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Molecular Characterization of Assam Hill Goat

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ABSTRACT

A total of 23 polymorphic microsatellite markers were used to evaluate genetic diversity and population structure in Assam Hill Goat (AHG). All the loci studied were polymorphic in nature. The number of observed alleles (N_a) detected ranged from 2 to 10 with an overall mean of 4.9±2.220. A total of 114 alleles were observed across all the loci. The effective number of alleles (N_e) ranged from 1.035 to 7.127 with a mean of 2.68±1.590. The allele frequency ranged from 0.013 to 0.982. The overall mean observed (H_O) and expected (H_e) heterozygosity were 0.43 and 0.48 respectively and this population was in Hardy-Weinberg equilibrium at most of the loci studied. The overall mean of within-population inbreeding estimate (F_{IS}) was 0.085. 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.

Keywords: Assam Hill Goat, Heterozygosity, PIC, Microsatellites

1. INTRODUCTION

Genetic diversity is necessary for the long-term survival of the species and populations because it provides the raw material for adoption and evolution, especially when environmental conditions have changed (Rajora and Mosseler, 2001). The genetic markers are playing important role in measuring genetic diversity. These have been used to find out evolutionary relationship within and between species, genera or higher taxonomic categories (Paterson *et al*., 1991). Goat is one of the significant food sources, because it can convert feed dry matter into milk as efficiently as other ruminants. The goat population in North East India was approximately 3.51% of the total India population (Feroze *et al*., 2010).

The Assam Hill goat (AHG) is an important meat type animal with high prolificacy from the North Eastern region of India. Most common colours of this goat is white, however, brown, black and mixed colour are not uncommon (**Fig. 1**). They are distributed in the hilly terrain of North Cachar hill, Karbi Anglong districts of Assam and also in the adjoining hilly tract of Meghalaya state.

The Network Project on Animal Genetic Resources-Core laboratory, Department of Animal Genetics and Breeding, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India has undertaken molecular characterization of livestock through microsatellite markers. Onto date no studies are conducted in AHG population from North East India using microsatellites.

In view of this the present study has been planned to investigate genetic variation and population structure within AHG population using 23 polymorphic microsatellite markers.

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Fig. 1. Figure showing a typical Assam Hill goat (Doe)

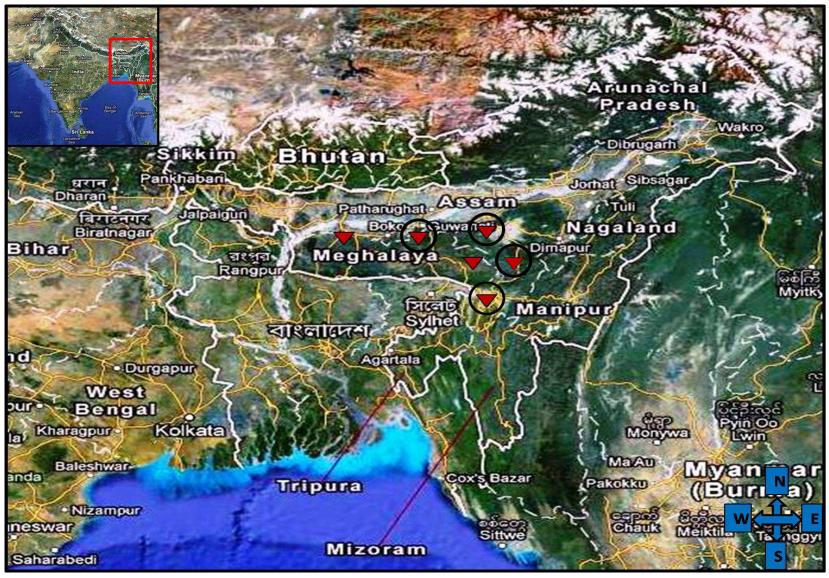

Fig. 2. Figure showing the breeding tract of Assam Hill Goat (AHG) and major sampling sites (Kindness to Google earth map)

2. MATERIALS AND METHODS

2.1. Sample Collection and DNA Isolation

A total of 40 blood samples of AHG were collected randomly from genetically unrelated individuals from their native breeding tract (**Fig. 2**).

