Indian Journal of Animal Sciences **87** (8): 982–990, August 2017/Article

Estimation of genetic diversity and relationship among goats of Maharashtra state

PRIYANKA MISHRA[1], S P DIXIT[2], R A K AGGARWAL[3], JYOTSNA BEHL[4], P S DANGI[5] and N K VERMA[6]

ICAR-National Bureau of Animal Genetic Resources, Karnal, Haryana 132 001 India

Received: 11 January 2017; Accepted: 26 April 2017

ABSTRACT

Sangamneri, Osmanabadi, Berari and Konkan Kanyal are the known breeds of Maharashtra state of India. DNA from 50 unrelated goats of each breed was analysed to know the genetic diversity using 25 microsatellite markers. A total of 479 alleles (192 in Sangamneri, 153 in Osmanabadi, 294 in Berari and 255 in Konkan Kanyal) were observed. Mean number of alleles per locus and observed heterozygosity were 7.68, 6.12, 11.76, 10.20 and 0.53, 0.42, 0.67 and 0.58 for Sangamneri, Osmanabadi, Berari and Kanyal goat populations respectively. The fixation coefficients of sub-populations within the total population (F_{ST}), varied from 0.04 (OarHH64) to 0.68 (OarJMP29) with a mean of 0.17. The L-shaped mode-shift curve indicated the absence of reduction in effective population size. The genetic distances between four breeds indicated the distinctness of Berari and Konkan Kanyal from Sangamneri and Osmanabadi. Berari and Konkan Kanyal although exists at different geographic locations but show some genetic overlapping. The Nei's genetic distance observed between breeds were 0.472 (Sangamneri and Osmanabadi), 0.667 (Sangamneri and Berari), 0.819 (Sangamneri and Konkan Kanyal), 0.797 (Osmanabadi and Berari), 0.994 (Osmanabadi and Konkan Kanyal) and 0.092 (Berari and Konkan Kanyal). Konkan Kanyal is genetically more distant from Osmanabadi and Sangamneri than Berari. Sangamneri and Osmanabadi goat breeds were assigned to cluster-3 where the proportion of membership for each breed was 0.975 (Sangamneri) and 0.992 (Osmanabadi). Kanyal and Berari goat populations were assigned to another cluster (Cluster 4). The proportion for their membership was 0.923 (Konkan Kanyal) and 0.869 (Berari).

Key words: Allele, Genetic diversity, Goat, Heterozygosity, Maharashtra, Microsatellite markers

India has 26 registered goat breeds (NBAGR 2016) constituting about 30–40% pedigreed vs crossbreds. The Indian goat displays a wide spectrum of genetic diversity that has been shaped by natural selection as well as human influence, the maintenance of which is a key to the long-term survival if the genetic variation between and within breeds is low. It is imperative to know the level and extent of diversity existing within a species/breed before proposing any programme for its conservation. The identification of microsatellite loci, because of their high degree of polymorphism and random distribution across the genome is one of the methods for inferring genetic diversity (Bruford *et al.* 1993). Diversity estimates provide useful information regarding the genetic structure of a population. This study presented the microsatellite markers based genetic diversity within and between Konkan Kanyal, Berari, Sangamneri and Osmanabadi goat breeds found in Maharashtra region of India (Fig. 1).

MATERIALS AND METHODS

The study locale is depicted in Fig. 2. DNA was extracted from whole blood using a standard phenol/chloroform extraction method (Sambrook *et al.* 1989). A battery of 25 microsatellite markers identified in ISAG (International Society for Animal Genetics) and FAO's (Food and Agricultural Organization) DAD-IS (Domestic Animal Diversity Information System) program was utilized to know the genetic diversity within and between breeds. Only forward primers at the 5' end of each pair were labeled with one of four fluorophores, i.e., FAM (blue), VIC (green), NED (yellow) and PET (red) supplied by Applied Biosystem, UK (Table 1). PCR reaction was carried out with mixture consisting of 50 ng DNA, 200 µM of each dATP, dCTP, dGTP and dTTP, 50 nM KCl, 10 mM Tris–HCl (pH 9.0), 0.1% Triton X-100, 2.0 mM $MgCl_2$, 0.75 unit Taq DNA polymerase and 4 pmol of each primer using PTC-200 PCR machine (M J Research). A touchdown PCR protocol was used with an initial denaturation of 95°C for 1 min; 3 cycles of 95°C for 45 sec., and 60°C for 1 min; 3 cycles of 95°C for 45 sec, and 57°C for 1 min; 3 cycles of 95°C for 45 sec, and 54°C for 1 min; 3 cycles 95°C for 45 sec and 51°C for 1 min; 20 cycles 95°C for 45 sec, and 48°C for 1 min with final extension at 72°C for 5 min. The reaction was stopped using 5.0 µl of a stop dye (95% formamide, 0.25% bromophenol blue and 0.25% xylene cyanol). PCR products (6 µl) were loaded on a 2% agarose gel, electrophoresed and visualized over UV light after ethidium bromide staining to detect the amplification.

Microsatellite genotyping was carried out using automated DNA sequencer of Applied Biosystems (ABI

Present address: [6]Principal Scientist (nkverma.497@gmail.com).

Fig. 1. Kanyal (A), Berari (B), Sangamneri (C) and Osmanabadi (D).

3100 Avant) with Liz 500 as internal lane standard. The electropherograms drawn through Gene Scan were used to extract DNA fragment sizing details using Gene Mapper software (version 3.0) of Applied Biosystems, U.S.A.

