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Molecular characterization of Bengal goats using microsatellite markers

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Bengal goat (BG) is a dwarf breed found almost in all villages of West Bengal and regaeded as a major meat producing animal in West Bengal along with the adjoining part of the Jharkhand, Odisha, Bihar, Tripura states of India (Zeshmarani *et al*. 2007). The Network Project on Animal Genetic Resources - Core laboratory, Department of Animal Genetics and Breeding, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Asom, India has undertaken molecular characterization of livestock through microsatellite markers. Onto date no published reports are available on microsatellite based characterization of BG goat. In view of this the present study was undertaken to investigate genetic variation and population structure within BG population using 20 polymorphic microsatellite markers.

Sampling: Blood samples (30) of BG were collected randomly from genetically unrelated individuals from their native breeding tract. Blood was collected aseptically into BD vacutainers (6 ml) containing K2 EDTA (10.8 mg) and samples were transported to the laboratory on ice and were stored at 4°C until use.

DNA extraction and quantification: Genomic DNA was extracted from the blood samples using standard phenol-chloroform method (Sambrook *et al*. 1989) with few modifications. All extracted samples were conformed through horizontal electrophoresis on 0.8% agarose gel containing ethidium bromide. The quantification of DNA was done by nano-drop spectrophotometer at 260 nm. The concentrated samples were diluted to reach appropriate concentrations (20–50 ng/µl) for the purpose of PCR amplification.

Selection of microsatellite markers: All the 20 microsatellite markers were selected from the list recommended by International Society for Animal Genetics (ISAG) and FAO's (DAD-IS) for Caprine (FAO 2004), based on their level of polymorphism, allele size range and reliability of allele calling. The forward primer of each marker was fluorescently labeled with either FAM, NED, PET or VIC dye. All microsatellite markers were first checked under single locus amplification conditions to evaluate their performance in the multiplex, and accordingly multiplex panels were prepared.

Multiplex PCR optimization: Multiplex PCR was used for multicolor fluorescence genotyping. Based on the guide lines of Henegariu *et al*. (1997) and Loffert *et al*. (1999) the initial parameters of multiplex PCR were set up. The basic PCR solution (15 µl) containing 20–50 ng of template DNA; 1.5 mM $MgCl_2$; 5 picomoles each of forward and reverse primers; 1 unit of taq DNA polymerase and 200 mM dNTPs was prepared. Amplification was carried out with initial denaturation at 95°C for 2 min followed by 30 cycles of denaturation (95°C for 30 sec), annealing (54°C to 60°C for 30 sec) and extension (72°C for 45 sec).

Genotyping

Genotyping was carried out on automated DNA sequencer. The resulting data were analyzed using standard software Gene Mapper to generate genotype calls for each locus by using GS 500 (- 250) LIZ as size standard.

Data analysis: POPGENE version 1.31 (Yeh *et al*. 1999) was used to calculate the allele frequencies, effective number of alleles (N_e), observed (H_o) and expected (H_e) heterozygosity, F-statistics, Shanon's information index (I) and to test Hardy-Weingberg equilibrium (HWE). Nei's formula (Nei 1978) was used to calculate polymorphic information content (PIC). The BOTTLENECK version 1.2.03 (Cornuet and Luikart 1996) analysis was performed to know whether this goat population exhibits a significant number of loci with excess of heterozygosity.

The various parameters of genetic differentiation in BG, such as allele number, effective number of allele, PIC, observed and expected heterozygosity, within- population inbreeding estimate (F_{IS}) and Shanon's information index are furnished in Table 1.

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The number of observed alleles (N_a) detected ranged from 2 (ILSTS044, ILSTS065 and OarVH72) to 12 (ILSTS34), with an overall mean of 5.5±2.645 and 110 alleles were observed at these loci in the population. The number and sizes of microsatellite alleles observed in this study fall within the range mentioned in the Secondary Guidelines for Development of National Farm Animal Genetic Resource Management Plans of FAO. The present result is in agreement with the reported mean number of alleles in Ganjam (6.29) goat (Sharma *et al*. 2009). However, mean number of alleles observed in the present investigation was less than the mean number reported for Gohilwari (10.12) goat (Kumar *et al*. 2009). The effective number of alleles (N_e) in the present study ranged from 1.039 (OarVH72) to 6.3452 (OarFCB304) with a mean of 3.03±1.493, while overall allele frequency ranged from 0.014 (at locus ILSTS33) to 0.983 (at locus OarJMP29).