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.

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^{-1}) for the purpose of PCR amplification.

Table 1. Details of microsatellite markers

Locus	Gene bank Accession Number	**Ch. No	*Repeat motif	Primer sequences (5' → 3')	Dye	T_a (°C)	Size range (bp) *in source reference	Size range (bp) in present study
ILSTS008	L23483	14	$(CA)_{12}$	F-GAATCATGGATTTTCTGGGG R-TAGCAGTGAGTGAGGTTGGC	FAM	58	167-195	168-178
ETH225	Z14043	14	$(CA)_{18}$	F-GATCACCTTGCCACTATTTCCT R-ACATGACAGCCAAGCTGCTACT	VIC	58	146-160	145-147
OarHH64	212[a]	4	Ann	F-CGTTCCCTCACTATGGAAAGTTATATATGC R-CACTCTATTGTAAGAATTTGAATGAGAGC	PET	60	120-138	121-131
ILSTS044	L37259	Ann	$(GT)_{20}$	F-AGTCACCCAAAAGTAACTGG R-ACATGTTGTATTCCAAGTGC	NED	54	145-177	153-171
ILSTS059	L37266	13	$(CA)_4(GT)_2$	F-GCTGAACAATGTGATAGTTCAGG R-GGGACAATACTGTCTTAGATGCTGC	FAM	54	105-135	106-120
OarAE129	L11051	5	Ann	F-AATCCAGTGTGTGAAAGACTAATCCAG RGTAGATCAAGATATAGAATATTTTTCAACACC	FAM	54	130-178	149-167
ILSTS002	L23479	Ann	$(CA)_{17}$	F-TCTATACACATGTGCTGTGC R-CTTAGGGGTGTATTCCAAGTGC	VIC	50	113-135	114-124
ILSTS065	L37269	24	$(CA)_{22}$	F-GCTGCAAAGAGTTGAACACC R-AACTATTACAGGAGGCTCCC	PET	60	105-135	116-118
OarJMP29	U30893	Ann	$(CA)_{21}$	F-GTATACACGTGGACACCGCTTTGTAC R-GAAGTGGCAAGATTCAGAGGGGAAG	NED	60	120-140	114-116
ILSTS019	L23492	Ann	$(TG)_{10}$	F-AAGGGACCTCATGTAGAAGC R-ACTTTTGGACCCTGTAGTGC	FAM	60	142-162	146-158
ILSTS033	L37213	12	$(CA)_{12}$	F-TATTAGAGTGGCTCAGTGCC R-ATGCAGACAGTTTTAGAGGG	PET	60	151-187	156-178
ILSTS005	L23481	10	$(nn)_{39}$	F-GGAAGCAATGAAATCTATAGCC R-TGTTCTGTGAGTTTGTAAGC	VIC	58	174-190	175-187
ILSTS058	Ann	Ann	Ann	F: GCCTTACTACCATTTCCAGC R: CATCCTGACTTTGGCTGTGG	PET	54	136-188	136-188
ILSTS087	L37279	Ann	$(CA)_{14}$	F-AGCAGACATGATGACTCAGC R-CTGCCTCTTTTCTTGAGAGC	NED	54	142-164	139-159
ILSTS030	L37212	2	$(CA)_{13}$	F-CTGCAGTTCTGCATATGTGG R-CTTAGACAACAGGGGTTTGG	FAM	60	159-179	161-173
ILSTS034	L37254	5	$(GT)_{29}$	F-AAGGGTCTAATGCCACTGGC R-GACCTGGTTTAGCAGAGAGC	VIC	58	153-185	157-161
ILSTS029	L37252	3	$(CA)_{19}$	F-TGTTTGATGGAACACAGCC R-TGGATTTAGACCAGGGTTGG	PET	60	148-191	153-177
ILSTS049	L37261	11	$(CA)_{26}$	F-CAATTTTCTTGTCTCTCCCC R-GCTGAATCTTGTCAAACAGG	NED	58	160-184	161-171
OarVH72	L12548	7	Ann	F-GGCCTCTCAAGGGGCAAGAGCAGG R-CTCTAGAGGATCTGGAATGCAAAGCTC	VIC	54	108-144	119-121
OarFCB48	M82875	17	$(CT)_{10}$	F-GAGTTAGTACAAGGATGACAAGAGGCAC R-GACTCTAGAGGATCGCAAAGAACCAG	VIC	54	149-181	146-164
OarHH35	L12554	7	Ann	F-AATTGCATTCAGTATCTTTAAACATCTGGC R-ATGAAAATATAAAGAGAATGAACCACACACGG	PET	54	92-112	96-98
OarFCB304	L01535	Ann	$(CT)_{11}(CT)_{15}$	F-CCCTAGGAGCTTTCAATAAAGAATCGG R-CGCTGCTGTCAACTGGGTCAGGG	FAM	54	119-179	124-172
OMHC1	228[a]	Ann	Ann	F-ATCTGGTGGGCTACAGTCCATG R-GCAATGCTTTCTAAATTCTGAGGAA	NED	58	179-209	184-200

