Molecular Characterisation of Nilagiri Sheep (*Ovis aries*) of South India Based on Microsatellites

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ABSTRACT: Genetic variation in Nilagiri sheep, the only apparel wool breed in South India was studied using 25 FAO recommended ovine-specific microsatellite markers. The number of observed alleles ranged from 3 to 8 with a mean of 5 across all loci. The size of alleles ranged from 72 to 228 bp. The frequency of alleles ranged from 0.0104 to 0.5781. In total, 125 alleles were observed at the 25 loci studied. The effective number of alleles ranged from 2.18 to 6.49. The mean number of effective alleles was 3.84 across all loci. All the 25 loci were found to be highly polymorphic. The PIC values ranged from 0.4587 to 0.8277 with a mean of 0.6485. Of 25 microsatellites studied, 17 were in Hardy-Weinberg Equilibrium proportions. The observed heterozygosity ranged from 0.4222 to 1.000 with a mean value of 0.7610 whereas the expected heterozygosity ranged from 0.5415 to 0.8459 with a mean value of 0.7213. Except six loci, the other loci revealed negative within-population inbreeding estimates (F_{IS}) indicating excess of heterozygotes in the population of Nilagiri sheep. (**Key Words :** Genetic Characterisation, Nilagiri Sheep, Microsatellites, PIC, Inbreeding Estimates)

INTRODUCTION

The erosion of domestic animal diversity due to natural causes and creative human activity is of serious concern if current production levels are to be sustained and the changing demands of future markets are to be addressed. There are more than 1,400 modern domestic sheep breeds. However the Food and Agricultural organization of the United Nations (FAO) estimates that at least one breed of traditional livestock becomes extinct every week. According to FAO, nearly 800 farm animal genetic resources have been lost and about 30% of all those remaining are associated with some degree of risk. To develop conservation strategies for the breeds, their genetic diversity and genetic distinctness must be determined. Thus, a molecular genetic study of population structure may improve the understanding of present day genetic resources.

Tamilnadu State of south India has eight recognized sheep breeds and Nilagiri is one among them. Nilagiri, evolved during the 19th century contains unknown levels of inheritance of Coimbatore (the local hairy breed),

Tasmanian Merino, Cheviot and South Down (Acharya, 1982). The breed is well adapted to the conditions of the Nilagiri hills and produces fine fleece. There is little organized shearing and marketing of wool, the sheep being mostly maintained for manure by tea planters and other flock owners. The population of this breed according to 1972 census was 8,000 and 1977 census it was 7,677. At present, the population is found to be less than 1,000. According to the conservationists, a combination of factors including loss of grazing ground in the Nilagiri district in the wake of agriculture taking a pointed diversion to horticulture and plantation crop is responsible for the decline in this breed. In addition, indiscriminate crossbreeding with a few exotic crossbred sheep during the last few decades at the field level has endangered the existence of this native breed. Considering its very small numbers, its adaptation and the need for meat and apparel wool in the area in which it is located, efforts have been initiated to conserve the Nilagiri breed of sheep. The present study on the molecular characterisation of Nilagiri sheep is one such effort towards their conservation, as microsatellite DNA markers are found suitable for the description of breeds due to their dense distribution in the genome, great variation, co-dominant inheritance and easy genotyping.

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Table 1. Number of observed and effective alleles, size of alleles, polymorphism information content, observed and expected heterozygosity at 25 microsatellite loci in Nilagiri sheep

