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MICROSATELLITE MARKER ANALYSIS IN NELLORE BREED OF SHEEP

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Abstract: The genetic diversity in the Nellore sheep, the highest mutton producing breed of Andhra Pradesh was analysed using ten microsatellite markers. A total of 75 alleles with a mean of 7.5 were observed across all loci which varied from four to ten. The mean effective number of alleles was 4.72. The size and frequencies of alleles ranged from 98 to 206 bp and from 0.013 to 0.447 respectively. The Polymorphism Information Content values varied from 0.637 to 0.815 with a mean value of 0.753. All the loci showed significant departure from Hardy-Weinberg Equilibrium proportions. The mean observed and expected heterozygosity was 0.49 and 0.79 respectively. Two out of ten loci revealed negative F_{IS} with a mean value of 0.19. Mode shift analysis for bottleneck excluded Nellore population from a genetic bottleneck.

Keywords: Genetic variation, Nellore Sheep, Microsatellites, PIC, Bottleneck.

Introduction

There are forty two indigenous breeds of sheep in India reared for meat and wool which play an important role in the biodiversity and livelihood of a large proportion of small and landless labourers. Nellore sheep is a tallest breed present in Nellore and Prakasam districts of Andhra Pradesh. It is a meat type breed known for heat tolerance, disease resistance and thrives well in harsh conditions. Considering the importance of meat in the area in which it is located, efforts have been initiated to conserve the Nellore breed of sheep at molecular level using microsatellites to understand the genetic constitution of population which will be helpful to breeders to formulate breeding strategy for the improvement of the breed.

Materials and methods

Blood samples were collected at random from 38 Nellore sheep unrelated by ancestry belonging to different villages of Nellore and Chittoor districts of Andhra Pradesh. Genomic DNA was isolated from the blood by phenol-chloroform method proposed by Sambrook *et al,* 1989[1]. The concentration and purity of DNA samples was estimated by UV Spectrophotometer. The yield of DNA ranged from 340 to 2465 µg/µl with a mean of

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950.421 µg/µl and the purity of DNA (OD ratio) ranged from 1.697 to 2.086 with a mean of 1.789.

PCR amplification and genotyping

A total of ten microsatellite primer sets specific for sheep were selected based on degree of polymorphism and genomic coverage (FAO, 2004)[2]. PCR amplification with a standard protocol was performed on Kyratec thermal cycler. Each 25 µl PCR reaction mixture contained 50 ng of genomic DNA, 10x PCR assay buffer, 1.5 mM of $MgCl_2$, dNTPs (each at 200 µM), 10 picomoles of each forward and reverse primer and 1 unit of Taq DNA polymerase. The annealing temperatures across different loci ranged from 47 to 70°C for different primers. The PCR products were checked on 2% agarose gel electrophoresis and the allele sizes were determined with the help of 20 bp DNA ladder as a standard marker. The genotypes of each animal were scored manually based on the presence of a single band (homozygotes) or double bands (heterozygotes) in the gel.

Molecular genetic analysis

Microsatellite allele frequencies, observed and effective number of alleles, observed and expected heterozygosity, test of Hardy-Weinberg equilibrium, and within breed heterozygosity deficiency were calculated using the POPGENE 1.31 version[3]. Polymorphism information content (PIC) was calculated using the PIC calculator. BOTTLENECK version 1.2.02[4] was used to identify the presence of bottleneck effect in the investigated sheep population.

Results & Discussion

The observed (n_a) and effective number of alleles (n_e), their sizes, frequencies, observed and expected heterozygosity values, HWE, PIC and within population inbreeding estimates obtained for the Nellore sheep are presented in table 1.

In the present study, a total of 75 alleles were observed at the 10 loci studied. The number of observed alleles ranged from 4 (OarVH72) to 10 (OarFCB128) with a mean of 7.5 per microsatellite locus. The effective number of alleles in Nellore sheep over 10 loci ranged from 3.21 (OarVH72) to 5.76 (OarCP34) with a mean of 4.72 alleles per locus that reflects higher level of genetic variability in the investigated population. The size of alleles ranged from 98 (OarFCB128) to 206 bp (BM757). The frequency of the alleles ranged from 0.013 to 0.447 across all loci.

The mean number of alleles observed in the present study was higher than those reported by Pramod *et al.* 2009[5] in Vembur (5.88) and Pandey *et al.* 2010[6] in Shahabadi (5.56) sheep

breeds and lower than those reported by Prema *et al.* 2008b[7] in Mecheri (9.8) sheep for this particular set of ten microsatellites. Genetic markers having a higher number of alleles per locus are more useful for population and individual typing.

The observed heterozygosity ranged from 0.237 (OarVH72) to 0.833 (BM8125) with a mean value of 0.49, while, the expected heterozygosity ranged from 0.688 (OarVH72) to 0.826 (OarCP34) with a mean value of 0.79. The mean observed heterozygosity value observed in the present study were in accordance with the reports on sheep breeds[8] (0.47 in Nali and Chokla and 0.44 in Garole) whereas the expected heterozygosity values were comparable with values reported by Girish *et al.* 2007[9] in Nilgiri (0.721), Pramod *et al.* 2009[5] in Vembur (0.734) sheep. Deficiency of observed heterozygosity in the present study might be attributable to non random mating that occurred within the population.