The PIC value ranged from 0.037 (OarVH72) to 0.824 (OarFCB304) with a mean of 0.53±0.230, which is in close agreement with reports of Sharma *et al*. (2009) in Ganjam goat and Kumar *et al*. (2009) in Gohilwari goat. Most of the studied loci were highly informative, indicating high polymorphism. Thus these markers strongly signified genetic diversity investigations of BG.

The overall means for observed (H_O) and expected (H_e) heterozygosities were 0.63±0.292 and 0.57±0.234 respectively, which ranged from 0.038 (OarVH72) to 1.000 (ILSTS30) and 0.037 (OarVH72) to 0.842 (OarFCB304) respectively. Though few loci exhibited lower heterozygosity values, most of the loci showed relatively higher expected heterozygosity, which reflects the existence of differentiation in the population (Karthickeyan *et al*. 2008).

The chi-square (χ^2) test for HWE revealed that 7 out of 20 loci deviated from equilibrium. These results established that the samples were drawn from large random mating population (Karthickeyan *et al*. 2008). Shannon's information index (I) (Lewontin 1972), which measures the level of diversity, was sufficiently high with a mean of 1.17 ±0.540 in the BG population.

The within population inbreeding estimates (F_{IS}) observed at 6 loci were positive which ranged from 0.037 (OarE129) to 0.416 (ILSTS058). Majority loci revealed negative F_{IS} values indicating absence of inbreeding. The mean F_{IS} value observed was –0.105 indicating that the BG population is an out-bred one, having the F_{IS} value on the negative side. The present findings of F_{IS} value is indicative of the fact that there is 10.5% excess of heterozygosity in the studied population. However, the heterozygote deficiency were reported in Ganjam goat 21.7% (Rekha Sharma *et al.* 2009); Gohilwari goat 26.4% (Kumar *et al*. 2009); Kutchi goat 26%,

Table 1. Microsatellite analysis in Bengal goat (BG)

Panel	Locus	Size range (bp)	Parameters							
			N_a	N_e	PIC	H_o	H_e	I	F_{IS}	HWE
Panel 1	ILSTS008	168–176	5	1.3824	0.2660	0.3077	0.2766	0.6225	–0.1123	0.73^{NS}
	OarHH64	121–131	5	2.9584	0.5982	0.8846	0.6620	1.2211	–0.3363	18.16^{NS}
	ILSTS044	153–169	2	1.1220	0.1028	0.1154	0.1087	0.2206	–0.0612	0.06^{NS}
Panel 2	ILSTS059	106–128	4	2.6316	0.5459	0.5600	0.6200	1.0736	0.0968	3.88^{NS}
	OarE129	149–167	6	3.7895	0.6929	0.7083	0.7361	1.4792	0.0377	19.14^{NS}
	ILSTS002	114–122	5	3.1515	0.6345	0.6538	0.6827	1.3353	0.0423	41.62**
	ILSTS065	116–118	2	1.8648	0.3562	0.7308	0.4638	0.6565	–0.5758	8.09**
Panel 3	ILSTS033	146–158	7	2.3558	0.5483	0.6250	0.5755	1.2425	–0.086	16.71^{NS}
Panel 4	ILSTS019	175–187	3	2.1589	0.4295	0.9200	0.5368	0.8325	–0.7139	16.85**
	ILSTS005	136–188	3	1.5152	0.3142	0.2400	0.3400	0.6390	0.2941	19.12**
	ILSTS058	139–159	6	4.6640	0.7796	0.4583	0.7856	1.6566	0.4166	42.9**
	ILSTS087	161–175	7	5.7870	0.8041	0.8800	0.8272	1.8346	–0.0638	29^{NS}
Panel 5	ILSTS30	157–177	6	3.5767	0.6780	1.0000	0.7204	1.4315	–0.3881	73.71**
	ILSTS34	153–179	12	3.7452	0.7154	0.8077	0.7330	1.8289	–0.1019	103.26*
	LSTS049	161–173	6	1.5850	0.3575	0.3462	0.3691	0.8532	0.0621	17.28^{NS}
	ILSTS029	119–121	5	3.1095	0.6201	0.8800	0.6784	1.2714	–0.2972	9.41^{NS}
Panel 6	OarVH72	146–164	2	1.0392	0.0370	0.0385	0.0377	0.0950	–0.0196	0^{NS}
	OarFCB304	184–200	11	6.3452	0.8241	0.9600	0.8424	2.0449	–0.1396	34.44^{NS}
	OarFCB48	156–178	6	3.4756	0.6659	0.7308	0.7123	1.4471	–0.026	10.13^{NS}
Panel 7	OMHC1	130–172	7	4.4620	0.7446	0.8846	0.7759	1.6749	–0.1401	22.96^{NS}
Mean overall loci			5.5±2.645	3.03±1.493	0.53±0.230	0.63±0.292	0.57±0.234	1.17±0.540	–0.1056	