Locus	Observed alleles	Effective alleles	Allele size range (bp)	PIC	Hardy-Weinberg equilibrium (x^2 value)	Heterozygosity		Within population inbreeding estimate
						Observed	Expected	
BM1314	8	6.49	144-170	0.8277	43.1043*	0.9787	0.8459	-0.1571
BM6506	5	3.51	214-226	0.6732	34.3445**	0.4222	0.7153	0.4097
BM757	4	3.38	176-196	0.6473	10.2703[NS]	0.7209	0.7039	-0.0242
BM8125	5	3.24	114-124	0.6413	26.7441**	0.7826	0.6914	-0.1319
BM827	5	3.57	216-228	0.6777	32.1210**	0.5581	0.7196	0.2244
CSSM31	5	2.94	134-164	0.6079	12.0846[NS]	0.7442	0.6601	-0.1274
CSSM47	3	2.59	162-174	0.5363	4.6208[NS]	0.5676	0.6136	0.0750
HUJ616	4	3.96	120-134	0.7000	41.2841**	1.0000	0.7473	-0.3381
OarAE129	3	2.18	150-160	0.4587	27.3697**	0.5938	0.5415	-0.0965
OarCP20	6	4.11	72-90	0.7180	21.6551[NS]	0.8000	0.7570	-0.0568
OarCP34	6	5.53	114-126	0.7939	22.1231[NS]	0.9744	0.8192	-0.1894
OarCP38	6	3.76	112-130	0.6904	48.6232**	0.9730	0.7337	-0.3260
OarFCB128	4	3.11	100-126	0.6188	10.8257[NS]	0.7419	0.6790	-0.0927
OarFCB20	6	4.01	124-148	0.7198	12.6400[NS]	0.8293	0.7507	-0.1046
OarFCB48	4	3.50	144-160	0.6602	1.5588[NS]	0.7234	0.7150	-0.0117
OarHH35	7	5.21	118-136	0.7805	21.1670[NS]	0.9167	0.8079	-0.1346
OarHH41	4	3.27	128-152	0.6334	8.2680[NS]	0.7027	0.6943	-0.0121
OarHH47	4	3.30	124-144	0.6474	9.9933[NS]	0.6341	0.6972	0.0904
OarHH64	7	5.70	104-128	0.8012	26.9856[NS]	0.9167	0.8247	-0.1160
OarJMP29	7	5.40	118-144	0.7901	45.9496**	0.6304	0.8147	0.2262
OarJMP8	5	4.25	144-168	0.6755	10.0223[NS]	0.8409	0.7647	-0.0996
OarVH72	5	3.51	128-140	0.6755	17.8340[NS]	0.8810	0.7157	-0.2309
RM4	4	2.81	138-148	0.5931	6.8404[NS]	0.6471	0.6449	-0.0034
TGLA137	4	3.83	136-156	0.6909	7.1812[NS]	0.8158	0.7393	-0.1035
TGLA377	4	2.75	86-100	0.5874	10.7604[NS]	0.6304	0.6363	0.0093
Mean	5.0	3.84		0.6485		0.7610	0.7213	-0.0551

[NS] Not significant, * Significant ($p<0.05$), ** Highly significant ($p<0.01$).

MATERIALS AND METHODS

Animal samples

Blood samples were collected from 50 Nilagiri sheep, unrelated by ancestry and the genomic DNA was isolated by a rapid non-enzymatic method as described by Lahiri and Nurnberger (1991).

PCR amplification and genotyping

A total of 25 microsatellite primer sets specific for sheep were used in the study as recommended by FAO (http://www.fao.org/dad-is). PCR amplification was performed on a PTC-200 thermal cycler (MJ Research Inc., USA). Each 20 µl PCR reaction mixture contained 50 ng of genomic DNA, PCR assay buffer 10×, 1.5 mM of $MgCl_2$, dNTPs (each at 100 µM) (Sigma-Aldrich, USA), 20 picomoles of each primer (Microsynth, Switzerland) and 0.75 units of Taq DNA polymerase (Biolabs, New England). The annealing temperature used was 50, 55, 57, 60, 62 or 63°C for different primers. The PCR products were separated on a 6% denaturing polyacrylamide gel and

silver-stained using a simplified method (Lujiang Qu et al., 2005). The silver-stained gels were analysed by Diversity Database software (Bio-Rad, USA) for sizing of various allelic products. The product sizes were determined with the help of 10 bp DNA ladder (Invitrogen, USA) as a standard marker.

Molecular genetic analysis

Microsatellite allele frequencies, effective number of alleles, polymorphism information content (PIC), test of Hardy-Weinberg equilibrium (HWE), observed and expected heterozygosity and F-statistics were calculated using the Popgene version 1.31 (Yeh et al., 1999).

