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Genetic diversity analysis of Mecheri sheep using microsatellite markers

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ABSTRACT

Genetic characterization of native breeds is the most significant step in safeguarding our germplasm. The present study aims to characterize Mecheri, a sheep mutton breed of Tamil Nadu, India based on microsatellite polymorphism. The animals of the breed are medium sized with compact body and covered with short hairs, which are usually not shorn. The animals are light brown in colour. Both males and females are polled and the breed occupies first position in terms of population and area of distribution in the state. Microsatellites are the markers of choice for genetic diversity analysis. The genomic DNA from 50 unrelated Mecheri sheep were PCR-amplified with a panel of 10 microsatellite markers and resolved through 6 per cent denaturing polyacrylamide gel electrophoresis followed by silver staining. The number of alleles ranged from 4 to 15 with allele sizes ranging from 96 to 154 bp. The allele frequencies ranged from 0.011 to 0.484. Polymorphism information content (PIC) ranged from 0.798 to 0.878. The population was deviating from Hardy-Weinberg equilibrium at most of the investigated loci, except two. The observed and expected heterozygosities ranged from 0.927 to 1.000 and 0.583 to 0.878. The set of microsatellite loci used in this study exhibited high genetic polymorphism and thus represented a useful panel of markers for population genetic studies in Indian sheep breeds.

Key words: Microsatellites, Mecheri, genetic diversity, Hardy-Weinberg equilibrium, Polymorphism information content.

INTRODUCTION

Genetic characterization of native breeds requires basic knowledge of genetic variation that can be measured within and between populations. Such variations may be of morphological or anatomical in origin and are called classical markers. Certain chromosomal abnormalities (numerical or structural) may also serve as markers, termed as chromosomal markers. In addition, the variations in molecules present in body fluids and tissues, that are detectable by either immunological (blood groups, MHC, etc.) or electrophoretic (isozymes, milk proteins, blood proteins, etc.) methods have also been exploited as markers, commonly referred to as biochemical markers. Classical and chromosomal markers usually have low degree of polymorphism and low heterozygosity. Though biochemical markers are polymorphic, they suffer from several disadvantages such as necessity in expression of the concerned genes or their sex-limited nature or effects of various degrees of dominance and pleiotrophy (Mommens *et al.* 1998). Microsatellites, on the other hand, are the markers of choice for genetic diversity analysis, because of their high informativeness, ubiquity in the genome and amenability to PCR. The microsatellite markers, due to their high levels of polymorphism, ubiquitous nature, codominant inheritance, ease and accuracy of typing have been successfully applied in population genetic studies (Buchanan *et al.* 1994, Mac-Hugh *et al.* 1998, Forbes *et al.* 1995) parentage testing (Coppieters *et al.* 1993) and linkage analyses (Barendse *et al.* 1994). Awareness of the value of genetic resources in livestock has stimulated the study of the genetic characterization of native breeds.

Mecheri sheep is one of the recognized breeds of sheep in Tamil Nadu, and belong to the hairy type, polled breeds of sheep (Acharya, 1982). The breed occupies first position in terms of population and area of distribution in the state. Mecheri sheep derives its name from the breeding tract, Mecheri block of Mettur taluka of Salem district. Mecheri sheep are also distributed in Salem, Erode, Karur, Namakkal and parts of Dharmapuri districts of Tamil Nadu. This tract lies in the northwestern agro-climatic zone of Tamil Nadu. Mecheri sheep are medium in size with a compact body and covered with short hairs, which are not shorn. Animals are light brown in colour. Their facial profile reveals a slightly Roman nose. Both males and females are polled. The pooled means for height at withers, chest girth and body length at above 24 months of age were 67±0.4, 74±0.4 and 66±0.4 cm, respectively. Mecheri sheep is famous for its meat producing ability with good growth rate and carcass characteristics. The body weight of the animals at birth 3, 6, 9 and 12 months of age was 2.82±0.01, 10.9±0.1, 15.6±0.1, 17.6±0.2 and 21.1±0.1, kg respectively. The dressing percentage in Mecheri sheep was found to be 54.4±0.4 and 51.8±0.5 in males and females respectively (Karunanithi *et al.* 2006). The molecular characterization is a desirable initial step to aid management and conservation strategies of Mecheri sheep breed, which helps to identify breeds that have unique genetic origin, and which therefore are important in preserving genetic diversity. The aim of the present study was to examine the genetic variability within Mecheri sheep breed of Tamil Nadu using FAO recommended microsatellite markers.