Statistical analysis: To determine the genetic variation between goat populations, parameters such as Nei unbiased expected heterozygosity (Nei 1973) were estimated for all loci. These parameters were statistically analyzed using POPGENE software package version 1.32. The F statistics parameters viz. F_{IT} (total inbreeding), F_{ST} (population differentiation) and small F_{IS} (within population inbreeding) were estimated using FSTAT software version 2.9.3.2 as per Wright (1978). The Nei's genetic distances (Nei 1987) were used for the comparison of breeds. Neighbour-Joining methodology was applied and a tree was built from the inter-individual distances by using the PHYLIP package. STRUCTURE version 2.2 (Pritchard *et al.* 2000) was employed to confirm the genetic pattern of each individual belonging to the different breeds and to reveal possible clustering substructures. The Bayesian assignment of individuals to populations considered an ancestry model with admixture and correlated allele frequencies. 100 independent runs with 100,000 MCMC (Markov Chain Monte Carlo) iterations and a burn-in of 10,000 were carried out for $2 \leq K \leq 4$ (K, number of clusters) to estimate the most likely number of clusters present in the data set.

RESULTS AND DISCUSSION

All the microsatellite markers mentioned in Table 1 were amplified successfully in all four goat breeds of Maharashtra and were found polymorphic. Estimates on total number of alleles, allele size, overall expected and observed heterozygosity, and observed and effective number of alleles for each locus in all four goat populations (Konkan Kanyal, Berari, Osmanabadi and Sangamneri) are presented in Table 2. The observed number of alleles per locus varied

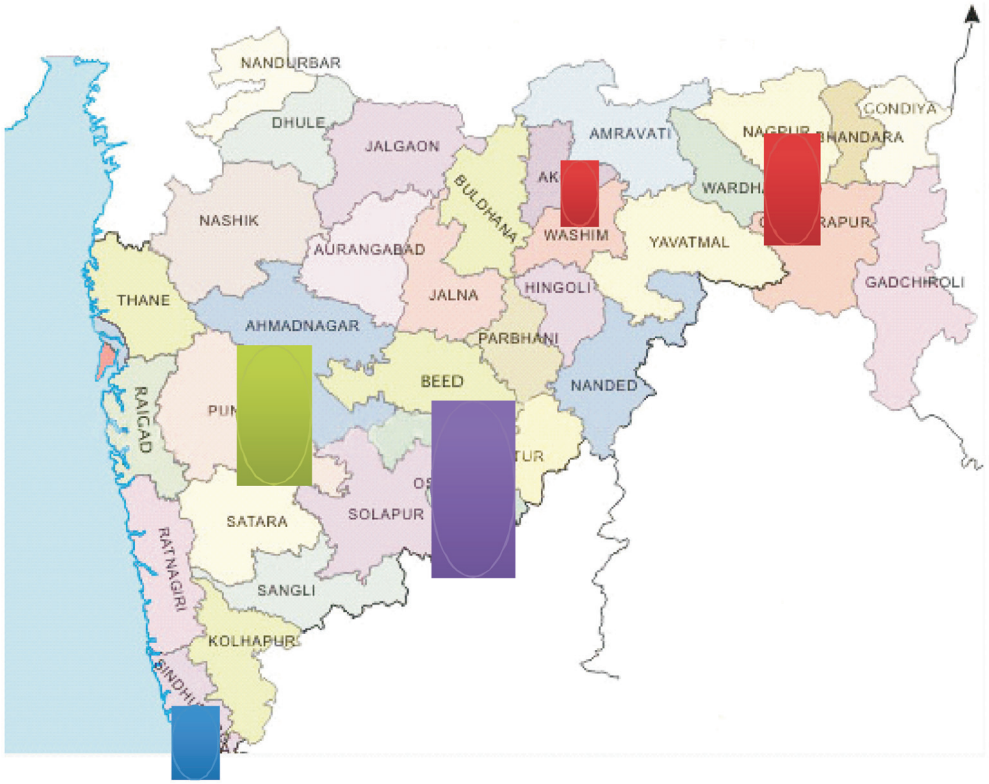

Fig. 2. Map of Maharashtra showing locations for animal sampling. Green indicates Sangamneri breed, purple indicates Osmanabadi breed, red indicates Berari population and blue indicates Kanyal.

Table 1. Microsatellite markers, their sequences, type of repeat, size range and dye

