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Analysis of Genetic Diversity in Berari Goat Population of Maharashtra State

Research Article

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ABSTRACT

Berari goats are medium sized animals reared mainly for meat purpose. They are predominantly brown and blackish brown in colour with glossy hair and found in Vidarbha region of Maharashtra state. Berari is not a recognized breed but a well established local population of goat which is yet to be fully explored for its phenotypic and genetic aspects. Within population genetic variation was examined in Berari goat population using microsatellite markers. The observed number of alleles varied from 4 (ILSTS05, OarJMP29) to 26 (OarFCB304) with a mean of 11.76 whereas the effective number of allele varied from 1.15 (OarJMP29) to 11 (ILSTS059) with a mean of 4.95. The effective number of allele is lesser than observed number at all the loci. Polymorphic information content ranged from 0.47 (OarJMP29) to 0.92 (OarFCB304) with the mean value of 0.82. Shannon's information index which measures the level of diversity ranged from 0.32 (OarJMP29) to 2.61 (ILSTS059) with average of 1.76 ± 0.55. Observed heterozygosity ranged from 0.10 (OarJMP29) to 1 (RM088) with the average of 0.67 and the expected heterozygosity ranged from 0.13 (OarJMP29) to 0.91 (ILSTS059) with the average of 0.73. Six of the twenty five loci showed the negative inbreeding coefficient as also depicted by the Hd value for the same loci. The unbiased expected gene diversity (Nei's) ranged from 0.14 (OarJMP29) to 0.92 (ILSTS059) with the overall mean 0.73 ± 0.19. The L-shaped curve obtained indicates that Berari goat population has not undergone any recent bottleneck. The genetic variability exhibited by the Berari goats can be suitably exploited for its genetic improvement and sustainable conservation.

KEY WORDS Berari, genetic variation, heterozygosity, microsatellite, polymorphism.

INTRODUCTION

Goats are found in all agro-ecological zones from hyper-arid to super-humid and over the whole range of production systems from intensive to very extensive nomadic pastoralists (Payne and Wilson, 1999).

Goat population of India is more than 140 million and 70-75% of this population consists of nondescript and crossbred goats. These nondescript goats which are result of unplanned and indiscriminate matings, have stabilized themselves in ecological zones and have a valuable genetic potential for sustainable agriculture and upliftment of the rural economy. Berari goat is one of such populations

Implication: Berari goat is a newly discovered and genetically investigated germplasm. The information generated on within population genetic variation of Berari goats will help in formulating the appropriate breeding strategies for breed improvement and sustainable conservation of these goats.

which are found in Vidarbha region of Maharashtra (Figure 1).

Figure 1 Breeding tract of Berari goat population

This region being very low rainfed necessitates improving livestock for sustainable improvement. Although sporadic information is available on the phenotype and performance of Berari goats, no information on its genetic characterization is available. Therefore, the present study was conducted to generate information on genetic variation existing within population of Berari goats using microsatellite markers. The microsatellites have been proved as potential genetic markers for diversity analysis because of their high degree of polymorphism, random distribution across the genome, co-dominance, possibility of automated scoring of genotypes and neutrality with respect to selection (Boyce *et al.* 1996).

MATERIALS AND METHODS

Blood collection

Blood samples were collected from 50 genetically unrelated animals having different parentage from different flocks existed in villages of Nagpur, Wardha and Akola districts of Maharashtra state (Figure 1). The blood was drawn from the jugular vein in vacutainer tubes coated with EDTA as blood anti-coagulants. The samples were kept at -20 ˚C in a deep freezer till subsequent processing.

DNA isolation

Genomic DNA was extracted using the standard phenol / chloroform / isoamyl alcohol extraction protocol with slight modifications (Sambrook, 1989). The extracted DNA was checked for its quantity and quality.

