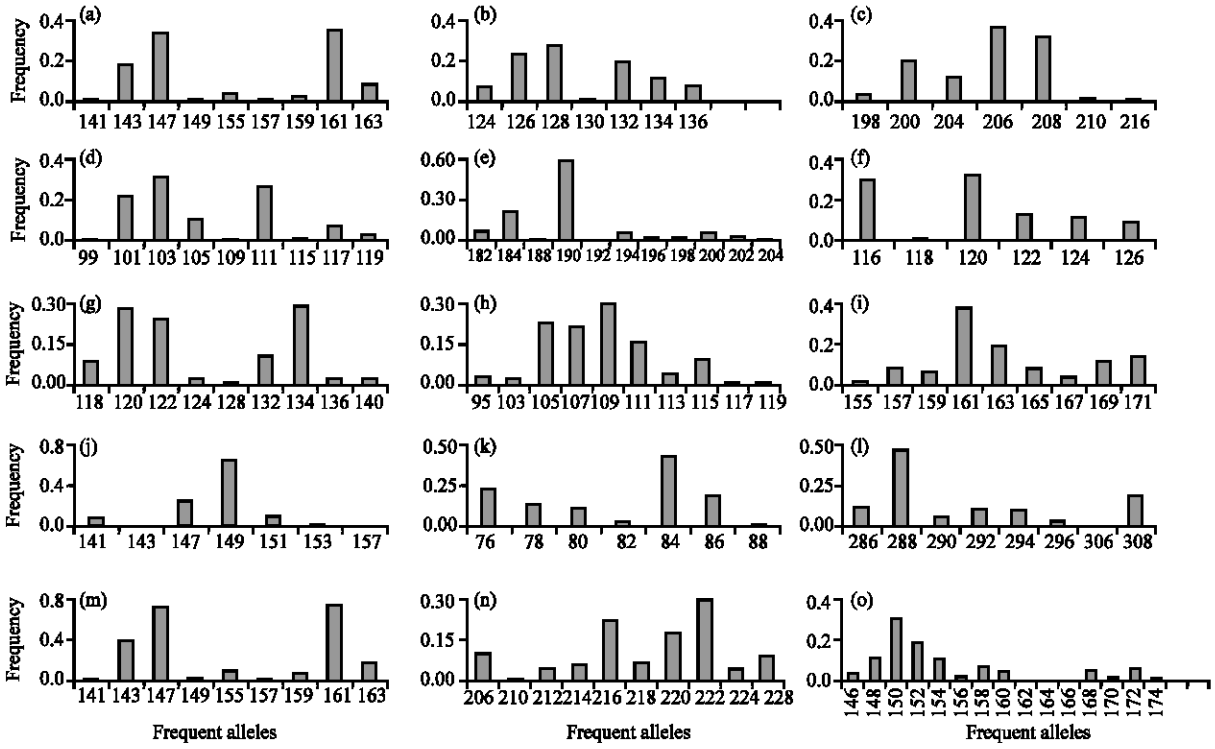

Fig. 1: Distributions of allele frequencies at 15 microsatellite markers in Liangshan Semi-wool Sheep, (a) BMS2263, (b) BMS1636, (c) ILSTS0049, (d) ILSTS004, (e) BMS835, (F) BM2830, (g) OarFOB11, (h) Oar OP0079, (I) BMS1126, (j) BMS0887, (k) OarOP0034, (l) BMC1009, (m) BM4513, (n) BMS1678 and (o) McM130

different studies. Goldstein and Pollock (1997) reviewed data in the literature and indicated that a positive asymmetry (a tendency to mutate to alleles of larger size) was first observed for large alleles at trinucleotide-expansion loci, but further investigations revealed asymmetry towards mutations that decrease size. They concluded that apart from differences among loci, allele size is an important factor in the determination of asymmetry effects. Another aspect that contributes to the irregularity of microsatellite distributions is the complex evolution at these loci. The high level of genetic variation was evident in the breed. The abundant genetic polymorphisms affords an opportunity to improve the defects that exist in Liangshan Semi-wool Sheep.

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