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Genetic diversity in Nilgiri sheep (*Ovis aries*): A molecular approach through microsatellite markers

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Abstract

Genetic variation in Nilgiri sheep, an apparel wool breed of South India, was studied using four microsatellite markers as recommended by FAO. The number of observed alleles ranged from 5 to 9 with a mean of 7 across all loci. The size of alleles ranged from 106 to 198 bp. In total, 28 alleles were observed in 4 loci. The frequency of alleles ranged from 0.0104 to 0.8085. The effective number of alleles ranged from 1.48 to 5.78 with a mean of 4.114 ± 1.888. The polymorphism information content (PIC) values varied from 0.2991 to 0.8062. The overall means for observed and expected heterozygosities were 0.5796 ± 0.3251 and 0.6781 ± 0.2367, respectively. The within population inbreeding estimate indicates heterozygote deficiency of 0.1451. Mode shift analysis revealed a normal L-shaped curve indicating that Nilgiri sheep population is non-bottlenecked. The markers used in the study were highly informative and high heterozygosity value is indicative of the high amount of genetic variability that can be exploited for their further improvement.

Keywords: allele frequency, genetic diversity, heterozygosity, Nilgiri sheep, PIC

INTRODUCTION

Among the domestic animal diversity, Tamilnadu is rich in sheep genetic resources with eight distinct breeds of sheep besides few lesser-known populations. Of eight recognized breeds, Nilgiri sheep is a unique, fine apparel wool breed, reported to be evolved during the 19th century, from the crossbred base, containing an unknown level of inheritance of Coimbatore, Tasmanian Merino, Cheviot and South Down breeds (Acharya, 1982). According to 1972 census, the population of this breed was 8,000 and subsequently reduced to 7,677 (Livestock census, 1977). At present, the estimated population would be very less about less than 2,000. The animals of true-to-type are found only in the organized farm (Sheep Breeding Research Station, Sandynallah, The Nilgiris, Tamil Nadu, South India). The sheep are meant for both meat and production of quality wool. It is also maintained for manure by tea planters and other flock owners. Considering the small population size, adaptation and the need for meat and apparel wool, immediate conservation strategies have to be initiated to safeguard this unique germplasm. Since genetic characterization is one of the pre-requisites for taking up decision on conservation of native germplasm, the present study was aimed at elucidating the genetic variation found within the Nilgiri sheep using microsatellite markers.

MATERIALS AND METHODS

Nilgiri sheep

The Nilgiri sheep is a medium-sized animal found only in uphills/undulating terrains of the Nilgiri district of Tamilnadu, South India. It is a fine apparel wool breed with white coat colour and exceptionally brown patches found on face and body. The face line is convex, giving a typical Roman nose. Males have horn buds while females are polled. The typical Nilgiri sheep is presented in Fig 1. Blood samples were collected from 48 Nilgiri sheep unrelated by ancestery and genomic DNA was isolated by phenol-chloroform method as described by Sambrook *et al.*, (1989). The purity and concentration of DNA samples were estimated by UV spectrophotometer.

Microsatellite analysis

As per the Secondary Guidelines of Food and Agriculture Organization of United Nations (FAO, 2004), a total of four microsatellite markers were selected based on degree of polymorphism and genome coverage. The basic details of microsatellite loci are presented in the Table 1.These markers were amplified using thermal cycler (Eppendorf mastercycle, epgradient S) with the PCR mixture of 15 µl. The mixture was prepared by adding 50-100 ng of template DNA; 1.5 µM $MgCl_2$; 5 picomoles each of forward and reverse primers; 0.75 units of taq polymerase and 100 µM dNTPs. The annealing temperature was ranging from 50°C to 63°C for different primers and the amplification was done

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Table 1: Basic details of microsatellite loci studied for genetic variation in Nilgiri Sheep *O.aries*

Locus	Primer sequence	Chromosome Number	Annealing temperature	Dye	Gene bank accession number
BM757	F 5′TGGAAACAATGTAAACCTGGG 3′ R 5′TTGAGCCACCAAGGAACC 3′	9	56°C	FAM	G18473
CSSM47	F 5′TCTCTGTCTCTATCACTATATGGC 3′ R 5′CTGGGCACCTGAAACTATCATCAT 5′	2	56°C	TET	U03821
OarJMP29	F 5′GTATACACGTGGACACCGCTTTGTAC 3′ R 5′GAAGTGGCAAGATTCAGAGGGGAAG 3′	24	58°C	FAM	U30893
OarCP34	F 5′GCTGAACAATGTGATATGTTCAGG 3′ R 5′GGGACAATACTGTCTTAGATGCTGC 3′	9	56°C	FAM	U15697

Table 2. Allele number, size and frequency at four microsatellite loci in Nilgiri sheep *O.aries*