**, Chromosome number; [a], Gene bank accession number of Arkdb data base (http://www.thearkdb.org); *, Kumar *et al*., 2009; T_a, Annealing temperature

2.2. Microsatellite Analysis

All the 23 microsatellite markers were selected from the list recommended by International Society for Animal Genetics (ISAG) and FAO's (DAD-IS) for Caprine, 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. Details of markers used in the present study are shown in **Table 1**.

Multiplex PCR has been used for multicolor fluorescence genotyping. Based on the guide lines of (Henegariu *et al*., 1997) 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 to 60°C for 30 sec) and extension (72°C for 45 sec). PCR conducted on an Applied Biosystems (Model #: 9902) Veriti™ 96-well thermal cycler.

After conformation of magnified PCR products on 2% agarose gel, genotyping was carried out on automated DNA Sequencer (ABI PRISM 3130 XL). The resulting data were analyzed using standard software Gene Mapper™ version 4.0 (Applied Biosystems Inc., California, USA) to generate genotype calls for each locus by using GS 500 (-250) LIZ as size standard.

2.3. Information 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 of 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.

3. RESULTS

The various parameters of genetic differentiation in AHG, 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 2**.

All the 23 loci investigated were polymorphic in nature. The number of observed alleles (N_a) detected ranged from 2 (ETH225, ILSTS065, OarJMP29 and ILSTS34) to 10 (OarFCB304), with an overall mean of 4.90±2.220 and a total of 114 alleles were observed at these loci in the population. However, the effective number of alleles (N_e) ranged from 1.035 to 7.127 with a mean of 2.68±1.590. Overall allele frequency ranged from 0.013 (at locus ILSTS33) to 0.982 (at locus ETH225). The PIC value ranged from 0.033 (ETH225) to 0.843 (OMHC1) with a mean of 0.44±0.263. The overall means for observed (H_O) and expected (H_e) heterozygosities were 0.43±0.285 and 0.48±0.281, respectively which ranged from 0.034 (ETH225) to 0.862 (ILSTS002) and 0.033 (ETH225) to 0.859 (OMHC1) respectively. The chi-square (χ^2) test for HWE revealed that 10 out of 23 loci deviated from equilibrium. Shannon's information index (I) (Lewontin, 1995), which measures the level of diversity, was sufficiently high with a mean of 1.00±0.606. The within population inbreeding estimates (F_{IS}) observed at 10 loci were positive which ranged from 0.012 (OarHH64) to 0.771 (OARE129). Only 13 loci revealed negative F_{IS} values indicating the absence of inbreeding in these loci. The mean F_{IS} value observed was 0.085. Though positive F_{IS} values were observed at 10 loci, only 8.5% of inbreeding was recorded in AHG.

