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Molecular Characterization of Nagaland Long Hair Goat

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Abstract

Nagaland Long hair goat comprises a small population of non-descript indigenous animals. Twenty microsatellite markers on Nagaland Long Hair goat (NLHG) were tested to understand the genetic diversity within the population. All the loci studied were polymorphic in nature. The number of observed alleles (N_a) detected ranged from 2 to 8 with an overall mean of 5.0±1.716. A total of 100 alleles were observed across all loci. The effective number of alleles (N_e) ranged from 1.2195 to 5.4340 with a mean of 2.93±1.330. The allele frequency ranged from 0.0312 to 0.9000. Overall mean observed (H_O) and expected (H_e) heterozygosity were 0.56 and 0.48 this population was in Hardy-Weinberg equilibrium at most of the loci studied. The overall mean within-population inbreeding estimate (F_{IS}) was 0.058. 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: Nagaland Long hair goat, Heterozygosity, PIC and Microsatellites

INTRODUCTION

Nagaland Long Hair goat (NLHG) locally known as Apu-Asu-Ne constitutes a small group of non-descript population, and is found only in the hilly tract of Zunheboto and Tuensang districts of Nagaland, India. These goats are reared mainly for meat, coarse fiber and skin. Moreover long coarse fibres obtained from this goat are used by the local tribes for making valuable items of traditional use. The coat colour of these goats is mostly white with long silky hair. The coat colour, however, is characterized by a specific black patch on the head and neck region. Black patches are, however, also seen on the legs below the knee joints in some animals (Fig. 1). 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. No study has so far been reported on this valuable germplasm. In view of this the present study has been planned to investigate genetic variation and population structure within NLHG population using 20 polymorphic microsatellite markers.

MATERIALS AND METHODS

Samples and DNA isolation

A total of 16 blood samples of NLHG were collected randomly from genetically unrelated individuals from Zunheboto and Tuensang districts of Nagaland (Fig. 2). Blood samples were collected aseptically into BD vacutainers (4 ml) containing K2 EDTA (7.2 mg) and samples were transported to the laboratory on ice and were stored at 4°C until use.

Genomic DNA was isolated from the blood samples using standard phenol-chloroform method [11] with few modifications. All Isolated samples were confirmed 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.

Fig 1. Figure showing a typical Nagaland Long Hair Buck

Magnification of microsatellite regions

A total of 20 microsatellite markers were selected from the list

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recommended by International Society for Animal Genetics (ISAG) and FAO's (DAD-IS) for Caprine [3]. Summary of microsatellite 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 [4] and [7] 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). 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 3130XL). 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.

Data analysis

POPGENE version 1.31 [12] was used to calculate the allele frequencies, effective number of alleles (N_e), test of Hardy-Weingberg equilibrium (HWE), observed (H_o) and expected (H_e) heterozygosity, F-statistics and Shanon's information index (I). Nei's formula [9] was used to calculate polymorphic information content (PIC). The BOTTLENECK version 1.2.03 [1] analysis was performed to know whether this goat population exhibits a significant number of loci with excess of heterozygosity.