MATERIALS AND METHODS

Blood samples were collected at random from 50 unrelated Mecheri sheep distributed in different villages of the breeding tract. Sampling was done as per the guidelines of the MOoDAD program of The Food and Agriculture Organization of United Nations (FAO, 2004). Blood was collected aseptically into vacutainers containing heparin from the anterior vena cava by holding the animal in dorsal recumbency. DNA was isolated by a rapid non-enzymatic method (Lahiri and Nurnberger, 1991). The DNA samples were stored at - 20°C and/or at 4°C.

Microsatellite markers were chosen based on the size of the product, level of polymorphic information content and easiness of scoring. Ten microsatellite markers were used in the present study. Genomic DNA was amplified by polymerase chain

reaction using the FAO recommended microsatellite primers (Table 1). PCR amplification was carried out in a 20µl final reaction volume containing 20-50 ng of genomic DNA, 20 picomoles of each primer, 1.5mM MgCl2, 100µM dNTPs, 0.25U Taq polymerase and 10x buffer. The PCR protocol was as follows, 3 min at 94°C followed by 30 cycles of 1 min at 94°C, 1 min at annealing temperature and 1 min at 72°C. The last elongation step was prolonged to 10 min. The reaction was stopped by soaking the tubes at 4°C. The amplified PCR products were electrophoresed in 6% denaturing urea polyacrylamide gel at a constant voltage of 1300 volts for a period of 1 to 2 h, depending upon the size of PCR products and then silver-stained (Comincini et al. 1995). The alleles were scored manually and analyzed by software-aided gel documentation system (Bio-Rad, USA). The exact allele sizes were determined by direct comparison with adjacent PCR bands and 10bp ladder. Allele frequencies were estimated by direct counting from the genotypes. The polymorphism information content was calculated using the individual frequencies in which the alleles occur at each locus. The heterozygosity at the locus was estimated as per Nei, 1973. The expected number of genotypes was compared with the observed genotypes using ?2 test for goodness of fit so as to assess whether the studied population was in Hardy-Weinberg equilibrium (HWE).

Table 1. List of primers used to amplify different microsatellite loci in Mecheri sheep

Locus	Primer Sequence	Chromosome Number	Annealing temp. (°C)
BM8125	P1: CTCTATCTGTGGAAAAGGTGGG P2: GGGGGTTAGACTTCAACATACG	17	60
CSSM31	P1: CCAAGTTTAGTRACTTGTAAGTAGA P2: GACTCTCTAGCACTTTATCTGTGT	23	60
OarAE129	P1:AATCCAGTGTGTGAAAGACTAATCCAG P2: GTAGATCAAGATATAGAATATTTTTCAACACC	5	60
OarCP34	P1: GCTGAAGAATGTGATATGTTCAGG P2: GGGACAATACTGTCTTAGATGCTGC	5	63
OarFCB128	P1: CAGCTGAGCAACTAAGACATACATGCG P2: ATTAAAGCATCTTCTCTTTATTTCCTCGC	2	63
OarHH35	P1: AATTGCATTCATATCTTTAACATC TGGC P2: ATGAAAATATAAAGAGAATGAACCACACGG	4	63
OarJMP29	P1: GTATACACGTGGACACCGCTTTGTAC P2: GAAGTGGCAAGATTCAGAGGGGAAG	24	55
OarJMP8	P1: CGGGATGATCTTCTGTCCAAATATGC P2: CATTTGCTTTGGCTTCAGAACCAGAG	6	63
RM4	P1: CAGCAAAATATCAGCAAACCT P2: CCACCTGGGAAGGCCTTTA	15	55
TGLA377	P1: GACTGTCATTATCTTCCAGCGGAC P2: GATCTCTGGTTGAAATGGCCAGCAG	2	55