Locus	Primer sequence	Type of repeat	Size range	Dye	*Chr. No.	**Acc. No.
ILSTS008	F-gaatcatggattttctgggg R-tagcagtgatgaggttggc	$(CA)_{12}$	167-195	FAM	14	L23483
ILSTS059	F-gctgaacaatgtgatatgttcagg R-gggacaatactgtcttagatgctgc	$(CA)_4\,(GT)_2$	105-135	FAM	13	L37266
ETH225	F- gatcaccttgccactatttcct R- acatgacagccagctgcttact	$(CA)_{18}$	146-160	VIC	14	Z14043
ILSTS044	F-agtcacccaaaagtaactgg R-acatgttgtattccaagtgc	$(GT)_{20}$	145-177	NED	Ann	L37259
ILSTS002	F-tctatacacatgtgctgtgc R-cttaggggtgaagtgacacg	$(CA)_{17}$	113-135	VIC	Ann	23479
Oar FCB304	F-ccctaggagctttcaataaagaatcgg R-cgctgctgtcaactgggtcaggg	$(CT)_{11}(CA)_{15}$	119-169	FAM	Ann	L01535
Oar FCB48	Fgagttagtacaaggatgacagaggcac	$(GT)_{10}$	149-181	VIC	17	M82875
Oar HH64	F-cgttccctcactaggaaagttatatatgc R-cactctattgtaagaatttgaatgaatgagagc	_	120-138	PET	4	212***
Oar JMP29	F-gtatacacgtggacaccgctttgtac R-gaagtggcaagattcagaggggaag	$(CA)_{21}$	120-140	NED	Ann	U30893
ILSTS005	F-ggaagcaatgaaatctatagcc R-tgttctgtgagtttgtaagc	$(nn)_{39}$	174-190	VIC	10	L23481
ILSTS019	F-aagggacctcatgtagaagc R-acttttggaccctgtagtgc	$(TG)_{10}$	142-162	FAM	Ann	L23492
OMHC1	F-atctggtgggctacagtccatg R-gcaatgctttctaaattctgaggaa	_	179-209	NED	Ann	228***
ILSTS087	F-agcagacatgatgactcagc R-ctgcctcttttcttgagagc	$(CA)_{14}$	142-164	NED	Ann	L37279
ILSTS030	F-ctgcagttctgcatatgtgg R-cttagacaacaggggtttgg	$(CA)_{13}$	159-179	FAM	2	L37212
ILSTS034	F-aagggtctaagtccacggc R-acctggtttagcagagagc	$(GT)_{29}$	153-185	VIC	5	L37254
ILSTS033	F-tattagagtggctcagtgcc R-atgcagacagttttagagg	$(CA)_{12}$	151-187	PET	12	L37213
ILSTS049	F-cattttcttgtctctcccc R-gctgaatcttgtcaaacagg	$(CA)_{26}$	160-184	NED	11	L37261
ILSTS065	F-gctgcaaagagttgaacacc R-aactattacaggaggctccc	$(CA)_{22}$	105-135	PET	24	L37269
ILSTS058	F-gccttactaccatttccagc R-catcctgactttggctgtgg	$(GT)_{15}$	136-188	PET	17	L37225
ILSTS029	F-tgttttgatggaacacagcc R-tggatttagaccagggttgg	$(CA)_{19}$	149-191	PET	3	L37252
RM088	F-gatcctcttctgggaaaaagagac R-cctgttgaagtgaaccttcagaa	$(CA)_{14}$	109-147	FAM	4	U10392
ILSTS022	F-agtctgaaggcctgagaacc R-cttacagtccttggggttgc	$(GT)_{21}$	186-202	PET	Ann	L37208
OARE129	F-aatcccagtgtgtgaaagactaatccag R-gtagatcaagatatatttttcaacac	$(CA)_{14}$	130-175	FAM	7	L11051
ILSTS082	F-ttcgttcctcatagtgctgg R-agaggattacaccaatcacc	$(GT)_{17}$	100-136	PET	2	L37236
RM4	F-cagcaaaatatcagcaaacct R-ccacctgggaaggccttta	$(CA)_{13}$	105-127	NED	15	U32910

*Chromosome no.; **Accession number; ***Accession number of Arkdb database (http://www.thearkdb.org); Ann, Annonymous microsatellite from other species

from 5.00 (ILSTS005, ILSTS044) to 19.00 (ILST059) with mean 10.20 in Konkan Kanyal goats; from 4.00 (ILSTS005, OarJMP 29) to 26.00 (OarFCB304) with mean 11.76 in Berari goats; from 2.00 (ILSTS059, RM4, OarJMP29) to 16.00 (OarFCB304) with mean 6.12 in Osmanabadi; and from 3.00 (ILSTS030, ILSTS022) to 17.00 (OarFCB304) with mean 3.00 in Sangamneri goats. The effective number of alleles ranged from 1.17 (OarJMP29) to 8.94 (ILSTS059) with mean 4.36 in Konkan Kanyal goats; from 1.15 (OarJMP29) to 11.00 (ILSTS059) with mean 4.95 in Berari; from 1.13 (OarJMP 29) to 7.43 (ETH225) with mean 3.27 in Osmanabadi; and from 1.36 (ILSTS030) to 6.09

(OarFCB304) with mean 3.45 in Sangamneri goats. Overall, a total of 479 alleles were detected in the four populations with varying allele frequencies (Table 3) where the number of alleles per locus varied from 7.00 (ILSTS30) to 32.00 (RM088) with a mean 19.16. The observed number of alleles were invariably higher than the effective number across all the loci in the breeds under study. The Konkan Kanyal and Berari exhibited more allelic polymorphism as compared to Osmanbadi and Sangamneri. The reason is obvious that Osmanabadi and Sangamneri are the descript breeds and the population is propagated with a planned breeding policy using the bucks of same breed whereas Konkan Kanyal and Berari are yet to be recognized as breed and hence no proper breeding policy is practiced. The indiscriminate mating between individuals may result in introduction of more allelic variability.

The observed value of heterozygosity varied from 0.13 (OarJMP29) to 0.95 (ILSTS034) with mean 0.58 in Konkan Kanyal goats; from 0.10 (OarJMP 29) to 1.00 (RM088) with mean 0.67 in Berari goats; from 0.00 (ILSTS059, RM4, ILSTS029) to 0.90 (ILSTS002) with mean 0.42 in Osmanabadi; and from 0.03 (ILSTS022) to 1.00 (ETH225) with mean 0.53 in Sangamneri goats. The expected heterozygosity ranged from 0.14 (OarJMP29) to 0.89 (ILSTS059, ILSTS082) with mean 0.67 in Konkan Kanyal goats; from 0.13 (OarJMP29) to 0.91 (ILSTS059) with mean 0.73 in Berari; from 0.12 (OarJMP29) to 0.88 (ETH225) with mean 0.61 in Osmanabadi; and from 0.33 (OarJMP29) to 0.84 (OarFCB304) with mean 0.66 in Sangamneri goats (Table 2). Across the populations, the observed heterozygosity was lowest (0.11) for OarJMP29 and highest (0.84) for ETH225 with an average of 0.61 whereas the expected heterozygosity was lowest (0.46) for OarJMP29 and highest (0.92) for ILST058 with mean 0.81 (Table 3). At most of the loci, the expected heterozygosity was significantly higher than the observed values. Among the breeds, the difference between the mean observed and expected heterozygosity was more (0.19) for Osmanabadi followed by Konkan Kanyal (0.11), Sangamneri (0.07) and Berari (0.06).