Table 1. Review of markers in the present study

Locus	Gene bank Accession Number	**Ch. No	*Repeat motif	Primer Sequences (5' → 3')	Dye	Ta (°C)	Size range(bp)	
							*in source reference	in present study
ILSTS008	L23483	14	(CA)12	F-GAATCATGGATTTTCTGGGG R-TAGCAGTGAGTGAGGTTGGC	6-FAM	58	167-195	168-176
ETH225	Z14043	14	(CA)18	F-GATCACCTTGCCACTATTTCCT R-ACATGACAGCCAAGCTGCTACT	VIC	58	146-160	145-147
OarHH64	212a	4	Ann	F-CGTTCCCTCACTATGGAAAGTTATATATGC R-CACTCTATTGTAAGAATTTGAATGAGAGC	PET	60	120-138	117-129
ILSTS044	L37259	Ann	(GT)20	F-AGTCACCCAAAAGTAACTGG R-ACATGTTGTATTCCAAGTGC	NED	54	145-177	151-169
ILSTS059	L37266	13	(CA)4(GT)2	F-GCTGAACAATGTGATAGTTCAGG R-GGGACAATACTGTCTTAGATGCTGC	6-FAM	54	105-135	112-120
OarAE129	L11051	5	Ann	F-AATCCAGTGTGTGAAAGACTAATCCAG RGTAGATCAAGATATAGAATATTTTTCAACACC	6-FAM	54	130-178	149-167
ILSTS002	L23479	Ann	(CA)17	F-TCTATACACATGTGCTGTGC R-CTTAGGGGTGTATTCCAAGTGC	VIC	58	113-135	116-124
ILSTS065	L37269	24	(CA)22	F-GCTGCAAAGAGTTGAACACC R-AACTATTACAGGAGGCTCCC	PET	60	105-135	118-120
ILSTS033	L37213	12	(CA)12	F-TATTAGAGTGGCTCAGTGCC R-ATGCAGACAGTTTTAGAGGG	PET	60	151-187	166-174
ILSTS019	L23492	Ann	(TG)10	F-AAGGGACCTCATGTAGAAGC R-ACTTTTGGACCCTGTAGTGC	6-FAM	60	142-162	146-158
ILSTS005	L23481	10	(nn)39	F-GGAAGCAATGAAATCTATAGCC R-TGTTCTGTGAGTTTGTAAGC	VIC	58	174-190	117-183
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	139-153
ILSTS030	L37212	2	(CA)13	F-CTGCAGTTCTGCATATGTGG R-CTTAGACAACAGGGGTTTGG	6-FAM	60	159-179	161-173
ILSTS034	L37254	5	(GT)29	F-AAGGGTCTAATGCCACTGGC R-GACCTGGTTTAGCAGAGAGC	VIC	58	153-185	157-179
ILSTS029	L37252	3	(CA)19	F-TGTTTGATGGAACACAGCC R-TGGATTTAGACCAGGGTTGG	PET	60	148-191	163-171
ILSTS049	L37261	11	(CA)26	F-CAATTTTCTTGTCTCTCCCC R-GCTGAATCTTGTCAAACAGG	NED	58	160-184	153-183
OarFCB48	M82875	17	(CT)10	F-GAGTTAGTACAAGGATGACAAGAGGCAC R-GACTCTAGAGGATCGCAAAGAACCAG	VIC	54	149-181	146-164
OarFCB304	L01535	Ann	(CT)11(CT)15	F-CCCTAGGAGCTTTCAATAAAGAATCGG R-CGCTGCTGTCAACTGGGTCAGGG	6-FAM	54	191-169	130-170
OMHC1	228a	Ann	Ann	F-ATCTGGTGGGCTACAGTCCATG R-GCAATGCTTTCTAAATTCTGAGGAA	NED	58	179-209	184-194

Ta, Annealing temperature.

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

RESULTS

The various parameters of genetic differentiation in NLHG 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 20 loci investigated were polymorphic in nature. The number of observed alleles (N_a) detected ranged from 2 (ETH225 and ILSTS065) to 8 (OarFCB48), with an overall mean of 5.0±1.716 and a total of 100 alleles were observed at these loci in the population. However, the effective number of alleles (N_e) ranged from 1.2195 (ETH225) to 5.4340 (ILSTS058) with a mean of 2.93±1.334. Overall allele frequency ranged from 0.0312 (at locus ILSTS029) to 0.9 000 (at loci ETH225 and ILSTS044).