PCR and agarose gel electrophoresis

A battery of 25 microsatellite markers indicated in table 1 was used for amplification of genomic DNA. Microsatellites have been used as markers for the genetic diversity estimation of many goat breeds: Mongolian goat (Takahashi *et al.* 2008), Lori goat (Mahmoudi *et al.* 2010), Southern Italian goat, Kutchi goat (Dixit *et al.* 2008) and Kanniadu goat (Dixit *et al.* 2011) etc. Amplification for each primer was performed in a 10 µL final reaction volume containing 50 ng of genomic DNA, 10 pmol of each primer, 10 m*M* dNTPs, 0.5 Ul Taq polymerase and 10X buffer. The amplification was carried out for 35 cycles with initial denaturation at 95 ˚C for 10 minutes, second denaturation at 95 ˚C for 30 seconds, Annealing with different temperatures upto 1 minute, extension for 45 second at 72 ˚C and final extension for 7 minutes at 72 ˚C. The amplified products were checked on electrophoresis in 2% (w/v) agarose gel.

Genotyping

The PCR products were mixed in a ratio of 1:1.5:2:2 of FAM, VIC, NED and PET labeled primers respectively. 0.5 µL of this multiplexed mixture was combined with 0.3 µL of Liz 500 as internal lane standard (Applied Biosystems, USA) and 9.20 µL of Hi-Di Formamide per sample. The resulting mixture was denatured by incubation for 5 min at 95 ˚C and run on automated DNA sequencer. The electropherogram drawn through GeneScan were used to extract DNA fragment sizing details using Gene Mapper software (version 3).

Statistical analysis

The genotypic data thus generated was statistically analysed using different softwares. Genetic variation was quantified by calculating number of alleles (Kimura and Crow, 1964), heterozygosities (Levene, 1949) and within breed heterozygotes deficiency (Fis) following the POPGENE (Yeh *et al.* 1999). The polymorphic information content (PIC) of microsatellite loci was estimated following Botstein *et al.* (1980) and Shannon information index as per Lewontin (1972).

Heterozygote deficiency (Hd) was estimated using the formula (Ho-He) / He. Within-population genetic diversity was assessed as per unbiased expected gene diversity (*H*) assuming Hardy-Weinberg equilibrium. Genetic bottleneck effect in the population was inferred using the qualitative graphical method (mode shift analysis) under the assumption of two phase microsatellite mutation model (TPM), implemented in the programme BOTTLENECK (Cornuet and Luikart, 1996).

RESULTS AND DISCUSSION

Microsatellite markers

The precision of microsatellite based genetic variability depends upon the type and number of markers and the

polymorphism they can detect at the loci. Therefore, the suitability of markers was studied by knowing the number of alleles at different loci, Shannon Information index (I) and Polymorphic Information Content (PIC). In the present investigation the markers used are neutral and are widely spread in the genome (Table 1).

The battery of microsatellites used for Berari goats has also been used earlier to study the genetic variability in other Indian goat breeds (Verma *et al.* 2007; Dixit *et al.* 2008; Kumar *et al.* 2009; Dixit *et al.* 2010; Mishra *et al.* 2010). All the microsatellite markers successfully amplified in Berari goats also and were found highly polymorphic.