Most of the 25 microsatellite markers utilized to generate allelic data of Maharashtra goat breeds was also used for the genetic diversity analysis of Marwari (Kumar *et al.* 2005), Kutchi (Dixit *et al.* 2008), Changthangi (Mishra *et al.* 2010), Mehsana (Aggarwal *et al.* 2007), Sirohi (Verma *et al.* 2007), Sangamneri (Verma *et al.* 2011), Singharey (Shivhare *et al.* 2017). The precision of estimated genetic diversity is a function of number of loci studied, heterozygosity of loci and the number of animals sampled in each population. The FAO recommended that at least 25 randomly selected markers should be used for diversity analysis. Barker (1994) suggested that loci with minimum 4 alleles should be taken into consideration for such studies. Both the conditions have been taken into consideration for the present investigation as obvious from the minimum number of loci per locus reported (Table 2).

The allelic number, heterozygosity per locus across the breeds/populations have been estimated and are given in Table 3. The total number of samples tested for each locus varied from 117 (ILSTS029) to 175 (ILSTS034). Alleles (479) were detected in the four populations with varying allele frequencies. The mean observed number of alleles per locus was 19.16 and varied from 7.00 (ILSTS30) to 47.00 (RM088) whereas the effective number varied from 1.86 (OarJMP29) to 13.69 (ILSTS058) with mean 7.10. Observed heterozygosity per locus ranged from 0.11 (OarJMP29) to 0.84 (ETH225, ILSTS002) with an average of 0.61 and expected heterozygosity from 0.46 (OarJMP29) to 0.92 (ILSTS058) with mean of 0.81. Gene diversity (Nei's expected heterozygosity) is an appropriate measure of genetic variation within a population (Nei 1987). Takezaki and Nei (1996) determined that markers to be used for diversity analysis should have an average heterozygosity ranging from 0.3 to 0.8 in the population. The average expected heterozygosity in the present study were within this range i.e. 0.67 (Konkan Kanyal), 0.73 (Berari), 0.61 (Osmanabadi) and 0.66 (Sangamneri). The overall expected heterozygosity across the breeds was 0.81. This again confirms the suitability of markers used for measuring the genetic variation. Although differing among populations, the expected heterozygosity was more than the observed heterozygosity based on all the microsatellite loci (Table 2) for all the 4 populations. This showed a positive deviation from Hardy-Weinberg equilibrium. Similar observation was made by Dixit *et al.* (2009) while studying the differentiation of Mehsana, Kutchi and Sirohi breeds of North Western India.

Genetic differentiation was explained by fixation indices (F_{IS}, F_{ST} and F_{IT}) for each locus across the populations and the values in four goat populations are presented in Table 4. F_{IS} is an estimate of genetic variation within population that measures the homozygosity or reduction in heterozygosity in an individual due to non random mating within the subpopulation. F_{ST} measures the variation due to differences among populations which is the reduction in heterozygosity of a subpopulation due to genetic drift. F_{IT} is the overall reduction in heterozygosity in an individual related to the total population. F_{ST} varied from 0.04 (OarHH64) to 0.68 (OarJMP29) with a mean of 0.17. All loci contributed to this differentiation significantly. The moderate value of F_{ST} (0.17) indicated that 17% variation is due to the breed whereas 83% of the total genetic variation corresponded to the differences among individuals within populations of four breeds. F_{ST} value of 0.13 was reported in three Indian goat breeds by Dixit *et al.* (2009), 0.10 in 12 Chinese indigenous goat populations by Li *et al.* (2008), 0.20 in goats from Korea and China by Kim *et al.* (2001) and 0.07 in Italian goat populations by Iamartino *et al.* (2005). The global deficit of heterozygotes across populations (F_{IT}) amounted to 28% ($P<0.05$). An overall significant deficit of heterozygotes (F_{IS}) of 13% occurred due to out breeding in some populations. Nineteen loci showed a significant deficit of heterozygosity and five loci (ETH225, ILSTS008, ILSTS034, ILSTS002 and RM088)

Table 2. The average number of observed (Na), effective (Ne), expected (He) and observed (Ho) heterozygosity of four populations

