

## Microsatellite based genetic diversity estimation in Sikkim Singharey goat population

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India has 24 registered goat breeds where Gujarat alone contributes 5 breeds (Gohilwadi, Jhalawadi, Surti, Mehsani and Kuchchi) but the North Eastern Himalayan (NEH) region of India in spite of being rich in flora and fauna is yet to contribute a well studied and documented goat population. Therefore, an attempt was made to study the various aspects of Sikkim goats by conducting surveys in different parts of Sikkim. Singharey is the main goat population spread throughout Sikkim, and is distinguished phenotypically from other populations of Sikkim in respect that its goats have stripes on face extending from base of horn to the muzzle and upper jaw (Fig. 1). The information collected on phenotypic, biometric and management aspects of Singharey goats has already been documented (Verma *et al.* 2015). However, to know the genetic diversity, characterization of these goats was carried out using a well tested battery of microsatellites (Verma *et al.* 2015b, Mishra *et al.* 2013, 2014, Dixit *et al.* 2012) and results are presented in this paper.

Singharey goats are spread throughout the Sikkim but concentration of these goats is more in West district of Sikkim (Verma *et al.* 2015a). Animals (60) having different parentage were selected for blood sampling. Genomic DNA was extracted from blood samples using a standard phenol-chloroform extraction method. A battery of 23 microsatellite markers based on the guidelines of ISAG and FAO's DAD-IS program was utilized to generate allelic data. DNA amplification was carried out using fluorescent-labeled primers. The amplified products were analyzed with a DNA capillary sequencer ABI Prism® 310 Genetic Analyzer.

The data were analysed to calculate allele frequencies, observed number of alleles, effective number of alleles, observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity at each

locus. Polymorphism information content (PIC) value for each locus was calculated as per Bostein *et al.* (1980). Finally the bottleneck hypothesis was investigated using BOTTLENECK 1.2.01 (Cornuet and Luikart 1996).

Efforts were made to collect the blood from Singharey goats of different genetic background. This was authenticated by applying the breed assignment test (Verma *et al.* 2015a) which assigned about 90% Singharey goats to Singharey group. The estimates for allele number, heterozygosities, inbreeding, polymorphic contents are given in Table 1. A total of 182 alleles were observed across the studied loci. The number of observed alleles varied from 3 (RM4) to 12 (ILSTS033, ILSTS029, ILSTS30 and ILSTS082) with an overall mean of  $7.913 \pm 0.576$  which corroborates with the mean number of alleles reported in Ganjam goat (6.29) by Sharma *et al.* (2009), however, it is less than Gohilwari goat (10.12) observed by Kumar *et al.* (2009). The observed number of alleles across the loci was more than the effective number of alleles (1.449 to 6.969) as per expectations. All the studied micro-satellite loci exhibited polymorphism, which indicated that the genetic markers used were also suitable for genetic studies of Singharey goats.



Fig. 1. A Singharey buck.

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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. The average observed heterozygosity was less than expected heterozygosity except ILSTS022 and RM088, which indicated significant heterozygotic deficiency. The observed heterozygosity values ranged from 0.196 (ETH225) to 0.927 (RM088) with an overall mean of  $0.507 \pm 0.042$  whereas expected heterozygosity ranged from 0.310 (ILSTS34) to 0.857 (ILSTS30) with mean heterozygosity  $0.649 \pm 0.034$ . The average genetic variation (0.507) observed in the present study was lower than that of Berari (Mishra *et al.* 2013), Zalawadi, Gohilwadi and Surti (Fatima *et al.* 2008) and of many Indian goat breeds studied by Dixit *et al.* (2012).

Shannon's Information index, which measures the level of biodiversity was also sufficiently high in Singharey goat population with overall mean of  $1.418 \pm 0.094$ . Polymorphic information content (PIC) showed that most of the loci were highly informative, indicating the polymorphism across the loci with an overall mean of  $0.611 \pm 0.036$ . The PIC values varied from 0.311 (ILSTS34) to 0.945 (ILSTS 058) across loci. Most of the studied loci showed the polymorphic information content value greater than 0.5 except ILST008, ILSTS065, OarJMP29, ILSTS029 and ILSTS34 locus. All the parameters (observed number of alleles, effective number of alleles, Shannon's information index and PIC

values) estimated to know the genetic variation showed that most of the microsatellite markers used were highly informative. Suitability of these markers was further strengthened as the numbers of alleles at different loci were greater than recommended number of alleles /locus which is at least 4 alleles/locus (Barker 2001).

To know the extent of inbreeding in the Singharey goat population  $F_{IS}$  was estimated. The value ranged from  $-0.205$  (RM088) to 0.682 (ETH225). The overall mean  $F_{IS}$  (0.225) observed in the present study indicate 22.5% deficiency of heterozygosity in Singharey goat population which is near to 21.7% in Ganjam goat (Sharma *et al.* 2009) but lesser than Gohilwadi goat (Kumar *et al.* 2009), Kutchi goat and Sirohi goat (Dixit *et al.* 2012). The chi-square ( $\chi^2$ ) test revealed that Singharey goat population was in Hardy Weinberg equilibrium sp. microsatellite loci ILSTS044, ILSTS087, ILSTS029, RM4 and ILSTS082 as shown in Table 1. The reason for HW disequilibrium at other loci could not be specified as population is subjected to so many genetic and non-genetic factors. This probably might be due to intermixing of populations (Verma *et al.* 2015a) or samples might have been drawn from the sub population or population having inbreeding.