Locus	Observed No. of alleles	No. Of Effective alleles	Allele sizes (bp) and their frequencies								
OarJMP29	9	5.7817	114	124	126	128	132	134	136	138	144
			0.0312	0.0625	0.1250	0.0833	0.1875	0.0625	0.2917	0.1458	0.0104
CSSM47	5	1.4835	126	128	130	132	134				
			0.0106	0.0106	0.1383	0.8085	0.0319				
OarCP34	7	5.1148	106	108	110	112	114	116	118		
			0.0116	0.2558	0.0930	0.1628	0.0465	0.2209	0.0213		
BM757	7	4.0779	178	180	182	184	188	196	198		
			0.3021	0.2083	0.0938	0.3125	0.0104	0.0625	0.0104		
Mean/ Range	7±1.633	4.1145± 1.8889	Allele frequency = 0.0104 to 0.8085; Allele range = 106 to 198 bp								

Table 3. Polymorphism information content, Chi-square values and heterozygote deficiency at four microsatellite loci in Nilgiri sheep *O.aries*

Locus	PIC	Hardy-Weinberg Equilibrium (x^2 value)	Heterozygosity		Within-population Inbreeding Estimate(F_{IS})
			Observed	Expected	
OarJMP29	0.8062	74.2638**	0.7292	0.8270	0.1183
CSSM47	0.2991	65.2601**	0.1064	0.3259	0.6736
OarCP34	0.7755	19.1215^{NS}	0.8372	0.8045	-0.0407
BM757	0.7145	32.9613*	0.6458	0.7548	0.1443
Mean	0.6488± 0.2362		0.5796± 0.3251	0.6781± 0.2367	0.1451

*Significant ($p \leq 0.05$), **Highly significant ($p \leq 0.01$), ^{NS} Not significant ($p \leq 0.05$)

Table 4. Bottleneck analysis in Nilgiri sheep *O.aries*

Model		IAM	TPM	SMM
Sign rank test Number of loci with heterozygosity excess	Expected	2.38	2.36	2.35
	Observed	0.533	0.5437	0.1926
Standardized differences test T2 values		-0.644	-2.734	-7.135
Wilcoxon test Probability of heterozygosity excess		0.8437	0.9062	0.9375

IAM-Infinite allele model; TPM-Two phase model; SMM-Stepwise mutation model

for 35 cycles. The genotyping was carried out on an automated ABI PRISM 3730XL Genetic Analyzer. Analysis of the fragment size and genotype of alleles were performed by the Gene Mapper™ software v4.0.

Statistical procedure

The genotyping of animals was done based on the size of PCR products. The genotypes were scored based on the presence of a homozygote or heterozygote in the gel. The genotypic data were subjected to analysis using software, POPGENE version 1.31 (Yeh *et al*., 1999) and the characteristics of microsatellites such as number of alleles, allele frequencies, observed and expected heterozygosities, Chi-square values and F-statistics were obtained. The polymorphic information content of each locus is estimated through PIC calculator. Since the

population size is very low, bottleneck analysis was performed using the programme, BOTTLENECK 1.2.02.

RESULTS AND DISCUSSION

In the present study, the number of observed alleles ranged from 5 (CSSM47) to 9 (OarJMP29) with a mean of 7 across all loci (Table 2). A total of 28 number of alleles were found in four polymorphic loci studied. The size of alleles ranged from 106 (OarCP34) to 198 bp (BM757). These microsatellite allele were found distributed with frequency ranging from 0.0104 to 0.8085. The allele 132 bp found in CSSM47 is predominant with the highest frequency of 0.8085 among all other alleles. This may be considered as one of the unique characteristics of microsatellites in the breed. The microsatellite alleles found in Nilgiri sheep of the present study is in agreemental with the earlier report (Kumarasamy *et al*., 2008) on Coimbatore sheep who found the number of alleles ranging from 3 to 8 with the mean of 6 and the sizes from 72 to 220 bp. Further, the number and mean of microsatellite alleles observed in the present study are in accordance with other sheep breeds of Tamilnadu *viz*., such as Mecheri (3 to 7 with a mean of 5; Prema *et al*., 2008); Kilakarsal sheep (3 to 13 with a mean of 7.6; Radha *et al*., 2011); Vembur sheep (2 to 9 with a mean of 5.88; Pramod *et al*., 2009); and Madras Red (7 to 19 with a mean of 9.8; Selvam *et al*., 2009).

