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Genetic diversity and relationship among three goat breed of Maharashtra state of India

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Berari, Osmanabadi and Sangamneri breeds of goat are found in Vidarbha, Marathwada and western region of Maharashtra, India. Animals of all three breeds are medium sized, reared for meat purpose and phenotypicaly these three breed appears distinct. Genetic variation within and between breeds is warranted to differentiate them on genetic basis. The resulting genetic information may assist in choosing the best conservation and improvement options for these genetic resources, for characterization of breeds and populations, including their genetic differentiation and relationships. The present investigation was undertaken to evaluate diversity within and between breeds, their phylogenetic relationship of these goat populations of Maharashtra state of India based on microsatellite loci.

Experimental material for the present study comprised of blood samples of 48 Berari goat, 31 Osmanabadi goat, 50 Sangamneri goat and collected at random from unrelated animals from their respective breeding tract. Samples were taken from different villages and owners were questioned in detail in order to avoid close relationships. 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 three goat breeds.

Amplification reactions were carried out after genomic DNA extraction by using touchdown PCR protocol. Each 25 µl PCR reaction contained of 3 µl (30 ng/µl) template DNA, 0.5 µl (10pmol/µl) of forward and reverse primers, 12.5 µl of 2 X PCR master mix and 8.5 µl of distilled water. The 'touchdown' PCR protocol was used with initial denaturation of 95°C for 1 min, 3 cycles of 95°C for 45 s and 60°C for 1 min, 3 cycles of 95°C for 45 s and 57°C for 1 min, 3 cycles of 95°C for 45 s and 54°C for 1 min, 3 cycles of 95°C for 45 s and 51°C for 1 min, 20 cycles of 95°C for 45 s and 48°C for 1 min with final extension at 72°C for 5 min. The amplified PCR products were resolved on 6% denaturing polyacrylamide gels, 10 bp and/or 100 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 BioRadTm 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 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 and gene diversity were calculated using FSTAT (Goudet 1995). Neighbor-joining (NJ) methodology (Saitou and Nei 1987) implemented in PHYLIP program was used to construct the phylogenic tree of breed relationships.

A total of 567 alleles were observed among 129 assayed animals across the three breeds (Table 1). The observed number of alleles per locus ranged from 12 (RM004) to 40 (ILSTS 058), with a mean value of 22.680±1.403 and the effective number of alleles ranged 5.505 (ILSTS 065) to 22.606 (ILSTS 058) with an average value of 13.128±0.841. Shannon's information index, a measure of polymorphism, across these loci for the populations varied from 2.097 (ILSTS065) to 3.355 (ILSTS 058) with an average of 2.726±0.063. All the studied microsatellite loci were polymorphic, which indicates that the microsatellites used were suitable for genetic diversity analysis. The observed heterozygosity and expected heterozygosity over the studied loci across all three populations ranged from 0.176 (ILSTS002) to 0.976 (ILSTS005) with an overall mean of

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0.751±0.204 and from 0.822 (ILSTS065) to 0.959 (ILSTS 058) with an average of 0.919±0.31, respectively. At 18 loci, the expected heterozygosity was significantly higher than the observed heterozygoity across three breeds. This indicated that most of the loci showed deviation from Hardy-Weiberg equilibrium. Out of 25 studied loci 24, 20 and 22 loci showed significant deviation from Hardy-Weinberg equilibrium in Berari, Osmanabadi and Sangamneri goat, respectively. The measures of genetic variation in the present study indicated a high level (0.919) of genetic diversity (average expected hetrozygocity) in goat breed of Maharashtra. The substantial genetic variation has also been observed in three north Indian goat breeds (Dixit *et al.* 2009), six southern Indian goat breeds (Dixit *et al.* 2010) and two goat breed of India (Vijh *et al.* 2010).

In terms of number of allele, the most diverse breed was Sangamneri (10.751±0.808) followed by Berari (10.214±0.602) and the least diverse breed was Osmanabadi (8.277±0.626). Among the breed, highest number of allele were reported for Sangamneri (420) followed by Berari (389) and the lowest for Osmanabadi (309).

The overall mean of gene diversity across the studied loci for the Berari, Osmanabadi and Sangamneri breeds were 0.906±0.006, 0.870±0.017 and 0.901±0.010, respectively. Out of 25 studied loci 14, 13 and 18 loci showed positive deviations (observed heterozygote deficiency) in the Berari, Osmanabadi and Sangamneri indicating departures from random mating and suggested that some of the studied loci were homozygous in the population. The majority of the loci (>50%) in each population showed heterozygote deficiency, which may be due to presence of low frequency alleles segregating at many of the loci and the significant genotypic linkage disequilibrium observed in these breeds.