Three mutation models namely, Infinite Allele Model (IAM), Two Phase Model (TPM), Stepwise Mutation Model (SMM) were used for Bottleneck analysis (**Table 3**). In AHG population, under Sign test, the expected number of loci with heterozygosity excess were 8.93 (TPM) and 9.07 (SMM) which are respectively higher than the observed number of loci 6 (TPM) and 4 (SMM) with heterozygosity excess. The expected number of loci (8.67) with heterozygosity excess was not significantly ($p>0.05$) higher than the observed number of loci (9) with heterozygosity excess under IAM. Standard difference test (T2 statistics) in this population provided the significant gene diversity deficit under the three mutation models IAM (-0.794), TPM (-2.751) and SMM (-6.447) respectively. Under Wilcoxon rank test, probability values of 0.701 (IAM), 0.974 (TPM) and 0.999 (SMM) were non-significant. The mode shift analysis revealed L-shaped curve indicating no mode-shift in the frequency distribution of alleles. The graphical representation of mode-shift has been shown in **Fig. 3**.

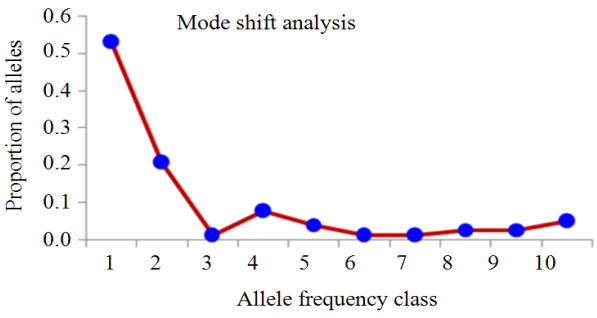

Fig. 3. Figure showing the graphical representation of allele proportions and their contribution in Assam Hill Goat (AHG)

Table 2. Microsatellite analysis in Assam Hill Goat (AHG)

		Parameters							
Panel	Locus	N_a	N_e	PIC	H_o	H_e	F_{IS}	HWE	I
Panel 1	ILSTS008	4	1.5898	0.3383	0.3793	0.3710	-0.0224	1.185^{NS}	0.7092
	ETH225	2	1.0351	0.0333	0.0345	0.0339	-0.0175	0^{NS}	0.0871
	OarHH64	6	3.3176	0.6472	0.6897	0.6986	0.0128	19.73^{NS}	1.3707
	ILSTS044	4	1.1110	0.0983	0.1034	0.0999	-0.0357	0.056^{NS}	0.2604
Panel 2	ILSTS059	5	2.2517	0.4860	0.4483	0.5559	0.1936	25.12**	1.0155
	OarE129	6	4.4122	0.5803	0.1765	0.7734	0.7718	79.25**	1.6226
	ILSTS002	6	3.7130	0.6872	0.8621	0.7307	-0.1798	79.69**	1.4740
	ILSTS065	2	1.3554	0.2278	0.3103	0.2622	-0.1837	0.85^{NS}	0.4316
	OarJMP29	2	1.0713	0.0644	0.0690	0.0666	-0.0357	0.018^{NS}	0.1500
Panel 3	ILSTS 033	8	1.9300	0.4652	0.5278	0.4819	-0.0953	10.21^{NS}	1.1178
Panel 4	ILSTS019	6	3.0036	0.6180	0.5172	0.6671	0.2246	27.32*	1.3451
	ILSTS005	3	1.2752	0.1988	0.2414	0.2158	-0.1185	0.46^{NS}	0.4178
	ILSTS058	6	3.0862	0.6179	0.4483	0.6760	0.3369	29.27*	1.3084
	ILSTS087	7	3.5262	0.6790	0.8276	0.7164	-0.1552	80.16**	1.4948
Panel 5	ILSTS030	7	5.1429	0.7792	0.7778	0.8056	0.0345	21.93^{NS}	1.7578
	ILSTS034	2	1.0571	0.0526	0.0556	0.0540	-0.0286	0^{NS}	0.1269
	ILSTS029	4	2.0313	0.4640	0.6111	0.5077	-0.2036	1.58^{NS}	0.9427
	ILSTS049	5	3.5801	0.6728	0.8333	0.7207	-0.1563	14.49^{NS}	1.4003
Panel 6	OarVH72	4	1.2675	0.2040	0.1429	0.2110	0.3230	69.06**	0.4791
	HH35	2	1.2462	0.1780	0.0556	0.1975	0.7188	21.22**	0.3488
	OarFCB48	5	3.2580	0.6330	0.4571	0.6931	0.3404	19.42*	1.2730
	OarFCB304	10	4.3272	0.7503	0.8056	0.7689	-0.0477	35.7^{NS}	1.8543
Panel 7	OMHC1	8	7.1271	0.8434	0.6207	0.8597	0.2780	65.33**	2.0129
Mean overall loci		4.95 ± 2.225	2.68 ± 1.590	0.44± 0.263	0.43 ± 0.285	0.48 ± 0.281	0.0850		1 ± 0.606