* Significant (P≤0.05); **highly significant (*P*≤0.01); ^{NS} not significant (P≤0.05); N_a, number of alleles; N_e, effective number of alleles; PIC, polymorphic information content; H_o, observed heterozygosity; H_e, expected heterozygosity; F_{IS}, deficit or excess of heterozygotes, HWE, Hardy-Weinberg equilibrium; I, Shannon's information index.

Table 2. Bottleneck analysis in Bengal goat (BG)

Model	Sign rank test - Number of loci with heterozygosity excess			Standardized differences test - T2 values (probability)	Wilcoxon test - Probability of heterozygosity excess
	Expected	Observed	Probability		
IAM	10.64	12	0.34209	0.860 (0.19503)	0.28388
TPM	10.88	12	0.38995	–1.415 (0.07858)	0.55472
SMM	10.94	8	0.12798	–5.259 (0.00000)	0.95629

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

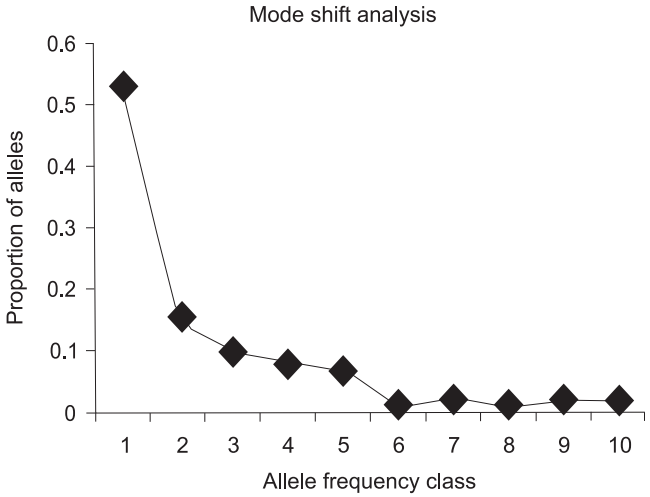

Fig. 1. Graphical representation of allele proportions and their contribution in Bengal goat (BG).

Mehsana goat 14% and Sirohi goat 36% (Dixit *et al*. 2009).

Three mutation models namely, infinite allele model (IAM), two phase model (TPM), stepwise mutation model (SMM) were estimated using the programme Bottleneck (Table 2). The results indicated that BG population is non-bottlenecked, i.e., it has not undergone any recent reduction in the effective population size and remained at mutation-drift equilibrium and no mode-shift was detected in the present investigation (Fig. 1).

In conclusion, the PIC values observed in the present study is indicative of the fact that the markers used are highly informative for characterization of BG diversity. The BG population is existing excess level of heterozygosity within the population which is indicated by the present study findings. The population has not under gone any reduction at least in the recent past.

SUMMARY

This study was conducted to understand the genetic diversity within the population of Bengal goat (BG) using 20 polymorphic microsatellite markers. All the loci studied were polymorphic in nature. The number of observed alleles (N_a) detected ranged from 2 to 12 with an overall mean of 5.50±2.645. A total of 110 alleles were observed across all the loci. The effective number of alleles (N_e) ranged from 1.0392 to 6.3452 with a mean of 3.03±1.493. The allele frequency ranged from 0.0143 to 0.9833. The overall mean observed (H_O) and expected (H_e) heterozygosity were 0.63 and 0.57 respectively and this population was in Hardy-Weinberg equilibrium at most of the loci studied. The overall mean F_{IS} (-0.105) indicated BG population is an outbred one. The population was stable with respect to size and was non-bottlenecked. The observed normal L-shaped curve indicated no mode shift in the population.

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