Table 1 Description of Microsatellite markers used

Locus	Primer sequence	Type of Repeat	Size range	Dye	* Chr. No.	** Acc. No.	PCR anealing temp and time
ILSTS008	F-gaatcatggattttctgggg R-tagcagtgatgaggttggc	$(CA)_{12}$	167-195	FAM	14	L23483	57 ˚C 30 s
ILSTS059	F-gctgaacaatgtgatatgttcagg R-gggacaatactgtcttagatgctgc	$(CA)_4(GT)_2$	105-135	FAM	13	L37266	57 ˚C 30 s
ETH225	F-gatcaccttgccactatttcct R-acatgacagccagctgcttact	$(CA)_{18}$	146-160	VIC	14	Z14043	57 ˚C 30 s
ILSTS044	F-agtcacccaaaagtaactgg R-acatgttgtattccaagtgc	$(GT)_{20}$	145-177	NED	Ann	L37259	55 ˚C 30 s
ILSTS002	F-tctatacacatgtgctgtgc R-cttaggggtgaagtgacacg	$(CA)_{17}$	113-135	VIC	Ann	23479	60 ˚C
Oar FCB304	F-ccctaggagctttcaataaagaatcgg R-cgctgctgtcaactgggtcaggg	$(CT)_{11}(CA)_{15}$	119-169	FAM	Ann	L01535	60 ˚C
Oar FCB48	Fgagttagtacaaggatgacagaggcac R-gactctagaggatcgc	$(GT)_{10}$	149-181	VIC	17	M82875	60 ˚C
Oar HH64	F-cgttccctcactaggaaagttatatatgc R-cactctattgtaagaatttgaatgaatgagagc	-	120-138	PET	4	*** 212	57 ˚C 30 s
Oar JMP29	F-gtatacacgtggacaccgctttgtac R-gaagtggcaagattcagaggggaag	$(CA)_{21}$	120-140	NED	Ann	U30893	60 ˚C
ILSTS005	F-ggaagcaatgaaatctatagcc R-tgttctgtgagtttgtaagc	$(nn)_{39}$	174-190	VIC	10	L23481	55 ˚C 30 s
ILSTS019	F-aagggacctcatgtagaagc R-acttttggaccctgtagtgc	$(TG)_{10}$	142-162	FAM	Ann	L23492	57 ˚C 30 s
OMHC1	F-atctggtgggctacagtccatg R-gcaatgctttctaaattctgaggaa	-	179-209	NED	Ann	*** 228	60 ˚C
ILSTS087	F-agcagacatgatgactcagc R-ctgcctcttttcttgagagc	$(CA)_{14}$	142-164	NED	Ann	L37279	55 ˚C 30 s
ILSTS030	F-ctgcagttctgcatatgtgg R-cttagacaacaggggtttgg	$(CA)_{13}$	159-179	FAM	2	L37212	55 ˚C 30 s
ILSTS034	F-aagggtctaagtccacggc R-acctggtttagcagagagc	$(GT)_{29}$	153-185	VIC	5	L37254	57 ˚C 30 s
ILSTS033	F-tattagagtggctcagtgcc R-atgcagacagttttagagg	$(CA)_{12}$	151-187	PET	12	L37213	55 ˚C 30 s
ILSTS049	F-cattttcttgtctctcccc R-gctgaatcttgtcaaacagg	$(CA)_{26}$	160-184	NED	11	L37261	55 ˚C 30 s
ILSTS065	F-gctgcaaagagttgaacacc R-aactattacaggaggctccc	$(CA)_{22}$	105-135	PET	24	L37269	55 ˚C 30 s
ILSTS058	F-gccttactaccatttccagc R-catcctgactttggctgtgg	$(GT)_{15}$	136-188	PET	17	L37225	57 ˚C 30 s
ILSTS029	F-tgttttgatggaacacagcc R-tggatttagaccagggttgg	$(CA)_{19}$	149-191	PET	3	L37252	55 ˚C 30 s
RM088	F-gatcctcttctgggaaaaagagac R-cctgttgaagtgaaccttcagaa	$(CA)_{14}$	109-147	FAM	4	U10392	60 ˚C
ILSTS022	F-agtctgaaggcctgagaacc R-cttacagtccttggggttgc	$(GT)_{21}$	186-202	PET	Ann	L37208	60 ˚C
OarAE129	F-aatcccagtgtgtgaaagactaatccag R-gtagatcaagatatatttttcaacac	$(CA)_{14}$	130-175	FAM	7	L11051	57 ˚C 30 s
ILSTS082	F-ttcgttcctcatagtgctgg R-agaggattacaccaatcacc	$(GT)_{17}$	100-136	PET	2	L37236	57 ˚C 30 s
RM4	F-cagcaaaatatcagcaaacct R-ccacctgggaaggccttta	$(CA)_{13}$	105-127	NED	15	U32910	57 ˚C 30 s

* Chromosome number; ** Accession number and *** Accession number of Arkdb data base.

