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Genetic diversity analysis of Sangamneri goat

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Goats comprise one of the most important domestic livestock species in India and play an important role in the livelihood of a large proportion of small and marginal farmers and landless labourers. Goat diversity in India is reflected by 23 descript breeds distributed throughout the country, and are well adapted to the harsh climate, long migration, tropical diseases, poor nutrition and shortage of drinking water. However, indiscriminate crossbreeding, uncontrolled intermixing and geographical overlap are leading to the endangerment of breed purity and potentially important caprine genetic material is being put to risk. Hence conservation of indigenous goat germplasm was recognized as a task of national concern. Sangamneri (Accession number: INDIA_Goat_1100_Sangamneri_06018) is an important dual-purpose goat breed of Maharashtra. The breed has derived its name from Sangamner town of Ahmednagar district of Maharashtra. Sangamneri goats are medium-sized animals. The coat colour is complete white, however, animals with admixture of black and brown colour were also seen. The farmers of rural area of this region keep small flocks of Sangamneri goats to meet out family requirements of milk and finally sell the live animals for meat. This breed has proved its adaptability in semi-arid region of Maharashtra and performs well under poor quality range condition. In view of the paramount importance of this breed in India, present research work was undertaken to characterize Sangamneri goat at molecular level using microsatellite markers.

Fifty animals confirming to typical phenotypic characteristics of Sangamneri goat breed were chosen randomly from different villages of Sangamner and Rahuri *Taluka* of Ahmednagar and owners were questioned in detail to avoid close relationships. The samples were also collected from All India Co-ordinated Research Project on Sangamneri Goat, Department of Animal and Husbandry, Mahatama Phule Krishi Vidyapeeth, Rahuri. Approximately 10 ml of venous blood samples from each animal were collected aseptically from jugular vein in the vacutainer tubes having EDTA as blood anticoagulant. The samples were brought to the laboratory in an ice-cooled box, where they were kept under –20°C in a deep freezer until DNA isolation. Genomic DNA was isolated from the blood samples using a standard phenol : chloroform extraction method (Sambrook and Russell 2001). A set of 25 heterologous polymorphic microsatellite markers were used for molecular characterization of Sangamneri goat.

Amplification reactions were carried out after genomic DNA extraction by using touchdown PCR protocol. Each 12.5 µl PCR reaction contained 30–80 ng template DNA; 5 pmol of each primer; 250 µM of each dNTPs; 0.5 unit of Taq DNA polymerase and 2 mM $MgCl_2$. The PCR reaction cycle was accomplished by initial denaturation of 95°C for 1 min, 3 cycles of 95°C for 45 s and 60°C for 1 min and 72°C for 1min, 3 cycles of 95°C for 45 s and 57°C for 1 min and 72°C for 1min, 3 cycles of 95°C for 45 s and 54°C for 1 min and 72°C for 1min, 3 cycles of 95°C for 45 s and 51°C for 1 min and 72°C for 1min, 20 cycles of 95°C for 45 s and 48°C for 1 min and 72°C for 5min. The amplified PCR products were resolved on 6% denaturing polyacrylamide gels, 10 bp ladder was used as a size standards for sizing PCR product. To visualize the PCR product, gels were stained using silver staining (Bassam *et al.* 1991) and dried between sheets of cellophone paper. The scoring of allele was carried out following gel documentation on a gel-doc system, where the image was captured and analysed by quantity oneTm software.

For the 25 microsatellites loci analysed, observed and effective number of alleles (Kimura and Crow 1964), observed and expected heterozygosity estimates were calculated using POPGENE software (Yeh *et al.* 1999) to determine genetic variation within the breed. The tests for

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deviation from Hardy–Weinberg equilibrium were also derived using the exact tests of POPGENE. Polymorphism information content values (PIC) for all the loci was estimated according to Botstein *et al.* (1980) and Shannon information index as per Lewontin (1972). Heterozygote deficiency, gene diversity and allelic richness were calculated using FSTAT (Goudet 1995).

Bottleneck hypothesis (Cornuet and Luikart 1996) was investigated using BOTTLENECK 1.2.01. The BOTTLENECK tests for the departure from mutation drift equilibrium were based on heterozygosity (not heterozygotes), excess or deficiency. Three models of mutation were used to calculate H_{eq} (mutation drift equilibrium): the strict one stepwise mutation model (SMM), the infinite allele model (IAM) and the two-phase model (TPM).

Results of the microsatellite analysis in terms of number of alleles observed, alleles size, polymorphism information content and heterozygosity of Sangamneri goats were furnished in Table 1.

Allele number: The observed number of alleles varied from 9 (ILSTS002, ILSTS 065) to 32 (ILSTS 008) with a mean of 16.800±1.109. The effective number of alleles varied from 3.663 (ILSTS 065) to 19.208 (ILSTS 008) with a mean 10.751±0.808. The mean observed and effective number of alleles were found to be higher in the present study than that reported by Dixit *et al.* (2011) in Kanniaddu goat, Thilagan *et al*. (2012) in Salem Black, Mishra *et al*. (2012) in Bundelkhandi, Dixit *et al*. (2013) in Surti and Bhat *et al.* (2013) in Osmanabadi goat. For 25 loci 420 alleles were observed.

