Wayamba Journal of Animal Science – ISSN: 2012-578X; P1044-P1050, 2014
First Submitted July 30, 2014; Number 1388595457

GENETIC DIVERSITY ESTIMATION IN BLACK BENGAL TYPE GOAT POPULATION USING MICROSATELLITE MARKERS

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Black Bengal Type goats are medium and small sized animals found in Mayurbhanj, Kendujhar and Baleshwar districts of Orissa. Genetic characterization of these animals was carried out using a battery of twenty five microsatellite markers. Microsatellite markers provided 255 alleles. The average observed and effective number of alleles were 10.20 and 4.29, respectively. Average expected and observed heterozygosity was 0.59 and 0.71, respectively. Polymorphic Information Content value varies from 0.55 to 0.91 with an overall mean of 0.81. Within population inbreeding estimate (F_{IS}= 0.17) showed moderate level of inbreeding. L-shaped curve showed that Black Bengal Type goat population had not faced any recent genetic bottleneck.

Keywords: Genetic diversity, Microsatellite markers, PIC, Heterozygosity

Goat rearing is the best choice for the rural people in developing countries like India because of their varied role and size of the herd, relative proportion to other animals if any, scale and intensity of production, low investment, high feed conservation and quick pay off and low risk involved. These aspects are closely associated with distinct socio-economic contribution to several millions of poor farmers, landless peasants and labourers to whom ownership of goats provides a definite means of livelihood and its sustainability (Devendra, 1980). Black Bengal type is a local goat population of Orissa found in Mayurbhanj, Kendujhar and Baleshwar districts and are famous for excellent skin quality. It gives high frequency of twins and the interval between kidding is less. These goats are yet to be characterization. Therefore, the present study was undertaken to characterize the Black Bengal Type goats genetically through microsatellite markers.

MATERIALS AND METHODS

Blood collection: Blood samples were collected from 50 genetically unrelated animals having different parentage from different flocks existed in villages of Mayurbhanj and Baleshwar districts of Maharashtra state (Figure 1). The blood was drawn from the jugular vein in the 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 Sambrook's standard phenol / chloroform / isoamyl alcohol extraction protocol with slight modifications (1989). The extracted DNA was checked for its quantity and quality. The DNA samples were dissolved in TE buffer (pH8.0) to make uniform concentration of 50 ng/ml.

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), Sauthern Italian goat (Iamartino *et al.,* 2005), Kutchi goat (Dixit *et al.,* 2008) and Kannaiadu 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 mM 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