	Kanyal				Berari				Osmanabadi				Sangamneri			
Locus	Na	Ne	Ho	He	Na	Ne	Ho	He	Na	Ne	Ho	He	Na	Ne	Ho	He
ILSTS030	8.00	5.22	0.75	0.81	12.00	7.18	0.75	0.86	5.00	3.05	0.44	0.67	3.00	1.36	0.20	0.27
ILSTS033	11.00	3.68	0.73	0.73	15.00	5.37	0.93	0.82	8.00	4.52	0.82	0.79	7.00	4.49	0.50	0.80
ILSTS005	5.00	2.36	0.44	0.58	4.00	3.00	0.57	0.67	3.00	1.78	0.23	0.44	4.00	2.48	0.43	0.60
ILSTS065	11.00	1.50	0.24	0.33	10.00	1.51	0.28	0.34	5.00	3.38	0.40	0.74	6.00	3.61	0.23	0.73
ILSTS087	10.00	5.63	0.71	0.83	10.00	5.83	0.79	0.83	9.00	2.88	0.56	0.66	10.00	4.72	0.70	0.79
OarAE129	13.00	5.74	0.80	0.83	14.00	7.00	0.66	0.86	3.00	1.61	0.25	0.38	5.00	3.04	0.08	0.68
ETH225	12.00	4.04	0.64	0.76	13.00	4.88	0.53	0.80	10.00	7.43	0.57	0.88	9.00	2.74	1.00	0.64
ILSTS058	15.00	4.50	0.91	00.78	16.00	6.04	0.79	0.84	8.00	4.15	0.76	0.76	8.00	4.50	0.59	0.79
ILSTS059	19.00	8.94	0.82	0.89	20.00	11.00	0.82	0.91	2.00	1.67	0.00	0.41	8.00	4.55	0.75	0.79
OARHH64	11.00	8.08	0.73	0.88	11.00	7.60	0.75	0.87	4.00	3.31	0.13	0.70	7.00	4.18	0.61	0.77
ILSTS008	6.00	3.33	0.93	0.70	10.00	5.14	0.90	0.81	6.00	1.92	0.38	0.48	8.00	2.03	0.36	0.51
ILSTS019	8.00	3.81	0.53	0.74	11.00	4.92	0.68	0.80	6.00	2.66	0.61	0.63	9.00	4.47	0.81	0.78
ILSTS034	10.00	3.75	0.95	0.74	13.00	3.10	0.78	0.68	5.00	1.46	0.30	0.33	10.00	2.14	0.53	0.53
ILSTS082	16.00	8.88	0.75	0.89	18.00	7.65	0.76	0.87	9.00	3.81	0.53	0.76	10.00	5.77	0.81	0.83
RM4	10.00	3.47	0.60	0.72	7.00	3.46	0.50	0.71	2.00	1.92	0.00	0.53	6.00	2.75	0.67	0.64
OarFCB304	15.00	4.13	0.66	0.76	26.00	6.61	0.90	0.85	16.00	6.91	0.74	0.86	17.00	6.09	0.65	0.84
OarFCB48	14.00	6.86	0.88	0.86	15.00	5.74	0.80	0.83	10.00	4.73	0.82	0.79	9.00	5.22	0.77	0.83
OarJMP29	6.00	1.17	0.13	0.14	4.00	1.15	0.10	0.13	2.00	1.13	0.12	0.12	5.00	1.48	0.08	0.33
ILSTS029	7.00	2.10	0.48	0.53	12.00	2.67	0.52	0.63	3.00	2.33	0.00	0.61	7.00	1.67	0.46	0.41
ILSTS044	5.00	2.51	0.27	0.60	6.00	1.60	0.28	0.38	7.00	1.34	0.18	0.25	8.00	2.91	0.43	0.66
ILSTS049	7.00	3.13	0.71	0.68	7.00	3.36	0.52	0.70	3.00	2.57	0.33	0.73	6.00	3.12	0.61	0.68
ILSTS002	6.00	5.05	0.80	0.81	8.00	4.71	0.84	0.79	6.00	5.80	0.90	0.84	7.00	4.33	0.85	0.78
RM088	13.00	2.92	0.91	0.66	13.00	5.04	1.00	0.80	10.00	6.11	0.62	0.85	8.00	2.97	0.66	0.67
OMHC1	11.00	5.17	0.84	0.81	12.00	6.21	0.94	0.84	8.00	3.85	0.63	0.75	12.00	3.90	0.50	0.76
ILSTS022	6.00	3.11	0.66	0.68	7.00	3.04	0.55	0.67	3.00	1.46	0.24	0.32	3.00	1.74	0.03	0.43
Mean	10.20	4.36	0.58	0.67	11.76	4.95	0.67	0.73	6.12	3.27	0.42	0.61	7.68	3.45	0.53	0.66

Table 3. The average number of observed (Na), effective (Ne), expected (He) and observed (Ho) heterozygosity in pooled population

Locus	Sample size	Na	Ne	Ho	He
ILSTS030	133	7.00	3.63	0.47	0.72
ILSTS033	171	22.00	10.65	0.73	0.90
ILSTS005	151	17.00	3.36	0.29	0.70
ILSTS065	172	12.00	6.33	0.61	0.84
ILSTS087	126	27.00	8.83	0.75	0.88
OarAE129	139	14.00	6.63	0.44	0.85
ETH225	145	24.00	8.87	0.84	0.89
ILSTS058	165	30.00	13.69	0.76	0.92
ILSTS059	165	18.00	10.08	0.64	0.90
OARHH64	157	13.00	6.43	0.56	0.84
ILSTS008	167	16.00	5.83	0.68	0.83
ILSTS019	169	19.00	8.07	0.66	0.87
ILSTS034	175	24.00	4.98	0.66	0.80
ILSTS082	153	22.00	9.53	0.75	0.89
RM4	143	12.00	3.36	0.56	0.70
OarFCB304	173	47.00	11.90	0.74	0.91
OarFCB48	158	27.00	11.32	0.82	0.91
OarJMP29	127	10.00	1.86	0.11	0.46
ILSTS029	117	15.00	3.08	0.47	0.67
ILSTS044	165	17.00	3.97	0.28	0.75
ILSTS049	145	8.00	3.78	0.60	0.73
ILSTS002	154	14.00	10.00	0.84	0.90
RM088	166	32.00	9.05	0.82	0.89
OMHC1	135	24.00	9.53	0.80	0.89
ILSTS022	150	8.00	2.74	0.43	0.63
Mean	153	19.16	7.10	0.61	0.81

exhibiting negative value showed an excess of heterozygosity.

The two phase mutation model under Wilcoxon's signed rank test and shift mode test were used to investigate any recent bottleneck (heterozygosity excess) in four goat populations. In a population at mutation-drift equilibrium, there is approximately an equal probability that a locus shows genetic diversity excess or deficit. Wilcoxon's signed rank test was used for evaluating bottleneck because of its relatively high statistical power (Luikart and Cornuet 1998). This test can be used with as few as four polymorphic loci and any number of individuals ranging from 15 to 40. Increased number of loci (10–20) is recommended to achieve more accuracy. The excess heterozygosity obtained was not significant ($P<0.05$) in the four populations using a two phase mutation model. The bottleneck test showed that there was significant deficiency of heterozygosity but the suspected genetic bottleneck was found to be absent as the mode-shift curve is a typical "L" shape in all the four populations (Fig. 3) as also described by Gour *et al.* (2006). It is important to conserve the genetic resources of seriously bottlenecked populations because such populations may be subjected to increased inbreeding depression, loss of genetic variation and fixation of deleterious alleles (Fatima *et al.* 2008). It is important to note that Berari and Konkan Kanyal goat populations have high genetic diversity as compared to Osmanabadi and Sangamneri.