Heterozygocity: Observed heterozygosity ranged from 0.000 (ILSTS 002) to 1.000 (ILSTS 033, ILSTS 058) with an average of 0.732±0.059, whereas the expected heterozygosity varied from 0.735 (ILSTS 065) to 0.958 (ILSTS 008) with an overall mean of 0.900±0.011. Takezaki and Nei (1996) determined that for the markers to be useful for measuring genetic variation, they should have expected average heterozygosity between 0.3 and 0.8 in the population. The average expected heterozygosity value of 0.900 was above the range in Sangamneri breed of goat which supports the suitability of selected microsatellite markers. Sangamneri possesses more genetic variability when compared to Black Bengal and Chegu goat (Vijh *et al.* 2010), Changthangi

Table 1. Measures of genetic variation in Sangamneri goat at studied microsatellites loci

Locus	Sample	Observed number of alleles	Effective number of alleles	Observed heterozygosity	Expected heterozygosity	PIC value	Heterozygote deficiency
	N	Na	Ne	Ho	He		Fis
ETH225	50	17	12.755	0.920	0.931	0.916	0.012
ILSTS002	44	9	4.939	0.000	0.807	0.775	1.000
ILSTS005	48	18	13.553	0.979	0.936	0.921	–0.047
ILSTS008	49	32	19.208	0.673	0.958	0.946	0.299
ILSTS019	50	19	13.624	0.780	0.936	0.922	0.168
ILSTS022	49	21	10.889	0.694	0.918	0.902	0.246
ILSTS029	50	19	11.494	0.900	0.922	0.907	0.024
ILSTS030	50	25	17.241	0.780	0.952	0.939	0.182
ILSTS033	50	13	10.482	1.000	0.914	0.897	–0.095
ILSTS034	50	13	7.022	0.980	0.866	0.842	–0.133
ILSTS044	46	25	15.970	0.913	0.948	0.934	0.037
ILSTS049	48	18	9.309	0.917	0.902	0.884	–0.016
ILSTS058	48	24	15.890	1.000	0.947	0.934	–0.057
ILSTS059	46	14	11.469	0.891	0.923	0.906	0.035
ILSTS065	47	9	3.663	0.447	0.735	0.701	0.395
ILSTS082	45	15	11.571	0.889	0.924	0.907	0.038
ILSTS087	50	17	9.980	0.980	0.909	0.891	–0.079
OarAE129	44	19	14.502	0.568	0.942	0.927	0.399
OarHH64	38	10	6.211	0.026	0.850	0.820	0.969
OarJMP29	50	14	6.840	0.480	0.862	0.842	0.446
OMHC1	45	18	10.743	0.533	0.917	0.900	0.421
RM004	50	12	7.837	0.820	0.881	0.860	0.070
OarFCB48	48	12	6.611	0.979	0.858	0.831	–0.143
RM088	46	14	11.346	0.891	0.922	0.905	0.034
OARFCB304	40	13	5.634	0.250	0.833	0.805	0.703
Mean	47.240	16.800	10.751	0.732	0.900	0.881	0.196
SE	0.646	1.109	0.808	0.059	0.011	0.012	0.064

(Mishra *et al*. 2010), Kanniaddu (Dixit *et al.* 2011), Bundelkhandi (Mishra *et al*. 2012), Surti (Dixit *et al*. 2013) goat breeds. The higher heterozygosity observed in the present investigation had resulted from retention of many alleles in small frequencies. Because of higher heterozygosity, there is further scope for improvement of Sangamneri goats. Thilagam *et al.* (2012) and Bhat *et al.* (2013) reported similar estimate of mean observed and expected heterozygosity in Salem Black goat and in Osmanabadi goat, respectively.

Shannon's information index (I) and polymorphic information contents (PIC): The Shannon's information index represents the relative abundance of genetic information of a specific locus to the total information available of the loci (Shannon and Weaver 1949). The values of Shannon's information index were found be in the range of 1.648 (ILSTS 065) to 3.204 (ILSTS 008) with an average of 2.487±0.076. The PIC values ranged from 0.701 (ILSTS 065) to 0.946 (ILSTS 008) with an average of 0.881±0.012. Thus all the markers under study were found to be highly informative. Vijh *et al.* (2010) in Black Bengal (0.80), Chegu goat (0.80), Mishra *et al*. (2012) in Bundelkhandi (0.82) and Bhat *et al.* (2013) in Osmanabadi (0.84) goat reported similar estimate of PIC, however Dixit *et al.* (2011) in Kanniaddu goat, Thilagan *et al*. (2012) in Salem Black and Dixit *et al*. (2013) in Surti breed reported lower estimate of PIC than the present study. The high value of Shannon's information index and PIC indicated the suitability of markers for studying the genetic variability in goat species and the high polymorphism across the loci.