The Fst values ranged from 0.008 (ILSTS008) to 0.1 (OarFCB48) with an overall genetic differential of 0.039 (3.9%) among breed. The lower level of genetic differentiation (Fst) among the three breeds (3.9%) under study implied that 96.1% of the total genetic variation corresponded to differences among individuals within breed and 3.9% due to unique allelic difference among the breed. This level of differentiation among the populations is lower in the range of Fst values reported in the literature. A value of 0.14 was reported in southern Indian goat breeds (Dixit *et al*. 2010), 0.13 was reported in the north–eastern breeds of India (Dixit *et al.* 2009); 0.073 in Italian goat populations

Table 1. Measures of genetic variation across three breed of goat at studied microsatellites loci

Locus	Observed number of alleles Na	Effective number of alleles Ne	Shannon's information index (I) I	PIC value	Observed heterozygosity Ho	Expected heterozygosity He	Nei expected heterozygosity Nei
ETH225	18	9.612	2.505	0.887	0.912	0.900	0.896
ILSTS002	18	8.651	2.420	0.875	0.176	0.888	0.884
ILSTS005	29	19.892	3.126	0.947	0.976	0.953	0.950
ILSTS008	33	18.548	3.134	0.943	0.806	0.950	0.946
ILSTS019	21	13.934	2.770	0.924	0.842	0.932	0.928
ILSTS022	23	12.030	2.757	0.912	0.571	0.920	0.917
ILSTS029	30	13.141	2.886	0.919	0.928	0.927	0.924
ILSTS030	27	17.486	3.002	0.940	0.867	0.947	0.943
ILSTS033	19	14.629	2.777	0.927	0.941	0.936	0.932
ILSTS034	22	9.746	2.553	0.889	0.946	0.901	0.897
ILSTS044	29	17.235	2.995	0.939	0.843	0.946	0.942
ILSTS049	19	12.340	2.653	0.913	0.927	0.923	0.919
ILSTS058	40	22.606	3.355	0.954	0.811	0.959	0.956
ILSTS059	15	10.138	2.444	0.893	0.695	0.905	0.901
ILSTS065	13	5.505	2.097	0.804	0.619	0.822	0.818
ILSTS082	16	13.353	2.666	0.920	0.765	0.929	0.925
ILSTS087	20	12.091	2.644	0.911	0.867	0.921	0.917
OarAE129	24	15.051	2.842	0.930	0.810	0.938	0.934
OarHH64	17	8.602	2.361	0.873	0.290	0.888	0.884
OarJMP29	27	13.528	2.915	0.922	0.600	0.930	0.926
OMHC1	21	11.094	2.613	0.903	0.458	0.914	0.910
RM004	12	7.446	2.140	0.851	0.906	0.869	0.866
OarFCB48	24	12.424	2.802	0.915	0.789	0.924	0.920
RM088	16	10.013	2.483	0.892	0.748	0.904	0.900
OARFCB304	34	19.098	3.200	0.945	0.693	0.952	0.948
Mean	22.680	13.128	2.726	0.909	0.751	0.919	0.915
SE	1.403	0.841	0.063	0.007	0.041	0.006	0.006

(Iamartino *et al.* 2005); 0.06 and 0.17, respectively, in Egyptian and Italian goat breeds (Agha *et al.* 2008). The result revealed that all the studied population were genetically quite distinct. The global deficit of heterozygotes across populations (Fit) amounted to 0.194. The Fis values were 0.125, 0.187 and 0.188 for Berari, Osmanabadi and Sangamneri goat breeds, respectively. The overall Fis value (0.161) was higher and significantly different from zero indicating departure from random mating. The measures of genetic differentiation revealed that the different populations remained genetically distinct, despite the fact that there is no breeding policy to create breeds or maintain the breed purity.

The genetic distance tended to be the least (0.4032) between Berari and Sangamneri, moderate (0.4834) between Sangamneri and Osmanabadi and the widest (0.6319) between Berari and Osmanabadi. The consensus phylogenetic tree revealed that Berari and Sangamneri clustered together and Osmanabadi remained as distinct breed. The UPGMA tree shows that two goat populations (Berari and Sangamneri) are distinct from the other goat population (Osmanabadi). This relationship may be contradictory to the geographic origin of the Berari and Sangamneri breeds, the close kinship might suggest some past crossing between these two geographically close populations. The inter individual distances were estimated using allele sharing and Nei's standard genetic distances and trees constructed (Fig. 1) using Neighbour Joining