The PIC value ranged from 0.1630 (ETH225) to 0.7910 (ILSTS058) with a mean of 0.54±0.189. The overall means for observed (H_O) and expected (H_e) heterozygosities were 0.56 ±0.295 and 0.48 ±0.239, respectively which ranged from 0.0660 (ETH225) to 1.0000 (ILSTS30) and 0.1800 (ETH225) to 0.8160 (ILSTS058) respectively. The chi-square (χ^2) test for HWE revealed that 7 out of 20 loci deviated from equilibrium. Shannon's information index (I) [8], which measures the level of diversity, was sufficiently high with a mean of 1.16±0.443. The within population inbreeding estimate (F_{IS}) observed at 12 loci were positive which ranged from 0.0300 (ILSTS002) to 0.6290 (ETH225). Only 8 loci revealed negative F_{IS} values indicating the absence of inbreeding in these loci. The mean F_{IS} value observed was 0.058. Though positive F_{IS} values were observed at 12 loci, only 5.8 per cent of inbreeding was recorded in NLHG.

Three mutation models namely, infinite allele model (IAM), two phase model (TPM), stepwise mutation model (SMM) were used for Bottleneck analysis (Table 3). The results are indicative of the fact that the NLHG population is non-bottlenecked. The graphical representation of mode-shift has been shown in Fig 3.

Table 2. Microsatellite analysis in Nagaland Long Hair goat (NLHG)

Panel	Locus	Parameters							
		Na	Ne	PIC	Ho	He	F_{IS}	HWE	I
Panel 1	ILSTS008	4	1.6484	0.3709	0.3333	0.3933	0.1525	9.41NS	0.7950
	ETH225	2	1.2195	0.1638	0.0667	0.1800	0.6296	8.98**	0.3251
	OarHH64	6	2.9801	0.6053	0.9333	0.6644	-0.4047	12.29NS	1.3006
	ILSTS044	4	1.2295	0.1813	0.1333	0.1867	0.2857	29.03**	0.4349
Panel 2	ILSTS059	5	3.2667	0.6368	0.6429	0.6939	0.0735	4.8NS	1.3000
	OarAE129	5	3.1429	0.6262	0.4545	0.6818	0.3333	43.3**	1.3100
	ILSTS002	4	2.9697	0.6003	0.6429	0.6633	0.0308	5.8NS	1.1973
	ILSTS065	2	1.6897	0.3249	0.5714	0.4082	-0.4	1.91NS	0.5983
Panel 3	ILSTS033	5	1.9922	0.4698	0.5000	0.4980	-0.0039	20.77*	1.0210
Panel 4	ILSTS019	3	2.2960	0.5026	0.6875	0.5645	-0.218	3.99NS	0.9559
	ILSTS005	3	2.2756	0.4962	0.4375	0.5605	0.2195	4.25NS	0.9461
	ILSTS058	7	5.4340	0.7910	0.5833	0.8160	0.2851	13.38NS	1.7983
	ILSTS087	7	5.0909	0.7764	0.6429	0.8036	0.2	34.09*	1.7565
Panel 5	ILSTS030	6	4.2609	0.7311	1.0000	0.7653	-0.3067	40**	1.5899
	ILSTS034	6	2.0417	0.4828	0.3571	0.5102	0.3	18.84NS	1.0866
	ILSTS029	4	1.9542	0.4182	0.3750	0.4883	0.232	4.43NS	0.8498
	ILSTS049	6	1.9481	0.4635	0.6000	0.4867	-0.2329	2.4NS	1.0503
Panel 6	Oar FCB48	8	5.0196	0.7743	0.6875	0.8008	0.1415	62.55**	1.7949
	OarFCB304	7	4.5918	0.7526	0.8000	0.7822	-0.0227	26.14NS	1.7095
Panel 7	OMHC1	6	3.5804	0.6918	0.8125	0.7207	-0.1274	24.44NS	1.5302
Mean overall loci		5.0 ± 1.716	2.93 ± 1.334	0.54 ± 0.189	0.56 ± 0.295	0.48 ± 0.239	0.0583		1.16 ± 0.443

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}, Inbreeding Coefficient, HWE, Hardy-Weinberg equilibrium; I, Shannon's Information Index.
* Significant ($P \leq 0.05$); **Highly significant ($P \leq 0.01$); NS Not significant ($P \geq 0.05$).