Genetic variability

Various genetic diversity measures estimated for each locus in Berari goat population are summarized in table 2. A total of 294 alleles distributed at different loci were observed. The observed number of alleles varied from 4 (ILSTS005, OarJMP29) to 26 (OarFCB304) with a mean of 11.76 whereas the effective number of allele varied from 1.15 (OarJMP29) to 11 (ILSTS059) with a mean of 4.95. The effective number of allele is lesser than observed number at all the loci. Barker (1994) suggested that loci with at least four alleles should be suitable for studying the genetic diversity. The alleles more than the recommended number observed in this study supports the suitability of microsatellite markers. The average number of alleles observed in Berari was found higher than that of Kannaiadu, Osmanabadi and Sangamneri (Dixit *et al.* 2010) indicating more allelic polymorphism in Berari goats. Polymorphic Information Content ranged from 0.47 (OarJMP29) to 0.92 (OarFCB304) with the mean value of 0.82. Shannon's information index which measures the level of diversity ranged from 0.32 (OarJMP29) to 2.61 (ILSTS059) with a moderate average of 1.76 ± 0.55 and further supports the suitability of markers and occurrence of genetic polymorphism in Berari goats.

Heterozygosity defines the probability that a given individual randomly selected from a population will be heterozygous at a given locus. To find this probability, the heterozygosity was determined for each locus. Observed heterozygosity ranged from 0.10 (OarJMP29) to 1 (RM088) with the average of 0.67 and the expected heterozygosity ranged from 0.13 (OarJMP29) to 0.91 (ILSTS034) with the average of 0.73. Except at ILSTS033, ILSTS008, OarFCB304, ILSTS002, RM088, OMHC1, the observed heterozygosities were lower than the expected (Table 2). This indicated significant heterozygotic deficiency at the remaining loci.

The average genetic variation (0.67) observed in the present study was lower than that of Black Bengal (Behl *et al.* 2003), but higher than those of many Indian breeds (Dixit *et al.* 2010; Dixit *et al.* 2011), Asian and Australian breeds (Barker *et al.* 2001), Swiss goats (Saitbekova *et al.* 1999), and Korean goats (Kim *et al.* 2002). This showed substantial genetic variation in Berari goats.

The unbiased expected gene diversity (Nei's) ranged from 0.14 (OarJMP29) to 0.92 (ILSTS059) with the overall mean 0.73 ± 0.19 (Table 2). The F-estimates was applied to all the 25 studied loci to determine the extent of inbreeding in the population.

Table 2 Genetic variability estimates

Locus	Allelic Number		Heterozygosity			Fis	PIC	I	Hd
	Na	Ne	Ho	He	Nei's				
ILSTS30	12.00	7.18	0.75	0.86	0.87	0.13	0.88	2.13	-0.128
ILSTS033	15.00	5.37	0.93	0.81	0.82	-0.14	0.82	2.02	0.148
ILSTS005	4.00	3.00	0.57	0.66	0.68	0.15	0.77	1.22	-0.136
ILSTS065	10.00	1.51	0.28	0.34	0.34	0.17	0.59	0.83	-0.176
ILSTS087	10.00	5.83	0.79	0.82	0.84	0.05	0.86	1.93	-0.037
OARE129	14.00	7.00	0.66	0.85	0.87	0.23	0.91	2.23	-0.224
ETH225	13.00	4.88	0.53	0.79	0.81	0.34	0.88	1.92	-0.329
ILSTS058	16.00	6.04	0.79	0.83	0.84	0.05	0.87	2.15	-0.048
ILSTS059	20.00	11.00	0.82	0.90	0.92	0.10	0.90	2.61	-0.089
OARHH64	11.00	7.60	0.75	0.86	0.88	0.14	0.90	2.17	-0.128
ILSTS008	10.00	5.14	0.90	0.80	0.81	-0.10	0.83	1.86	0.125
ILSTS019	11.00	4.92	0.68	0.79	0.81	0.15	0.86	1.83	-0.139
ILSTS034	13.00	3.10	0.78	0.67	0.68	0.14	0.85	1.69	-0.98
ILSTS082	18.00	7.65	0.76	0.86	0.88	0.13	0.92	2.37	-0.116
RM4	7.00	3.46	0.50	0.71	0.72	0.30	0.82	1.47	-0.296
OarFCB304	26.00	6.61	0.90	0.84	0.86	-0.05	0.92	2.54	0.071
OarFCB48	15.00	5.74	0.80	0.82	0.83	0.04	0.91	2.10	-0.024
OarJMP29	4.00	1.15	0.10	0.13	0.14	0.25	0.47	0.32	-0.231
ILSTS029	12.00	2.67	0.52	0.62	0.63	0.17	0.79	1.49	-0.161
ILSTS044	6.00	1.60	0.28	0.37	0.38	0.25	0.62	0.78	-0.243
ILSTS049	7.00	3.36	0.52	0.70	0.71	0.26	0.81	1.39	-0.257
ILSTS002	8.00	4.71	0.84	0.78	0.80	-0.05	0.85	1.71	0.077
RM088	13.00	5.04	1.00	0.80	0.81	-0.23	0.81	1.97	0.250
OMHC1	12.00	6.21	0.94	0.83	0.85	-0.11	0.88	2.03	0.133
ILSTS022	7.00	3.04	0.55	0.67	0.68	0.19	0.77	1.30	-0.179
Mean	11.76±5.01	4.95±2.28	0.67±0.18	0.73±0.18	0.73±0.19	0.15	0.82	1.76±0.55	-