Allele frequencies were utilized to measure the genetic distances between each pair of the studied breeds and distance matrices were used to build phylogenetic tree based on neighbour-joining algorithm. The matrix showing genetic identity and distances between the four populations is given in Table 5. The Nei's genetic distance observed between breeds were 0.472 (Sangamneri and Osmanabadi), 0.667 (Sangamneri and Berari), 0.819 (Sangamneri and Konkan Kanyal), 0.797 (Osmanabadi and Berari), 0.994 (Osmanabadi and Konkan Kanyal) and 0.092 (Berari and Konkan Kanyal). Konkan Kanyal is genetically more distant from Osmanabadi and Sangamneri than Berari. This is more obvious and expected looking at the geographical distance between their breeding tracts. But Konkan Kanyal and Berari showed more genetic similarity as compared to any

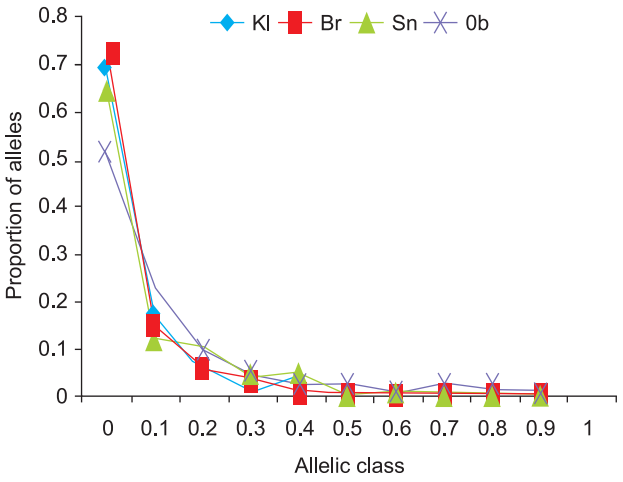

Fig. 3. Graphical representation of proportions of alleles in four goat populations.

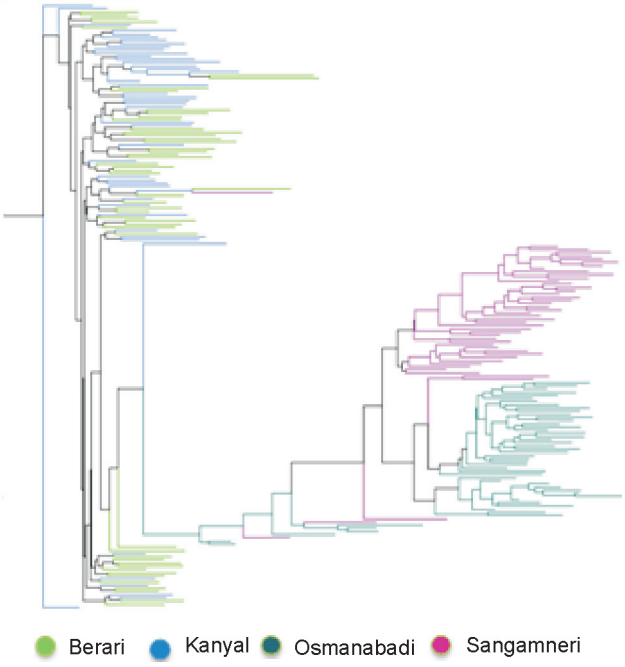

Fig. 4. Phylogenetic tree showing relationship between four goat populations.

Table 4. F estimates in Maharashtra goat populations

Locus	Population wise F_{IS} estimates						
	F_{IS} (Kl)	F_{IS} (Br)	F_{IS} (Ob)	F_{IS} (Sn)	F_{IT}	F_{ST}	F_{IS}
ILSTS030	0.07	0.13	0.34	0.29	0.38	0.19	0.20
ILSTS033	0.004	-0.14	-0.03	0.38	0.22	0.11	0.12
ILSTS005	0.24	0.15	0.46	0.28	0.57	0.40	0.29
ILSTS065	0.28	0.17	0.47	0.69	0.35	0.06	0.30
ILSTS087	0.14	0.05	0.14	0.12	0.16	0.15	0.01
OaeAE129	0.04	0.23	0.35	0.87	0.55	0.23	0.41
ETH225	0.15	0.34	0.35	-0.56	0.08	0.13	-0.05
ILSTS058	-0.16	0.05	0.00	0.25	0.18	0.09	0.09
ILSTS059	0.08	0.10	1.00	0.05	0.38	0.20	0.22
OARHH64	0.17	0.14	0.80	0.21	0.32	0.04	0.30
ILSTS008	-0.32	-0.10	0.21	0.29	0.20	0.23	-0.03
ILSTS019	0.28	0.15	0.03	-0.03	0.24	0.15	0.10
ILSTS034	-0.29	-0.14	0.06	0.01	0.18	0.28	-0.13
ILSTS082	0.16	0.13	0.30	0.02	0.19	0.06	0.13
RM4	0.16	0.30	1.00	-0.04	0.35	0.08	0.30
OarFCB304	0.13	-0.05	0.14	0.22	0.19	0.10	0.10
OarFCB48	-0.02	0.04	-0.03	0.06	0.09	0.10	-0.00
JMP29	0.11	0.25	0.00	0.75	0.81	0.68	0.39
ILSTS029	0.08	0.17	1.00	-0.12	0.51	0.29	0.30
ILSTS044	0.54	0.25	0.28	0.35	0.61	0.38	0.37
ILSTS049	-0.03	0.26	0.60	0.10	0.25	0.09	0.18
ILSTS002	0.01	-0.05	-0.07	-0.09	0.06	0.12	-0.06
RM088	-0.37	-0.23	0.27	0.00	0.11	0.18	-0.08
OMHC1	-0.03	-0.11	0.16	0.35	0.18	0.12	0.06
ILSTS022	0.29	0.19	0.25	0.91	0.38	0.13	0.28
Mean	0.05	0.15	0.32	0.21	0.28	0.17	0.13