RESULTS AND DISCUSSION

The microsatellite allele number, size and frequency data are presented in Table 2. The number of alleles was in the range of 4 to 15. A total of 86 alleles were observed at the10 different microsatellite loci in Mecheri sheep. In general, the number and sizes of microsatellite alleles observed in this study fall within the range mentioned in the Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans, published by FAO 2004. Apart from the loci TGLA 377 where only four alleles were detected, a fairly high degree of genetic variation was observed in terms of number of alleles per locus. The frequency of the alleles ranged from 0.011 (Oar FCB 128 and Oar JMP 29) to 0.484 (TGLA 377). The number of alleles observed at a locus is an indication of genetic variability at that locus having direct impact on differentiation of breeds within a species (Buchanan et al. 1994). The average number of alleles found in the investigated breed was, however, lower than most of the Baltic (Grigaliunaite *et al.* 2003), Austrian (Baumung *et al.* 2006) and Caucasian (Hirbo *et al.* 2006) sheep breeds. However, a direct comparison cannot be made, as the microsatellite loci used in those studies were different from that of the present study.

The mean number of alleles observed in the present study was generally higher than those reported for Nilgiri and Coimbatore sheep, the other two breeds of sheep from the State of Tamilnadu. In Nilgiri sheep, the number of alleles across all the loci studied ranged from 3 to 8 with a mean of 5 and the size of alleles ranged from 72 to 228 bp. The frequency of alleles ranged from 0.010 to 0.578 (Girish *et al.* 2007). Kumarasamy *et al* (2008) analyzed genetic structure of Coimbatore breed with 27 sheep specific markers and observed that the number of alleles ranged from 3 to 8 with a mean of 6 and the size of alleles ranged from 72 to 220 bp. The frequency of the alleles ranged from 0.017 to 0.725. In another study with 25 markers on Ganjam sheep, distributed in Orissa state of India, the mean number of allele was found to be 5.48 (Arora *et al.* 2008).

Table 2. Microsatellite alleles and their frequency in Mecheri Sheepa

S. No.	Locus	Allele No.	Allele size (bp) and frequency							
1	BM 8125	6	114	116	118	120	122	124		
			0.0813	0.3953	0.4418	0.0581	0.0116	0.0116		
			122	124	130	132	134	136	140	144
			0.0330	0.0330	0.0166	0.0330	0.0166	0.0166	0.0166	0.0166
2	CSSM 31	15	146	148	150	152	154	160	164	
			0.0500	0.2125	0.2125	0.0250	0.0250	0.0750	0.0125	
3	Oar AE 129	6	144	146	148	150	152	154		
			0.0813	0.1510	0.2441	0.2209	0.2250	0.0930		
4	Oar CP 34	13	111	113	115	117	119	121	123	125
			0.0450	0.0450	0.0303	0.0450	0.1818	0.0757	0.2575	0.0152
			127	129	131	145	147			
			0.1212	0.0454	0.0450	0.1515	0.4540			
5	Oar FCB 128	6	108	110	112	118	120	122		
			0.0640	0.3870	0.3064	0.0645	0.1129	0.0645		
6	Oar HH 35	9	114	116	118	122	124	126	128	132
			0.0444	0.0555	0.0666	0.0666	0.2444	0.3333	0.1777	0.0333
			134							
			0.0333							
7	Oar JMP 29	12	124	126	128	130	132	134	136	138
			0.0555	0.1777	0.0666	0.1000	0.0333	0.0444	0.0222	0.1666
			140	142	144	148				
			0.1888	0.0777	0.0555	0.0111				
8	Oar JMP 8	9	119	121	123	127	129	131	133	135
			0.0125	0.0375	0.0500	0.2250	0.2250	0.1125	0.2000	0.1250
			137							
			0.0125							
9	RM 4	6	134	136	138	140	142	144		
			0.0348	0.1162	0.2325	0.3488	0.2325	0.0348		
10	TGLA 377	4	96	98	100	102				
			0.0810	0.4840	0.4190	0.0160				