Table 3. Bottleneck analysis in Nagaland Long Hair goat (NLHG)

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	10.88	12	0.39131	0.505 (0.30670)	0.24498
TPM	11.08	11	0.57237	-1.554 (0.06013)	0.67463
SMM	11.19	7	0.04342	-4.531 (0.00000)	0.94331

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

DISCUSSION

Most of the studied loci were highly informative, indicating high polymorphism. Thus these markers strongly signified genetic diversity investigations of NLHG. 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 (5.0) in the present study corroborates with the mean number of alleles reported in

Ganjam (6.29) goat [10]. However, mean number of alleles observed in the present investigation was less than the mean number reported for Gohilwari (10.12) goat [6]. This goat population showed that low effective number of alleles than the observed number of alleles due to very low frequency of most of the alleles at each locus and a very few alleles might have contributed the major part of the allelic frequency at each locus.

The PIC value in the present investigation ranged from 0.1630 to 0.7910 which is in close agreement with the reports of Ganjam goat [10] and Gohilwari goat [6]. Most of the loci possessed high PIC values (above 0.05) signifying that these markers are highly informative for characterization of NLHG. Thus these markers strongly signified genetic diversity investigations of NLHG. The low observed heterozygosity 0.0660 (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 [5]. The chi-square (χ^2) test revealed that 13 microsatellite loci in the NLHG population are in equilibrium. These results established that the samples were drawn from the large random mating population [5].

The overall mean F_{IS} (0.058) observed in the present study indicated a 5.8 per cent deficiency of heterozygosity in NLHG population which is not significant as compared to heterozygote shortfall observed in Ganjam goat 21.7 per cent [10]; Gohilwari goat 26.4 per cent [6]; Kutchi goat 26 per cent, Mehsana goat 14 per cent and Sirohi goat 36 per cent [2]. The present findings of F_{IS} value indicates that the population under reference is random bred.

The NLHG population is non-bottlenecked as evident from the quantitative graphical method [1]. The population has not undergone any recent and/or sudden reduction in the effective population size and remained at mutation-drift equilibrium. In the present study, no mode-shift was detected in the frequency distribution of alleles and a norm al L-shaped curve was observed.

Fig 2. Figure showing the breeding tract and sampling sites of NLHG (Politeness to Google earth map)

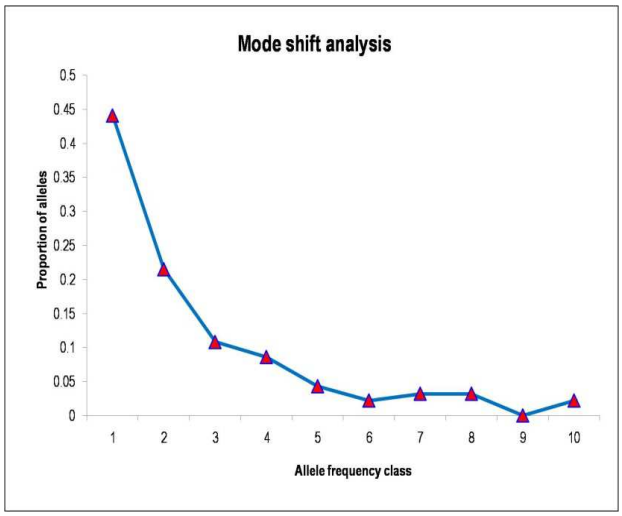

Fig 3. Figure showing the graphical representation of allele proportions and their participation in Nagaland Long Hair goat (NLHG)

CONCLUSION

The PIC values observed in the present study is indicative of the fact that the markers used are highly informative for characterization of NLHG diversity. The population has not undergone any reduction at least in the recent past. The significant level of genetic diversity observed in the present study indicates the population may be improved through suitable breeding strategies.

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