RESULTS AND DISCUSSION

The genotyping of animals was done based on the size of PCR products resolved through polyacrylamide gels and their sizes were assessed on the basis of 10 bp molecular DNA marker. The genotypes were scored based on the presence of a single band (homozygotes) or double bands (heterozygotes) in the gel.

Number of observed alleles

In the present study, the number of observed alleles ranged from 3 (CSSM47 and OarAE129) to 8 (BM1314) with a mean of 5 across all loci (Table 1). The most frequent number of alleles was 4 (36 per cent) and the least frequent number of alleles was 8 (4 per cent). The size of alleles ranged from 72 (OarCP20) to 228 bp (BM827). In total, 125 alleles were observed at the 25 loci studied. The frequency of the alleles ranged from 0.0104 to 0.5781 across all loci. Similarly, 126 alleles were observed at 25 microsatellite in Muzzafarnagri sheep breed with the number of observed allele varying between 2 and 8, the average being 5.04 (Arora and Bhatia, 2004). In Garole sheep, the observed number of alleles reported was 6.2 (Sodhi et al., 2003), which is little higher than that of the present study. In Nali and Chokla sheep breeds (carpet wool breeds of Northwestern India), a total of 138 and 133 alleles respectively were reported. The number of observed allele ranged from 3 to 10 in Nali and 2 to 8 in Chokla with the respective means of 5.52 and 5.32 alleles (Sodhi et al., 2005), which is similar to the observation of the present study. In Magra breed of sheep, another carpet wool breed of Northwestern India, the number of observed alleles ranged from 3 to 10 with a mean of 5.7 (Arora and Bhatia, 2006). In exotic sheep breeds, a wide range of number of observed alleles were reported (2 to 4, Gutierrez-Espelata et al., 2000; 7 to 28, Pariset et al., 2003).

Effective number of alleles

Of the 25 loci studied, the effective number of alleles ranged from 2.18 (OarAE129) to 6.49 (BM1314). The mean number of effective alleles was 3.84 across all loci (Table 1). The effective number of alleles at each locus provides information on predominant alleles. The results of the present study are similar to the earlier reports of 3.73 effective number of alleles in Garole sheep (Sodhi et al., 2003), 3.64 alleles in Muzzafarnagri sheep (Arora and Bhatia, 2004), 3.34 in Nali and 3.27 in Chokla sheep (Sodhi et al., 2005) and 3.8 in Magra sheep (Arora and Bhatia, 2006).

Polymorphism information content

The polymorphism information content (PIC) values for the 25 loci are presented in Table 1. In general, the PIC values are suggestive of high polymorphic nature of the microsatellite loci analyzed. The PIC values in the present study ranged from 0.4587 (OarAE129) to 0.8277 (BM1314). The mean PIC value for all the 25 loci was found to be 0.6485. Based on the PIC values, it was found that 96 per cent of these markers showed values of more than 0.5, indicating that these microsatellite markers can effectively be used for molecular characterisation and genetic diversity

studies. Among the Indian sheep breeds, the PIC values were estimated to range from 0.533 to 0.808 in Muzzafarnagri sheep (Arora and Bhatia, 2004), 0.210 to 0.831 and 0.346 to 0.768 in Nali and Chokla sheep breeds respectively (Sodhi et al., 2005) and 0.347 to 0.849 in Magra sheep (Arora and Bhatia, 2006)using ovine-specific microsatellite markers. Among the exotic sheep breeds, the lowest PIC value of 0.20 (Jandurova et al., 2005) and the highest of 0.89 (Arranz et al., 2001) were observed.