Table1. Description of Microsatellite markers used

Locus	Primer sequence	Type of Repeat	Size range	Dye	*Chr. No.	**Acc. No.	PCR anealing temp&time
ILSTS008	F-gaatcatggattttctgggg R-tagcagtgatgaggttggc	$(CA)_{12}$	167-195	FAM	14	L23483	57°C/30Sec.
ILSTS059	F- gctgaacaatgtgatatgttcagg R- gggacaatactgtcttagatgctgc	$(CA)_4$ $(GT)_2$	105-135	FAM	13	L37266	57°C/30Sec.
ETH225	F- gatcaccttgccactatttcct R- acatgacagccagctgcttact	$(CA)_{18}$	146-160	VIC	14	Z14043	57°C/30Sec.
ILSTS044	F-agtcacccaaaagtaactgg R-acatgttgtattccaagtgc	$(GT)_{20}$	145-177	NED	Ann	L37259	55°C/30Sec.
ILSTS002	F-tctatacacatgtgctgtgc R-cttaggggtgaagtgacacg	$(CA)_{17}$	113-135	VIC	Ann	23479	60°C /45 Sec.
Oar FCB304	F-ccctaggagctttcaataaagaatcgg R-cgctgctgtcaactgggtcaggg	$(CT)_{11}(CA)_{15}$	119-169	FAM	Ann	L01535	60°C/45 Sec.
Oar FCB48	Fgagttagtacaaggatgacagaggcac R-gactctagaggatcgc	$(GT)_{10}$	149-181	VIC	17	M82875	60°C/45 Sec.
Oar HH64	F-cgttccctcactaggaaagttatatatgc R-actctattgtaagaatttgaatgaatgagagc	_	120-138	PET	4	212***	57°C/30Sec.
Oar JMP29	F-gtatacacgtggacaccgctttgtac R-gaagtggcaagattcagaggggaag	$(CA)_{21}$	120-140	NED	Ann	U30893	60°C/45 Sec.
ILSTS005	F-ggaagcaatgaaatctatagcc R-tgttctgtgagtttgtaagc	$(nn)_{39}$	174-190	VIC	10	L23481	55°C/30Sec.
ILSTS019	F-aagggacctcatgtagaagc R-acttttggaccctgtagtgc	$(TG)_{10}$	142-162	FAM	Ann	L23492	57°C/30Sec.
OMHC1	F-atctggtgggctacagtccatg R-gcaatgctttctaaattctgaggaa	_	179-209	NED	Ann	228***	60°C/45 Sec.
ILSTS087	F-agcagacatgatgactcagc R-ctgcctcttttcttgagagc	$(CA)_{14}$	142-164	NED	Ann	L37279	55°C/30Sec.
ILSTS30	F-ctgcagttctgcatatgtgg R-cttagacaacaggggtttgg	$(CA)_{13}$	159-179	FAM	2	L37212	55°C/30Sec.
ILSTS34	F-aagggtctaagtccacggc R-acctggtttagcagagagc	$(GT)_{29}$	153-185	VIC	5	L37254	57°C/30Sec.
ILSTS033	F-tattagagtggctcagtgcc R-atgcagacagttttagagg	$(CA)_{12}$	151-187	PET	12	L37213	55°C/30Sec.
ILSTS049	F-cattttcttgtctctcccc R-gctgaatcttgtcaaacagg	$(CA)_{26}$	160-184	NED	11	L37261	55°C/30Sec.
ILSTS065	F-gctgcaaagagttgaacacc R-aactattacaggaggctccc	$(CA)_{22}$	105-135	PET	24	L37269	55°C/30Sec.
ILSTS058	F-gccttactaccatttccagc R-catcctgactttggctgtgg	$(GT)_{15}$	136-188	PET	17	L37225	57°C/30Sec.
ILSTS029	F-tgttttgatggaacacagcc R-tggatttagaccagggttgg	$(CA)_{19}$	149-191	PET	3	L37252	55°C/30Sec.
RM088	F-gatcctcttctgggaaaaagagac R-cctgttgaagtgaaccttcagaa	$(CA)_{14}$	109-147	FAM	4	U10392	60°C/45 Sec.
ILSTS022	F-agtctgaaggcctgagaacc R-cttacagtccttggggttgc	$(GT)_{21}$	186-202	PET	Ann	L37208	60°C/45 Sec.
OarAE129	F-aatcccagtgtgtgaaagactaatccag R-gtagatcaagatatatttttcaacac	$(CA)_{14}$	130-175	FAM	7	L11051	57°C/30Sec.
ILSTS082	F-ttcgttcctcatagtgctgg R-agaggattacaccaatcacc	$(GT)_{17}$	100-136	PET	2	L37236	57°C/30Sec.
RM4	F-cagcaaaatatcagcaaacct R-ccacctgggaaggccttta	$(CA)_{13}$	105-127	NED	15	U32910	57°C/30Sec.