F-statistics: The F_{is} values provide the non-random union of gametes in the population, i.e. the mating among the individuals in the population which are related more than the average relationship. More the values of F_{is}, more the inbreeding in the population. The negative values of F_{is} point towards out-breeding. Heterozygote deficiency (positive F_{is} value) was observed in eighteen out of twenty five studied loci indicating departures from random mating. The positive value of F_{is} ranged from 1.00 (ILSTS 002) to 0.012 (ETH 225) with an average of 0.196±0.064. Significant heterozygote deficiencies have also been reported in Indian goat populations, viz. Changthangi (Mishra *et al*. 2010), Kanniadu (Dixit *et al.* 2011), Surti (Dixit *et al*. 2013) and Osmanabadi (Bhat *et al.* 2013). The gene diversity across the studied loci for Sangamneri breed of goat varied from 0.738 (ILSTS 065) to 0.961 (ILSTS 008) with average value 0.902. The allelic richness across the studied loci varied from 8.732 (ILSTS 065) to 29.657 (ILSTS 008). This reflected the sufficient number of the alleles per locus in the population independent of the sample size and thus can be used for comparing the different populations. Lower estimate of gene diversity and allelic richness was reported by Verma *et al*. (2009) in Malbari goat and Dixit *et al.* (2011) in Kanniadu goat than the present study.

Mutation drift equilibrium: Heterozygosity excess/ deficiency under different models generated by the BOTTLENECK under IAM, TPM and SMM showed deficiencies in 5, 12 and 17 loci, respectively. In heterozygosity methods IAM, TPM and SMM showed deficiencies in 10, 15 and 20 loci, respectively. The difference of values in different models is as per the expectation. The higher heterozygosity deficiency exhibited by SMM in both methods is due to over sensitivity of this model for microsatellite mutation. The TPM having proportion of 70% SMM at 1000 replication showed deficiency of heterozygosity in 12 and 15 loci by frequency and heterozygosity methods, respectively. The sign test, standardized difference and wilcoxon test revealed significant heterozygosity excess under IAM model, mutation drift equilibrium under SMM model and heterozygosity deficiency under TPM models under frequency method. In heterozygosity methods the sign test, standardized difference and wilcoxon test revealed mutation drift equilibrium under IAM models, while sign test and standardized difference test revealed significant heterozygosity deficiency under TPM and SMM models. Standardized difference test indicated significant departure of the population from mutation drift equilibrium under two-phase and single-step mutation models. The deviation was negative in heterozygosity based methods under two-phase and single-step mutation models (T_2 = -2.392, -6.749, respectively, P<0.01). Similar deviation was also found in frequency method under single-step mutation model (T_2 = - 3.668). The mode shift analysis generated a L–shaped distribution curve (Fig. 1) indicating that the population has not undergone any bottle neck in the recent past.

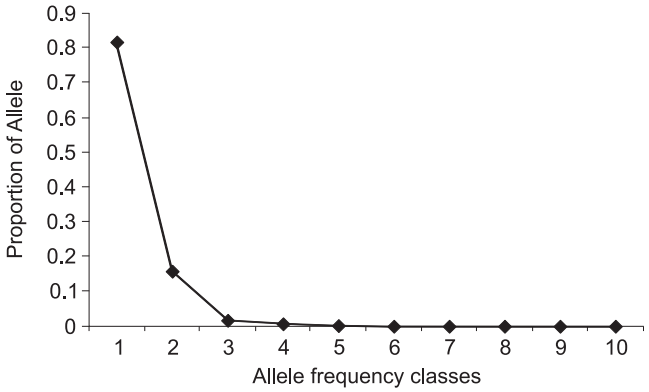

Fig. 1. L-shaped mode-shift graph showing lack of bottleneck in the Sangamneri goat.

The high level of genetic variability suggested the scope for further genetic improvement of Sangamneri goats, avoiding unplanned and indiscriminate mating.

SUMMARY

Sangamneri, one of the important dual-purpose goat breed

of Maharashtra existing in the villages of Nasik, Ahmednagar and Pune district was investigated genetically utilizing 25 microsatellite markers. The observed number of alleles varied from 8 (ILSTS 002, ILSTS 065) to 32 (ILSTS 008) with an overall mean of 16.800±1.109. The effective number of alleles ranged from 3.663 (ILSTS 065) to 19.208 (ILSTS 008) with a mean of 10.751±0.808. The observed heterozygosity ranged from 0.000 (ILSTS 002) to 1.000 (ILSTS 033, ILSTS 058) with an average of 0.732±0.059, whereas the expected heterozygosity varied from 0.735 (ILSTS 065) to 0.958 (ILSTS 008) with an overall mean of 0.900±0.011. All of the studied loci showed the polymorphic information content (PIC) value greater than 0.5 with an average value of 0.881±0.012, these high values of PIC indicated higher polymorphism in the breed. The values Shannon's information index ranged from 1.648 (ILSTS065) to 3.204 (ILSTS008) with an average of 2.487±0.076. Heterozygosity deficiency (positive F_{is} value) was observed in 18 out of 25 studied loci indicating departures from random mating. The mode shift analysis generated L–shaped distribution curve indicating that the population has not undergone any bottleneck in the recent past.

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