Figure 1. A typical Nilgiri sheep (*Ewe*)

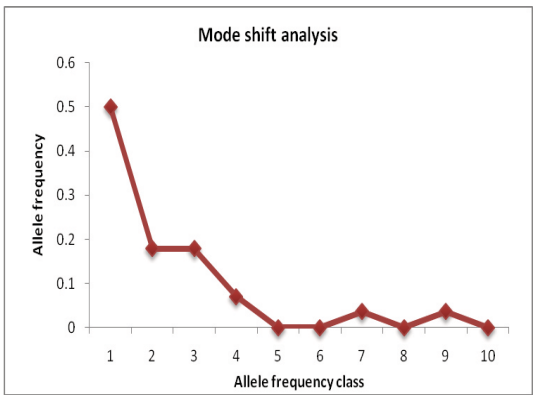

Figure 2. Graphical representation of allele frequency and their contribution in Nilgiri sheep at four microsatellite loci

The effective number of alleles ranged from 1.48 (CSSM47) to 5.78 (OarJMP29) with a mean of 4.11 across all loci. 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 3.61 in Mecheri (Prema *et al*., 2008), 4.93 in Coimbatore sheep (Kumarasamy *et al*., 2008) and 3.88 in Kilakarsal sheep (Radha *et al*., 2011).

The results such as PIC, Chi-square values, heterozygosities and within population inbreeding are furnished in Table 3. The PIC values in the Nilgiri sheep ranged from 0.2991 (CSSM47) to 0.8062 (OarJMP29). Based on the PIC value, it was found that all the markers showed values of more than 0.5, indicating that these markers are highly informative for characterization of Nilgiri sheep. Among the South Indian breeds, the PIC values were reported to range from 0.3966 to 0.8096 in Coimbatore sheep (Kumarasamy *et al*., 2008), 0.523 to 0.791 in Mecheri sheep (Prema *et al*., 2008), 0.591 to 0.917 in Kilakarsal sheep (Radha *et al*., 2011), 0.737 to 0.902 in Madras Red sheep (Selvam *et al*., 2009), and from 0.3712 to 0.8360 in Vembur sheep (Pramod *et al*., 2009), which corroborated with our findings. Among the exotic breeds, the lowest PIC value of 0.20 (Jandurova *et al*., 2005) and the highest of 0.89 (Arranz *et al*., 2001) were also reported.

The Chi-square test of goodness fit revealed that the population was in HWE proportion for only one locus (OarCP34) and the remaining three loci departed from HWE, which might be due to presence of non-amplifying alleles in those loci. However, the influence of systematic and/or dispersive forces could not be ruled out considering the small size of the population. Similar departure of the population from HWE at various loci was also reported in many exotic sheep breeds (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).

The observed heterozygosity ranged from 0.1064 (CSSM47) to 0.8372 (OarCP34) with a mean of 0.5796±0.32 while the expected heterozygosity ranged from 0.3259 (CSSM47) to 0.8270 (OarJMP29) with a mean of 0.6781±0.23. Such high observed and expected heterozygosity values were also reported earlier, *viz*., 0.669 and 0.706 for Mecheri sheep (Prema *et al*., 2008) 0.740 and 0.810 for Coimbatore sheep (Kumarasamy *et al*., 2008) 0.618 and 0.725 for Kilakarsal sheep (Radha *et al*., 2011) and 0.520 and 0.733 for Vembur sheep (Pramod *et al*., 2009), respectively.

The mean within breed diversity (F_{IS}) was found to be 0.1451 with the range of -0.0407 to 0.6736. This F_{IS} value observed in Nilgiri sheep could be attributed to a moderate level of inbreeding, as a result of reduction in the population size in the breeding tract. Such levels of inbreeding through positive F_{IS} value were reported in Kilakarsal sheep (0.147; Radha *et al*., 2011) and Vembur sheep (0.295; Pramod *et al*., 2009). However the breed contributed for the development of Nilgiri sheep (i.e. Coimbatore breed) had considerably low F_{IS} value (0.066; Kumarasamy *et al.*, 2008).

The result of bottleneck analysis using three tests *viz.*,. Sign rank test, Standardized differences test and Wilcoxon test in each of three models of mutation, namely, infinite allele model (IAM), two phase model (TPM) and stepwise mutation model (SMM) revealed that the Nilgiri sheep population is non-bottlenecked; it has not undergone any recent reduction in the effective population size and remained at mutation-drift equilibrium (Figure 2). This finding indicates that the population has not suffered very seriously to exhibit the charges at molecular level. The results of bottleneck analyses are summarized in Table 4.

CONCLUSION

Despite smaller in size of population, the study revealed that the Nilgiri sheep has not undergone any significant reduction in the recent past. The higher heterozygosities found within the microsatellite loci revealed the genetic variability existed still in the population which open up the scope for further improvement. Detailed studies on comparative analysis with other Indian sheep breeds will determine the genetic distance and relationship of Nilgiri sheep breed. However, immediate conservation measures have to be initiated in order to prevent further loss of genetic variability in the breed.

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