Na: observed number of alleles; Ne: effective number of alleles [Kimura and Crow (1964)]; He and Ho: expected homozygosty and heterozygosity were computed using Levene (1949); Hd: heterozygotic deficiency and I: shannon's Information index.

The Fis values provide the information on non-random union of gametes in the population i.e. the matings among the individuals which are related more than the average relationship in the population. The positive value of Fis indicate inbreeding in the population whereas the negative values point towards out breeding i.e. mating of individuals who are less related than the average relationship of the population.

Six loci (ILSTS033, ILSTS008, ILSTS34, OarFCB304, ILSTS002, RM088 and OMHC1) showed the negative inbreeding coefficient (Table 2). The values ranged from -0.23 (RM088) to -0.05 (OarFCB 304, ILSTS002). Among the positive Fis, values ranged from 0.05 (ILSTS087) to 0.34 (ETH225). The overall mean Fis was 0.15. The negative Fis indicated the excess of heterozygote whereas the significant positive Fis indicated the increased homozygosity or heterozygotic deficit.

The marker loci ETH 225, RM4, OarJMP29, ILSTS044 and ILSTS049 showed moderately high Fis. The negative values of Fis exhibited by the six loci were also depicted by the positive Hd or excess of heterozygotes (Table 2). The festimates obtained in this study differ from those reported in many livestock populations where f generally was not significant from zero (Behara *et al.* 1998; Cannon *et al.* 2000).

However, significant heterozygote deficiency have also been reported in many goat breeds (Luikart *et al.* 1999; Kumar *et al.* 2005; Verma *et al.* 2007; Kumar *et al.* 2009; Dixit *et al.* 2011; Mishra *et al.* 2012). In mutation drift equilibrium heterozygosity excess / deficiency under different mutation models: Infinite allele model (IAM), Two phase model (TPM) and Stepwise mutation model (SMM) generated by BOTTLENECK showed that there was significant deficiency of heterozygosity (Table 3).

In frequency method, under the sign test expected numbers of loci with heterozygosity excess were 14.99, 14.83 and 14.73 respectively whereas the observed numbers of loci with heterozygosity excess were 17, 11 and 2 for IAM, TPM and SMM respectively. The observed numbers of loci with heterozygosity deficiencies were 8, 14 and 23 respectively. The higher deficiency exhibited under SMM may be due to the oversensitivity of the method.

The difference between expected and observed heterozygosity excess was not significant in IAM and TPM, whereas this was significant in SMM showing lack of reduction in effective population size.

The sign test under Infinite Allele model proved the null hypothesis that the population is in mutation drift equilibrium. The Standardized difference test (statistic T_2) is equal to -0.217, -6.120 and -16.01 respectively for IAM, TPM and SMM models in Berari goat population (Table 3). The values of IAM, TPM and SMM are less than 1.645 (tabulated value of normal distribution) and thus supports the acceptance of the null hypothesis of mutation-drift equilibrium.