The results of the microsatellite analysis of Mecheri sheep in terms of polymorphism information content equilibrium and heterozygosity are furnished in Table 3. The polymorphic information content is a parameter, indicative of the degree of informativeness of a marker. The degree of informativeness of a marker reveals its usefulness in diversity analysis of a breed. In the present study polymorphism information content revealed an average of 0.798 with a range of 0.609 (TGLA 377) to 0.878 (Oar JMP 29). Except TGLA 377, all other loci possessed very high PIC values indicating that these markers are highly informative for characterization of Mecheri sheep breed. The PIC values observed in most of the loci are comparable with Garole sheep (Sodhi *et al.* 2003), Nilgiri sheep (Girish *et al.* 2007) of India and those observed in other indigenous breeds of sheep breeds by Arora and Bhatia (2004, 2006) and Mukesh *et al* (2006).

Table 3. Polymorphic information content (PIC) equilibrium and heterozygosity in Mecheri sheep

S. No.	Locus	PIC	HWE (χ^2)	Heterozygosity	
				Observed	Expected
1	BM 8125	0.705	19.05 [NS]	0.638	1.000
2	CSSM 31	0.867	249.269**	0.855	1.000
3	Oar AE 129	0.848	51.131**	0.826	0.977
4	Oar CP 34	0.871	251.343**	0.867	0.939
5	Oar FCB 128	0.762	95.570**	0.731	1.000
6	Oar HH 35	0.810	151.838**	0.786	1.000
7	Oar JMP 29	0.878	225.649**	0.878	0.978
8	Oar JMP 8	0.846	35.011 [NS]	0.826	0.927
9	RM 4	0.782	565.876**	0.754	1.000
10	TGLA 377	0.609	15.831 [NS]	0.583	1.000

[NS]- Not significant, ** - Highly significant ($P<0.01$)

The Chi-square test for Hardy-Weinberg equilibrium revealed that except in 2 loci (Oar JMP 8 and TGLA 377), the Mecheri sheep population was deviating from equilibrium at most of the investigated microsatellite loci. The disequilibria proportion observed in the population can be attributed to the existence of null alleles, inherently high mutation rate of microsatellites and size homoplasy of loci, besides the small sample size (although representative of the entire breeding tract) taken in the present study. The disequilibria proportions observed in most of the loci in the study might be due to both the systematic and dispersive processes operating in the population. Similar population disequilibria were noticed for Garole (Sodhi *et al.* 2003), Muzzafarnagri (Arora, 2004), Bellary (Kumar *et al.* 2007) and Nilgiri sheep (Girish *et al.* 2007) of India. Similar disequilibria

were also reported in previous study (Satyanarayana, 2001).

The heterozygosity is an appropriate measure of genetic variability within a population. The observed and expected heterozygosities ranged from 0.583 (TGLA 377) to 0.878 (Oar JMP 29) and 0.927 (Oar JMP 8) to 1.000 (BM 8125, CSSM 31, Oar FCB 128, Oar HH 35, RM 4, TGLA 377), respectively. Though few loci exhibited lower heterozygosity values, majority of the loci had relatively higher expected heterozygosity, which reflects the existence of substantial variation in Mecheri sheep in the breeding tract. The average observed heterozygosity in this breed was higher than 0.7610 (range 0.4222 to 1.000) reported for Nilgiri sheep (Girish *et al.* 2007). The heterozygosity ranging from 0.6250 to 0.8462 was observed in Coimbatore sheep (Kumarasamy *et al.* 2007). The average observed heterozygosity was 0.623 in Ganjam sheep (Arora *et al.* 2008) of India. The result of the present study harmonizes with the same sheep breed using different sets of microsatellite markers (Satyanarayana, 2001).

In conclusion all the 10 microsatellite loci investigated in the present study were highly polymorphic in nature and the overall mean polymorphism information content of 79.8 per cent suggests that these markers are highly informative for genetic characterization of Mecheri breed. A high degree of genetic variability was observed in Mecheri Sheep population, which was evident from the allelic polymorphism and observed heterozygosity. The polymorphism study and characterization of Mecheri sheep will facilitate the identification and analysis of genetic variability in the population for conservation

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