The results of χ^2 test of goodness of fit revealed that all the ten loci were showing significant deviation from Hardy-Weinberg Equilibrium which might be due to both the systematic (migration, mutation and selection) and dispersive (genetic drift & inbreeding) forces operating in the population.

The inbreeding coefficient measures the reduction of heterozygosity within the population. In the present study only two loci out of ten had negative F_{IS} values indicating an absence of inbreeding at these loci. The mean F_{IS} value 0.19 over all the ten loci revealed that there is a shortage of heterozygotes (19%) in the population. The reason for deficiency of heterozygosity in the present study might be attributable to a number of causes like non-random mating, heterozygote deficiencies within the selected loci. Inbreeding estimates similar to present study were also reported by Arora and Bhatia (2004) [10] in Magra sheep (0.16) sheep.

All the loci are highly polymorphic with the PIC values ranged from 0.637 (CSSM47) to 0.815 (OarCP34) with a mean PIC value of 0.753. PIC values more than 0.5 indicates the suitability of these markers for molecular characterization & genetic studies in sheep. Similarly higher PIC values were also reported by Girish et al. 2007[9] in Nilgiri (0.648) and Pramod et al. 2009[5] in Vembur (0.690) sheep breeds.

The molecular data was subjected to genetic bottleneck analysis by using three tests viz., Sign rank test, Standardized differences test and Wilcoxon test in each of three models of mutations namely Infinite Allele Model (IAM), Stepwise Mutation Model (SMM) and Two Phase Model (TPM) and the results were summarized in table 2. Among all these models, SMM which is the most suited model for microsatellites evolution, neither the sign rank test

and standardized difference test nor the wilcoxon test revealed any significant results (P>0.05) indicating the absence of significant heterozygote excess in the population accepting the null hypothesis of mutation drift equilibrium. In addition, qualitative graphical method based on mode-shift distortion was used to visualize the spectra of allele frequencies as a check for genetic bottleneck that gave typical L-shaped graph shown in figure.1 which supported that there is no recent bottleneck or reduction in effective population size observed in the Nellore sheep. Similar results were also reported by Radha *et al.* 2011[11] in Kilakarsal, and Kavitha *et al.* 2015[12] in Tiruchy black sheep breeds.

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Table 1: Observed and effective number of alleles, allele size and frequencies, observed and expected heterozygosity, test of Hardy-Weinberg equilibrium, and within breed heterozygosity deficiency of Nellore sheep for 10 microsatellite markers.

Locus	Observed alleles (n_a)	Effective number of alleles (n_e)	Allele size	Allele frequency	PIC	HWE	Heterozygosity		Within population inbreeding
							Observed	Expected	
BM757	9	4.95	176-206	0.013-0.272	0.803	119.11**	0.50	0.82	0.37
BM8125	8	5.12	128-144	0.125-0.347	0.783	279.30**	0.83	0.81	-0.09
BM1314	7	5.30	156-174	0.077-0.295	0.786	223.74**	0.60	0.81	0.28
BM6526	7	4.91	150-200	0.060-0.232	0.689	292.38**	0.30	0.82	0.20
OarFCB128	10	5.07	98-130	0.013-0.289	0.735	255.62**	0.73	0.80	0.08
OarCP34	8	5.76	110-130	0.026-0.263	0.816	297.73**	0.39	0.83	0.42
CSSM47	8	4.44	140-176	0.026-0.355	0.637	307.55**	0.28	0.77	0.16
OarFCB48	7	4.76	160-194	0.024-0.317	0.744	311.85**	0.80	0.79	-0.07
OarHH41	7	3.65	120-162	0.028-0.431	0.761	238.17**	0.28	0.73	0.18
OarVH72	4	3.21	130-146	0.105-0.447	0.775	80.13**	0.24	0.69	0.36
Mean	7.5 ± 0.5	4.72 ± 0.24	-	-	0.753± 0.17		0.49 ± 0.07	0.79 ± 0.01	0.19 ± 0.0562
Range	4-10	3.21-5.76	98-206	0.013-0.447	0.637-0.82		0.2368-0.8330	0.6887-0.8265	-0.09 to 0.42

** - Highly significant (P<0.01)

Table 2: Bottle neck analysis in three models of mutation

	Models of microsatellite evolution		
	IAM	TPM	SMM
Sign test:			
Expected no. of loci with Heterozygosity excess	6.06	5.94	5.90
Observed no. of loci with Heterozygosity excess	10	8	5
Standardized differences test:			
T2 values	2.841	1.741	-0.025
Probability	0.00225	0.04085	0.48990
Wilcoxon rank test:			
Probability of het. Excess	0.00049	0.009280	0.50

*Bottleneck (rejection of null hypothesis of mutation drift equilibrium)

Figure 1: L-shaped mode-shift graph showing lack of bottleneck in the Nellore sheep.