Wilcoxon Rank test which is a nonparametric test, the probability values were 0.280, 0.981 and 1 for IAM, TPM and SMM respectively. These values are more than 0.05 for IAM, TPM and SMM respectively, thus favour the null hypothesis of mutation-drift equilibrium. In a population at mutation drift equilibrium, the effective population size remains constant and there is approximately an equal probability that a locus shows heterozygosity excess or deficit. Under Wilcoxon Rank test, the probability values were 0.280, 0.981 and 1.00 for IAM.

TPM and SMM respectively. These values are more than 0.05, thus supports the null hypothesis of mutation drift equilibrium.

Mode Shift test allows to check whether the distribution followed the normal L-shaped curve depicted in the graph (Figure 2).

Qualitative graphical method based on the allele frequency spectra detected no shift in the frequency distribution of alleles, where the alleles with the lowest frequencies (0.01-0.1) were found to be most abundant.

This reflects that the population had not undergone bottleneck in recent past. Genetic bottleneck occurs when population experiences some temporary reduction in its effective size. This may influence distribution of genetic variation within and among populations.

Loss of genetic diversity may reduce the potential of small populations to respond to selective pressure (Allendorf *et al.* 1986) and increased inbreeding may reduce population viability.

Table 3 Heterozygosity excess/deficiency, probability under frequency method

Test	Heterozygosity excess / deficiency, probability	IAM	TPM	SMM
Sign test	Expected no. of loci with He excess	14.99	14.83	14.73
	Observed no. of loci with He excess	17	11	2
	Heterozygosity deficiency	8	14	23
	P-value	0.272	0.088	0.000
Standardized difference test	P-value	0.414	*** 0.000	*** 0.000
	T2-value	-0.217	-6.120	-16.01
Wilcoxon test	P-value (one tail for He excess)	0.280	0.981	1.000

IAM: infinite allele model; TPM: two phase model and SMM: stepwise mutation model.

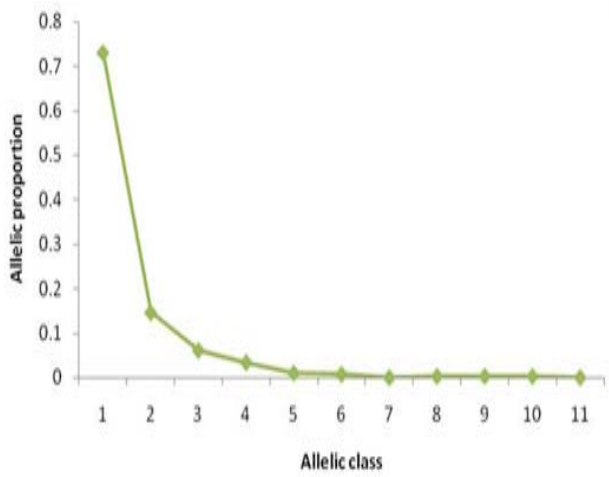

Figure 2 Allele frequency distribution

The three tests (Sign test, standard difference test and Wilcoxon rank test) under these three models (infinite allele model, two phase model and stepwise mutation model) for heterozygosity excess can detect the bottleneck for only a short duration of time after a bottleneck has been initiated. The L-shaped curve obtained indicates that Berari goat population has not undergone any recent bottleneck.

CONCLUSION

To conclude, the present study shows that all the microsatellite markers used were suitable for genetic characterization of Berari goats as they amplified successfully and exhibited high PIC and allelic polymorphism. Based on allele numbers, observed and expected heterozygosities and Nei's values, the Berari goat's exhibit good amount of genetic variability. The population has not undergone any recent bottleneck indicating that minor alleles are intact in the population. The characterization of Berari goat will facilitate the recognition and maintenance of this unique indigenous gene pool for current and future generations through implication of suitable genetic improvement programmes and ensure its sustainable conservation.

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