other breed combination. Using Nei's standard genetic distances and the neighbour joining method of clustering, the dendrogram of relationships among the four goat population was obtained (Fig. 4). The Sangamneri and Osmanabadi goat breeds had unique branch, but Berari and Konkan Kanyal goat populations showed genetic closeness indicating gene introgression in spite of having geographically distant native tracts. On the other hand, Sangamneri and Osmanabadi goat breeds are inhabited in the regions which are far from the breeding tracts of Berari and Konkan Kanyal goats. Thus, the genetic relationship of these four populations corresponds to their breeding history and geographic origins. The population clustering according to geographic locations was also observed in microsatellite analysis of humans (Bowcock *et al.* 1994), cattle (MacHugh *et al.* 1997), buffalo (Vijh *et al.* 2008), chickens (Wimmers *et al.* 2000) and goats (Agha *et al.* 2008, Muema *et al.* 2009). Geographic clustering of breeds based on mitochondrial DNA analysis was shown (Joshi *et al.* 2004).

The assignment of cluster analysis can effectively resolve the genetic similarity of a group of highly diverged breeds and has great potential for identifying individuals with different or similar multi-locus genotypes (Ibeagha-Awemu *et al.* 2005). The proportion of membership of each defined population to a cluster is given in Table 6 and Fig. 5. As obvious from the values given in the Table 6 at $K = 4$, the four goat populations were divided into two main clusters.

Table 5. Genetic similarity and genetic distances among four populations.

Population	Sangamneri	Osmanabadi	Berari	Kanyal
Sangemneri	****	0.472	0.667	0.819
Osmanabadi	0.472	****	0.797	0.994
Berari	0.667	0.797	****	0.936
Kanyal	0.819	0.994	0.092	****

Sangamneri and Osmanabadi goat breeds were assigned to cluster-3 where the proportion of membership for each breed was 0.975 (Sangamneri) and 0.992 (Osmanabadi). Kanyal and Berari goat populations were assigned to another cluster (Cluster 4). The proportion for their membership was 0.923 (Konkan Kanyal) and 0.869 (Berari).

In conclusion, the microsatellite markers selected for this study suitably amplified to generate information on genetic variability. The mean number of alleles per locus and the average Nei's heterozygosity supported the justifiable use of selected battery of markers. The analysis revealed that there was substantial genetic diversity among the Konkan Kanyal, Berari, Osmanabadi and Sangamneri breeds. Most of the loci in these breeds were not in Hardy–Weinberg equilibrium and exhibited heterozygote deficiency. There was significant genetic differentiation (F_{ST}) among breeds which indicated that 17% of the total genetic variation corresponds to differences among populations and the remaining 83% is the result of differences among

Table 6. Proportion of membership of each pre-defined population in each of the 4 clusters

Population	Cluster1	Cluster2	Cluster3	Cluster4
Kanyal	0.026	0.050	0.001	0.923
Berari	0.053	0.077	0.001	0.869
Sangamneri	0.006	0.002	0.975	0.016
Osmanabadi	0.005	0.002	0.992	0.002

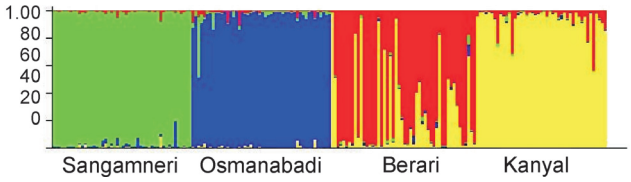

Fig. 5. Cluster analysis of studied goat breeds at K=4.

individuals. The genetic distance between four breeds/ populations indicated the distinctness of Berari and Konkan Kanyal from Sangamneri and Osmanabadi. Berari and Konkan Kanyal although existing at different geographic locations show some genetic overlapping. But based on their phenotype and geographical locations of their breeding tracts, the two populations can be treated as distinct populations.

ACKNOWLEDGEMENTS

Authors sincerely thank the Director, NBAGR for providing the facilities to carry out this work. We also acknowledge the technical support of Sh. Subhash Chander, T-3. The support and cooperation provided by the goat keepers in the field are duly acknowledged.

REFERENCES

Aggarwal R A K, Dixit S P, Verma N K, Ahlawat S P S, Kumar Y, Kumar S, Chander R and Singh K P. 2007. Population genetics analysis of Mehsana goat based on microsatellite markers. *Current Science* **92**(8): 1133–37.

Agha S H, Pilla F, Galal S, Shaat I, Andrea M D, Reale S, Abdalesalam A Z A and Lee M H. 2008. Genetic diversity in Egyptian and Italian goat breeds measured with microsatellite polymorphism. *Journal of Animal Genetics and Breeding* **125**: 194–200.

Barkar J S F. 1994. A global protocol for determining genetic distances among domestic livestock breeds. *Proceedings of 5th World Congress of Genetics Applied to Livestock Production* **21**: 501–08.

Bowcock A M, Ruiz-Linares A, Tomfohrde J, Minch E, Kidd J R, Cavalli L L and Forz S. 1994. High resolution of human evolutionary trees with polymorphic microsatellites. *Nature* **368**: 455–57.

Bruford M W and Wayne R K. 1993. Microsatellites and their application to population genetic studies. *Current Opinion in Genetics and Development* **3**: 939–43.

Dixit S P, Verma N K, Ahlawat S P S, Aggarwal R A K, Kumar S and Singh K P. 2008. Molecular genetic characterization of Kutchi breed of goat. *Current Science* **95**(7): 946–51.