Bottleneck influences the distribution of genetic variation within and among populations. In recently bottlenecked populations, the majority of loci exhibited an excess of heterozygotes, exceeding the heterozygosity expected in a population at mutation drift equilibrium. To estimate the

Table 1. Measures of genetic variation in Singharey breed of goat

Locus	Allelic number		Hetrozygosity		Shannon's information index (I)	F	PIC	HWE	
	N <sub>a</sub>	N <sub>e</sub>	H <sub>o</sub>	H <sub>e</sub>				$\chi^2$	Sign.
ETH225	5.000	2.606	0.196	0.616	1.125	0.682	0.616	70.531	***
ILSTS044	5.000	1.948	0.449	0.487	0.972	0.077	0.504	12.602	ns
ILSTS008	5.000	1.811	0.280	0.448	0.890	0.375	0.448	42.266	***
OarHH64	7.000	3.241	0.604	0.691	1.513	0.126	0.692	74.193	***
ILSTS059	8.000	3.947	0.667	0.747	1.572	0.107	0.794	72.216	***
ILSTS065	5.000	1.809	0.214	0.447	0.788	0.521	0.447	81.524	***
OarJMP29	5.000	1.569	0.211	0.363	0.770	0.420	0.363	107.220	***
ILSTS033	12.000	4.622	0.719	0.784	1.881	0.082	0.784	90.715	*
OarFCB48	8.000	4.281	0.500	0.766	1.666	0.348	0.767	110.890	***
OMHC1	11.000	4.645	0.563	0.785	1.870	0.283	0.785	96.925	***
ILSTS005	7.000	2.463	0.478	0.594	1.178	0.195	0.594	103.704	***
ILSTS019	6.000	4.325	0.453	0.769	1.581	0.411	0.774	60.155	***
ILSTS058	11.000	3.934	0.270	0.746	1.764	0.638	0.945	242.767	***
ILSTS087	10.000	6.696	0.741	0.851	2.066	0.129	0.853	60.073	ns
ILSTS029	12.000	1.948	0.480	0.487	1.266	0.014	0.488	77.995	ns
ILSTS049	8.000	2.494	0.574	0.599	1.219	0.042	0.599	135.815	***
ILSTS30	12.000	6.969	0.519	0.857	2.129	0.394	0.857	136.310	***
ILSTS34	6.000	1.449	0.250	0.310	0.732	0.193	0.311	37.292	**
ILSTS022	9.000	3.542	0.731	0.718	1.538	-0.018	0.722	77.491	***
RM088	7.000	4.332	0.927	0.769	1.667	-0.205	0.771	39.615	**
RM4	3.000	2.032	0.467	0.508	0.735	0.081	0.508	5.941	ns
ILSTS082	12.000	5.023	0.755	0.801	1.941	0.057	0.802	84.751	ns
OarAE129	8.000	4.711	0.613	0.788	1.750	0.222	0.788	41.586	*
Mean	7.913	3.496	0.507	0.649	1.418	0.225	0.661		
SE	0.576	0.328	0.042	0.034	0.094	0.046	0.036		

Table 2. Population bottle neck analysis in Singharey goat

Model used		IAM	TPM	SMM
Sign test (No. of with hetrozygosity loci excess)	Exp	13.55	13.62	13.63
	Obs	14	8*	4*
	P-value	0.51371	0.01560	0.00005
Standardized differences test	T2 value	0.568	-3.722*	-12.041*
	P-value	0.28488	0.00010	0.0000
Wilcoxon test (one tail for H excess)	P-value	0.10008	0.97187	0.99999

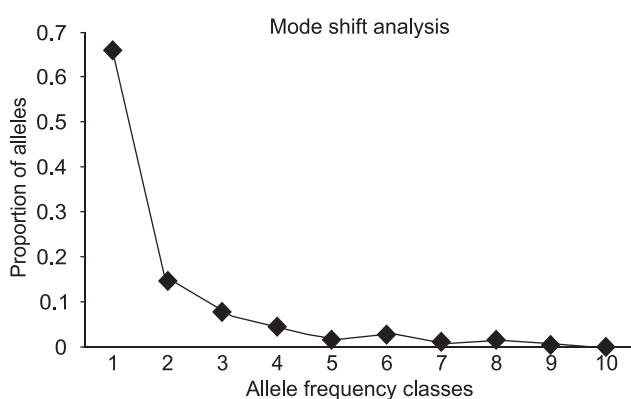


Fig. 2. Graphic representation of proportion of alleles and their distribution in Singharey goat.

excess of such heterozygosity, standardized differences and Wilcoxon sign rank tests were utilized. The actual mutation model of evolution followed by our microsatellites is not known, thus all the three models; Infinite allele model (IAM), stepwise mutation model (SMM) and two-phase model of mutation (TPM) were applied (Table 2). A normal 'L' shaped distribution of mode-shift test (Fig. 2), nonsignificant heterozygote excess on the basis of different models, as revealed from Sign, Standardized differences and Wilcoxon sign rank tests suggested that there was no recent bottleneck in the existing Singharey population.

#### SUMMARY

Singharey goats of Sikkim is a lesser known population of goat. The study was undertaken to know the genetic diversity occurring in these goats. Alleles (182) were observed across the microsatellite loci. The average observed and effective numbers of alleles were  $7.913 \pm 0.576$  and  $3.496 \pm 0.328$ , respectively. A polymorphic information content (PIC) value varied from 0.311 to 0.945 with an overall mean of  $0.661 \pm 0.036$ . Mean observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosities were  $0.507 \pm 0.042$  and  $0.649 \pm 0.034$ , respectively. The inbreeding estimate ( $F_{IS}$ ) was  $0.225 \pm 0.046$  indicated moderate level of inbreeding. 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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