Hardy-Weinberg equilibrium

The results of the x^2 test of goodness of fit (Table 1) revealed that the population was in Hardy-Weinberg Equilibrium proportions for 17 microsatellite loci. The remaining 8 loci departed from HWE, which might be either due to the presence of unobserved null alleles (non-amplifying alleles) or the consequences of several years of intensive selection practised in the population of Nilagiri sheep. On the contrary, the systematic or dispersive forces have not influenced those loci under HWE. The reports on exotic sheep breeds revealed similar departure of the populations from HWE at various microsatellite loci (Diez-Tascon et al., 2000; Tomasco et al., 2002; Alvarez et al., 2004; Ivankovic et al., 2005; Jandurova et al., 2005; Calvo et al., 2006). On the other hand, some of the reports showed that the sheep populations studied were in HWE for the respective microsatellite loci (Gutierrez-Espelata et al., 2000; Soysal et al., 2005).

Estimation of observed and expected heterozygosity

Genetic diversity can be measured as the amount of actual or potential heterozygosity. The estimates of heterozygosity for each locus are given in Table 1. The observed heterozygosity ranged from 0.4222 (BM6506) to 1.000 (HUJ616) with a mean value of 0.7610 while the expected heterozygosity ranged from 0.5415 (OarAE129) to 0.8459 (BM1314) with a mean value of 0.7213. The high mean heterozygosity values estimated in the present study could be attributed to low level of inbreeding, low selection pressure and large number of alleles. The genetic markers having a higher number of alleles per locus and a higher degree of heterozygosity are more useful for population and individual typing. The genetic variability of the Nilagiri sheep is relatively high, supported by the mean number of alleles per microsatellite locus and mean expected heterozygosity. The level of genetic variation indicated that the genome of Nilagiri sheep has diverse origins, as a consequence of improvement of Coimbatore breed of sheep (the local breed) by exotic sheep breeds such as Tasmanian Merino, Cheviot and South Down during the 19th century. The very low level of inbreeding related to the population size estimated in the present study could not lead to the

possibility of fixation of alleles. The high heterozygosity values obtained in the Nilagiri sheep were in accordance with those reported in French Mutton Merino (0.679; Diez-Tascon et al., 2000), Swiss sheep breeds (0.67; Saitbekova et al., 2001), Garole (0.673; Sodhi et al., 2003), Muzzafarnagri (0.697; Arrora and Bhatia, 2004), Spanish sheep (0.776; Calvo et al., 2006), Czech and Slovak sheep breeds (0.736; Jandurova et al., 2005), Baltic sheep breeds (0.71; Tapio et al., 2005), Pag Island sheep (0.833; Ivankovic et al., 2005), Turkish sheep breeds (0.75; Soysal et al., 2005), Nali and Chokla sheep breeds (0.67; Sodhi et al., 2005) and Magra sheep (0.69; Arora and Bhatia, 2006).

Within-population inbreeding estimate

The overall within-population inbreeding estimates or heterozygotes deficiency within-population (F_{IS}) calculated in the present study was -0.0551 indicating excess of heterozygotes in the population of Nilagiri sheep. Though positive F_{IS} values were observed in six loci, the excess of heterozygotes found in the majority of the loci (80 per cent) reflects outbreeding and wide genetic variability as a result of admixture of various breeds in the formation of Nilagiri sheep. The F_{IS} reported in most of the literature indicated various levels of inbreeding as 0.19 in Sarda sheep (Pariset et al., 2003), 0.066 in Spanish breeds (Alvarez et al., 2004), 0.009 in Spanish sheep (Calvo et al., 2006) and 0.033 in Turkish sheep breeds (Soysal et al., 2005). Among the Indian sheep breeds, Arora and Bhatia (2004) reported a mean F_{IS} value of 0.058 in Muzzafarnagri indicating a very low rate of inbreeding in that population. However, a high rate of inbreeding was reported in Nali and Chokla (0.397 and 0.299 respectively; Sodhi et al., 2005) and Magra (0.159; Arora and Bhatia, 2006) sheep breeds.

Conclusions

The results of the present study reveal high genetic variation in the population and hence, there is scope for further improvement. The high within-breed variation existing in this small population proves the fact that the breed has evolved from a broad genetic base. Owing to its small population size, greater variation, dual utility (meat and wool) and their genetic isolation (confined only to the hilly terrains of the Nilgiris), it is highly imperative to strengthen the conservation measures.

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