Dixit S P, Verma N K, Aggarwal R A K, Kumar S, Ramesh Chander, Vyas M K and Singh K P. 2009. Genetic structure and differentiation of three Indian goat breeds. *Asian Australasian Journal of Animal Sciences* **22**(9): 1234–40.

Dixit S P, Verma N K, Aggarwal R A K, Vyas M K, Rana Jyoti and Sharma A. 2012. Genetic diversity and relationship among Indian goat breeds based on microsatellite markers. *Small Ruminant Research* **105**: 38–45.

Fatima S, Bhaug C D, Rank D N and Joshi C G. 2008. Genetic variability and bottleneck studies in Zalawadi, Gohilwadi and Surti goat breeds of Gujarat (India) using microsatellites. *Small Ruminant Research* **71**: 58–64.

Gour D S, Malik G, Ahlawat S P S, Pandey A K, Sharma R, Gupta N, Gupta S C, Bisen P S and Kumar D. 2006. Analysis of genetic structure of Jamunapari goats by microsatellite markers. *Small Ruminant Research* **66**: 140–49.

Ibeagha-Awemu E M and Erhardt G. 2005. Genetic structure and differentiation of 12 African *Bos indicus* and *Bos taurus* cattle breeds, inferred from protein and microsatellite polymorphisms. *Journal of Animal Breeding and Genetics* **122**: 12–20.

Iamartino D A, Bruzzone A, Lanza M, Blasi and Pilla F. 2005. Genetic diversity of Southern Italian goat populations assessed by microsatellite markers. *Small Ruminant Research* **57**: 249–55.

Joshi M S, Rout P K, Mandal A, Singh L and Thangraj K. 2004. Phylogeography and origin of Indian domestic goats. *Molecular Biology and Evolution* **21**: 454–62.

Kim K S, Yeo J S, Lee J W, Kim J W and Choi C B. 2001. Genetic diversity of goats from Korea and China using microsatellite analysis. Department of Animal Science, Yeungnam University, Daedong 214–1, Kyongsan 712–749, Korea.

Kumar D, Dixit S P, Sharma R, Pandey A K and Sirohi G. 2005. Population structure, genetic variation and management of Marwari goats. *Small Ruminant Research* **59**: 41–48.

Li J Y, Chen J, Lan X Y, Kong X J and Min L J. 2008. Genetic diversity of five Chinese goat breeds assessed by microsatellite markers. *Czechoslovakian Journal of Animal Sciences* **53**: 315–19.

Luikart G and Cornuet J M. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conservation Biology* **12**: 228–37.

MacHugh D E, Shriver M D, Loftus R T, Cunningham P and Bradley D G. 1997. Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle (*Bos taurus* and *Bos indicus*), *Genetics* **146**: 1071–86.

Mishra P, Verma N K, Aggarwal R A K and Dixit S P. 2010. Breed characteristics and genetic variability in Changthangi goats. *Indian Journal of Animal Sciences* **80**(12): 43–48.

Muema E K, Wakhungu J W, Hanotti O and Jianlin H. 2009. Genetic diversity and relationship of Indian goats of sub-saharan Africa using microsatellite DNA markers. *Livestock Research and Rural Development* 21. NBAGR.2016. *ICAR-NBAGR News Letter* 13(1) : 2.

Nei M 1973. Analysis of gene diversity in subdivided populations. *Proceedings of National Academy of Sciences USA* **70**: 3321–23.

Nei M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, USA.

Pepin L, Amigues Y, Lepingle A, Berthier J L, Bensaid A and Vaiman D. 1995. Sequence conservation of microsatellites between *Bos taurus* (cattle), *Capra hircus* (goat) and related species: examples of use in parentage testing and phylogeny

analysis. *Heredity* **74**: 53–61.

Pritchard J K, Stephens M and Donnely P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–59.

Sambrook J, Fritsch E F and Maniatis T. 1989. *Molecular Cloning: A Laboratory Manual*. 2nd edn. Cold Spring Harbor, Cold Laboratory Press, New York, USA.

Shivhare P, Verma N K, Aggarwal R A K, Sharma Rekha, Dangi P S, Bhutia N and Ahlawat Sonika. 2017. Microsatellite based genetic diversity estimation in Sikkim Singharey goat population. *Indian Journal of Animal Sciences* **87** (1): 125–27.

Takezaki N and Nei M. 1996. Genetic distances and reconstruction of phylogenetic tree from microsatellite DNA. *Genetics* **144**: 389–99.

Vaiman D, Schibler L, Bourgeois F, Oustry A, Amigues Y and Cribiu E P. 1996. A genetic linkage map of the male goat genome. *Genetics* **144**: 279–305.

Verma N K, Dixit S P, Aggarwal R A K, Chander R, Kumar S and Ahlawat S P S. 2007. Genetic analysis of the Sirohi breed of Indian goat (*Capra hircus*). *Korean Journal of Genetics* **29**: 129–36.

Verma N K, Dixit S P, Aggarwal R A K, Dangi P S and Joshi B K. 2011. Phenotypic and genetic characterization of Sangamneri goat breed. *Indian Journal of Animal Sciences* **80 (11)**: 1109–14.

Vijh R K, Tantia M S, Mishra B, Bharani Kumar S T. 2008. Genetic relationship and diversity analysis of Indian buffaloes. *Journal of Animal Sciences* **86**: 1495–1502,

Wimmers K, Ponsuksili S, Hardge T, valle-Zarate A, Mathur P K and Horst P. 2000. Genetic distinctness of African, Asian and Americal local chicken, *Animal Genetics* **31**: 159–65.

Wright S. 1978. Evolution and the Genetics of Populations. *Variability Within and Among Natural Populations*. University of Chicago Press, Chicago, IL, USA.4 pp.