* Significant ($p \leq 0.05$); **Highly significant ($p \leq 0.01$); ^{NS} Not significant ($p \geq 0.05$); Na, Number of alleles; Ne, Effective number of alleles; PIC, Polymorphic information content; Ho, Observed Heterozygosity; He, Expected Heterozygosity; F_{IS}, Deficit or excess of Heterozygotes; HWE, Hardy-Weinberg equilibrium; I, Shannon's Information Index

Table 3. Bottleneck analysis in Assam Hill Goat (AHG)

	Sign rank test-Number of loci with heterozygosity excess			Standardized differences test-T2 values (probability)	Wilcoxon test-Probability of heterozygosity excess
Model	Expected	Observed	Probability		
IAM	8.67	9	0.53699	-0.794 (0.21351)	0.70171
TPM	8.93	6	0.10894	-2.751 (0.00297)	0.97467
SMM	9.07	4	0.01015	-6.447 (0.00000)	0.99958

IAM-Infinite allele model; TPM-Two phase model; SMM-Stepwise mutation model

4. DISCUSSION

The present study revealed that the most of the studied loci were highly informative, indicating high polymorphism. Thus these markers strongly signified genetic diversity investigations of AHG. 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 mean number of alleles observed (4.90) in the present investigation was less than the mean number of alleles reported in Ganjam (6.29) goat (Sharma *et al*., 2009) and Gohilwari (10.12) goat (Kumar *et al*., 2009).

The PIC value in the present investigation ranged from 0.033 to 0.843 which is in close agreement with the reports of (Sharma *et al*., 2009) in Ganjam goat and (Kumar *et al*., 2009) in Gohilwari goat. The low observed heterozygosity 0.034 (ETH225) was observed in the present study may be due to the presence of more homozygote individual in the samples analyzed. 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 revealed that 13 microsatellite loci in the AHG population are in equilibrium. These results established that the samples were drawn from the large random mating population (Karthickeyan *et al*., 2008).

The overall mean F_{IS} (0.085) observed in the present study indicated a 8.5% shortfall of heterozygosity in AHG population which is not significant as compared to heterozygote deficiency reported in Ganjam goat 21.7% (Sharma *et al*., 2009); Gohilwari goat 26.4% (Kumar *et al*., 2009); Kutchi goat 26%, Mehsana goat 14% and Sirohi goat 36% (Dixit *et al*., 2009). The present findings of F_{IS} value supports random mating in the studied population. The main reasons for the random mating are wide range of native breeding tract and sufficient availability of breeding bucks in the population.

Bottleneck analysis revealed that the breed is non-bottelnecked where the mode-shift for the frequency distribution of alleles had a normal L-shaped curve stating that there was no recent and/or sudden reduction in the population.

5. CONCLUSION

The PIC values observed in the present study is indicative of the fact that the markers used are highly informative for characterization of AHG diversity. The significant level of variability in this population reflects that the AHG population contains a valuable genetic diversity. The population has not undergone any reduction at least in the recent past. Hence, this population could provide a valuable source of genetic material that may be used for meeting the demands of future breeding programmes.

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