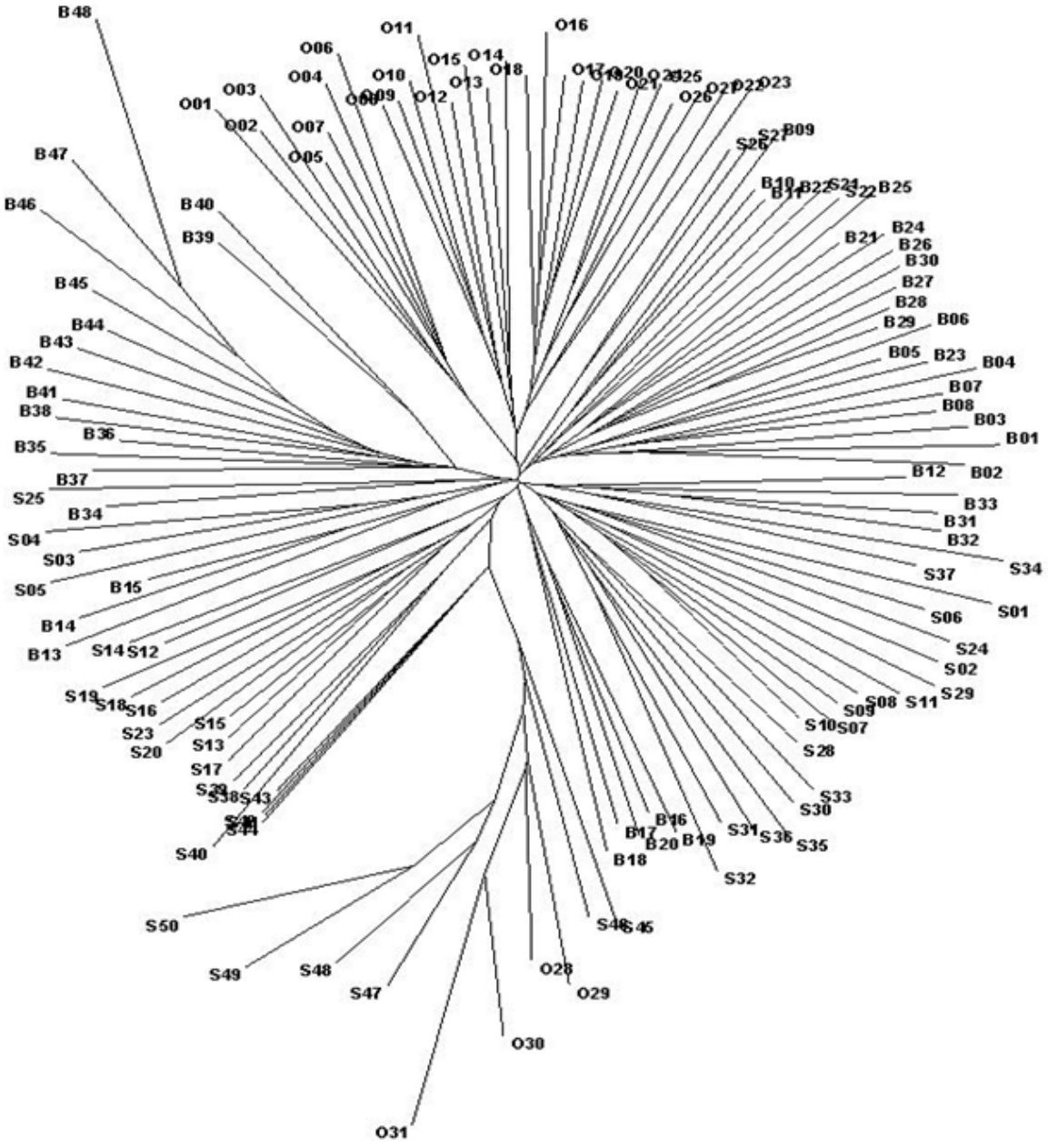

Fig 1. Neighbour joining tree based on inter individual distances based on allele sharing.

Method (Saitou and Nei 1987).

Results suggested that there was substantial genetic diversity among the studied breed. Lower level of genetic differentiation among the three breeds showed intermixing of the populations as breeding tract of three bred overlap hence, this may pose a danger to the purity of the breed in the long run. Therefore, suitable breeding strategies are required for maintaining breed purity and improving these breeds.

SUMMARY

Genetic diversity and relationship among in the three goat breed of Maharashtra, India namely Berari, Osmanabadi and Sangamneri were evaluated based on 25 microsatellite markers. A total of 567 alleles were observed among 129 assayed animals across the three breeds. The most diverse breed was Sangamneri followed by Berari and the least diverse breed was Osmanabadi in terms of number of allele. The overall mean of gene diversity across the studied loci for the Berari, Osmanabadi and Sangamneri breeds were 0.906±0.006, 0.870±0.017 and 0.901±0.010, respectively. The overall Fis value (0.161) was higher and significantly different from zero indicating departure from random mating and suggested that some of the studied loci were homozygous in the populations. The lower level of genetic differentiation (Fst) among the three breeds (3.9%) under study implied that 96.1% of the total genetic variation corresponded to differences among individuals within breed. The genetic distance tended to be the least (0.4032) between Berari and Sangamneri, moderate (0.4834) between Sangamneri and Osmanabadi and the widest (0.6319) between Berari and Osmanabadi. Lower level of genetic differentiation among the three breeds showed intermixing of the populations as breeding tract of three bred overlap hence, this may pose a danger to the purity of the breed in the long run.

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