*Chromosome number, **Accession number, (http//www.thearkdb.org)

Table 2. Observed and effective number of allele (Na, Ne)

Locus	Na	Ne	Ho	He	PIC	F_{IS}
ILSTS30	11.00	4.81	0.51	o.80	0.85	0.36
ILSTS065	4.00	1.36	0.20	0.26	0.55	0.25
ILSTS005	10.00	2.26	0.46	0.56	0.72	0.17
ILSTS087	11.00	6.55	0.70	0.85	0.88	0.18
ILSTS033	13.00	4.67	0.71	0.79	0.87	0.10
OarAE129	15.00	7.27	0.77	0.87	0.91	0.10
ETH225	4.00	2.32	0.31	0.57	0.74	0.46
ILSTS58	15.00	5.37	0.68	0.82	0.86	0.16
OarHH64	7.00	5.05	0.62	0.81	0.87	0.23
ILSTS059	6.00	2.85	0.45	0.65	0.78	0.30
ILSTS34	14.00	4.53	0.58	0.78	0.89	0.26
ILSTS082	16.00	6.51	0.76	0.85	0.91	0.11
RM4	6.00	2.22	0.52	0.55	0.68	0.06
ILSTS08	6.00	1.15	0.28	0.34	0.60	0.16
ILSTS19	9.00	4.59	0.44	0.79	0.87	0.44
OarFCB304	13.00	3.47	0.51	0.71	0.83	0.29
OarFCB48	10.00	6.20	0.78	0.84	0.89	0.08
ILSTS022	5.00	2.19	0.45	0.55	0.70	0.17
OarJMP29	13.00	4.82	0.52	0.80	0.84	0.35
OMHC1	10.00	6.21	0.91	0.84	0.88	-0.07
ILSTS044	11.00	4.76	0.85	0.79	0.86	-0.06
ILSTS049	12.00	3.66	0.82	0.73	0.81	-0.12
ILSTS029	8.00	1.83	0.30	0.45	0.68	0.34
RM088	9.00	4.48	0.78	0.78	0.85	0.06
ILSTS002	17.00	7.68	0.93	0.87	0.91	-0.06
Mean	10.20	4.29	0.59	0.71	0.81	0.17

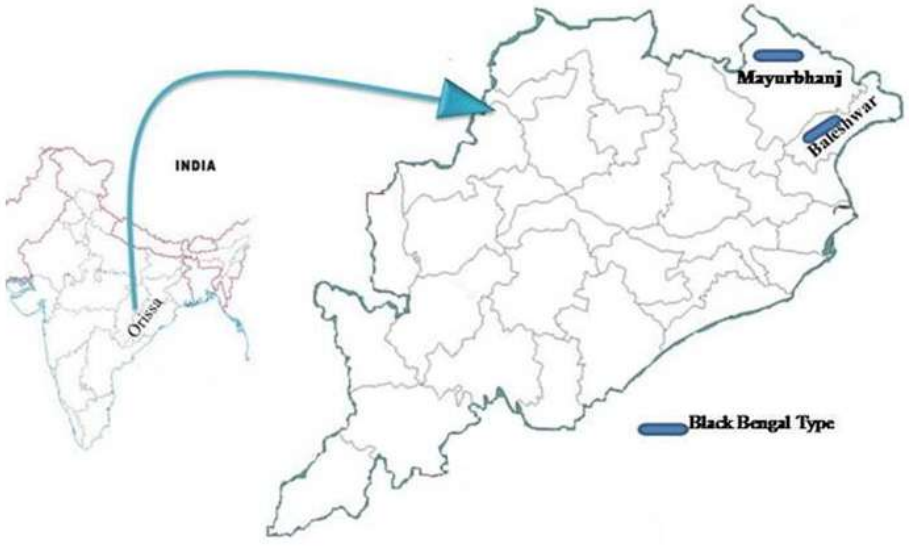

Figure 1. Breeding tract of Black Bengal Type Goat population

temperatures up to 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 3.0).

Statistical analysis

Microsatellite data on genotyping was analyzed to estimate observed number of alleles, expected number of alleles, observed heterozygosity and expected heterozygosity using POPGEN 3.2 diversity analysis software. The test of the departure from Hardy-Weinberg proportions was performed using exact probability tests provided in GENEPOP. Within population inbreeding estimates (F_{IS}) at each microsatellite loci were estimated using F-STAT (version 2.9.3). Neighbour-Joining diagrams were constructed on genetic distances within individuals using the Nei's distance (Saitou and Nei, 1987) by the PHYLIP package.

Finally the bottleneck hypothesis was investigated using BOTTLENECK 1.2.01 (Cornuet and Luikart, 1996). The BOTTLENECK tests for the departure from mutation drift equilibrium based on heterozygosity (not heterozygote), excess or deficiency. This does not require information on historical population sizes or level of genetic variations. It requires only measurement of allele frequencies from 5 to 20 polymorphic loci in a sample of approximately 20–30 individuals. The bottleneck compares heterozygosity expected (*H*E) at Hardy–Weinberg equilibrium to the heterozygosity expected (*H*eq) at mutation drift equilibrium in the same sample, that has the same size and the same numbe of alleles. All the three models of mutation were used to calculate *H*eq: the strict one stepwise mutation model (Kimura and Ohta, 1973), the infinite allele model (Kimura and Crow, 1964) and two-phase model (Di Rienzo *et al.*, 1994).

RESULTS

Various measures of genetic variability in Black Bengal Type goat population are presented in table 2. The observed number of alleles across studied loci ranged from 4.00 (ILSTS065 & ETH225) to 17.00 (ILSTS002) with an overall mean of 10.20 and effective number of allele ranged from 1.15 (ILSTS008) to 7.27 (OarAE129) with an overall mean of 4.29. Observed number of allele was more than effective number of alleles. The average observed heterozygosity (0.59) was lower than expected heterozygosity (0.71). The PIC (Polymorphic Information Content) varied from 0.55 (ILSTS065) to 0.91 (ILSTS002, ILSTS082 and OarAE129) with an overall mean of 0.81. Out of twenty five loci four loci showed negative F_{IS} value and positive F_{IS} value ranged from 0.06 (ILSIS044 and RM4) to 0.46 (ETH225) with an overall mean of 0.17.

DISCUSSION

The Black Bengal Type goat had substantial genetic variation based on its gene diversity and average number of alleles per locus. The genetic analysis of 25 microsatellite loci in Black Bengal Type Goat population revealed a reasonable level of diversity. The mean observed number of allele was estimated in Black Bengal Type goat population (table 2) were higher than that of Jakhrana goat breed (Verma *et al.,* 2007) and Marwari goat breed (Kumar *et al.,* 2005). However higher values were reported by Mishra *et al.,* 2010, Verma *et al.,* 2007 and Kumar *et al.,* 2009.

The average genetic variation (0.59) in this population lower than the genetic variation found in Changthangi (0.60, Mishra *et al.,* 2010), Mehsana, Beetal (0.64, 0.60 Dixit *et al.,* 2011) and Zalawadi (0.60, Fatima *et al.,* 2008) goat breeds of India and Higher than Barbari, Gaddi and Surti studied by Dixit *et al.,* 2011, Marwari studied by Kumar *et al.,* 2005 and Jakhrana studied by and Verma *et al.,* 2007.

The PIC value showed that all the markers were highly polymorphic with an overall mean of 0.81 and this suggested the suitability of these markers for genetic diversity studies in *Capra hircus*. All of 25

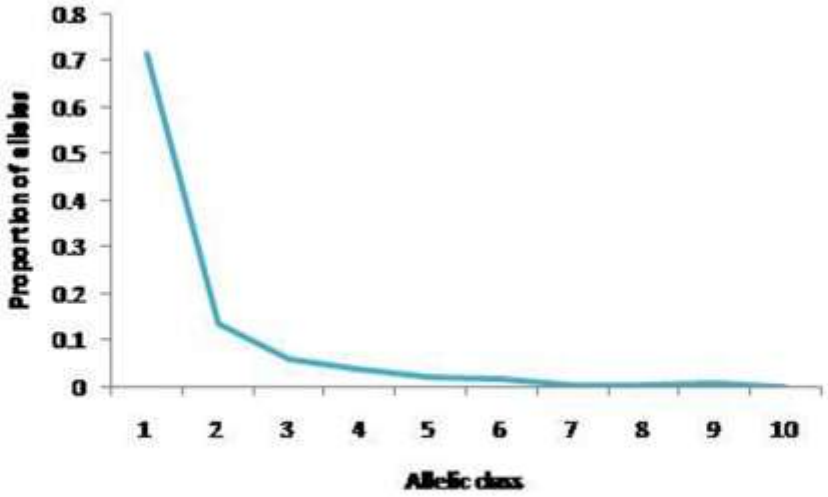

Figure 2. L-shaped curve of Black Benga Type goat

Table 3. Test for null hypothesis for mutation drift equilibrium under three mutation models (IAM, TPM and SMM) using sign rank, standardized differences and Wilcoxon tests

	IAM		**TPM**		**SMM**	
	Expected	Observed	Expected	Observed	Expected	Observed
Sign rank test	14.98	15	14.85	8	14.65	3
Standardized differences test (T2 values)	0.201		-5.025*		-14.73*	
Wilcoxon Test (Probability of heterozygote excess)	0.316		0.997		1.00	

* $P < 0.05$ (rejection of null hypothesis of mutation drift equilibrium).

microsatellite loci had PIC values greater than 0.5 indicating their high degree of informativeness for population genetic studies (Botstein *et al.,* 1980). Some of the loci exhibited positive values which ranged from -0.06 to 0.46 However, the overall mean F_{IS} value observed across all the loci in the present study was 0.17 indicating an excess of heterozygotes in the population. The Black Bengal Type goat population was evaluated for mutation drift equilibrium (table 3 and figure 2) to detect the occurrence of genetic bottleneck in the recent past.

The bottleneck analysis supported for the non-existence of any bottleneck in Black Bengal Type goat population in recent past. In Black Bengal Type goat population, sign rank test under IAM mutation model, expected number of loci with heterozygotic excess was 14.98 while the observed number of loci with heterozygosity excess was 15 (Table 2). With TPM and SMM, the expected and observed loci with heterozygosity excess were 14.85 and 8. The probability values for sign rank test under IAM ($p = 0.583$), not significantly different. Thus the outcome for IAM and TPM supported the absence of any bottleneck in

this goat population, as none of the estimated *P* values were significant (*P* > 0.05).

However, under TPM and SMM 14.65 and 3, , the null hypothesis of mutation drift equilibrium was rejected as only 3 and 10 loci with heterozygote excess was observed while 17 and 22 loci showed significant heterozygote deficiency ($p < 0.05$). The standardized difference test provided the T2 statistics equal to 0.201, -5.025 and –14.737 for IAM, TPM and SMM, respectively. The probability values were significant for IAM ($p = 0.420$) and non-significant for SMM ($p = 0.0000$) and TPM ($p = 0.000$). Thus, null hypothesis was accepted under SMM and TPM and rejected under IAM model. The probability values with Wilcoxon rank test under 3 models IAM ($p = 0.316$), TPM ($p = 0.997$) and SMM ($p = 1.000$) indicated acceptance of null hypothesis under SMM and TPM while rejection under IAM model in Black Bengal Type goats.

Thus all the three tests (sign rank, standardized differences and Wilcoxon tests) indicated the acceptance of mutation drift equilibrium (*P* > 0.05) in Black Bengal Type Goat population under one or the other mutation model. Another powerful test of qualitative graphical method based on the allele frequency spectra detected no shift in the frequency distribution of alleles and a normal L-shaped curve was observed (figure 2).

CONCLUSION

The result of this study suggests that there is substantial genetic variation in the Black Bengal Type goat population. All the studied loci were highly polymorphic.

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