

Sikkim Black goats – characters, management and microsatellite based genetic profile

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ABSTRACT

Sikkim Black goats are distinct from Singharey goats, the main population of Sikkim state, in respect of coat colour, type of horns and their size. Data on phenotypic and biometric traits was generated on 93 animals belonging to different flocks in their native tract. Blood samples were collected from genetically unrelated animals. Sikkim Black goats are of medium body size. Head is proportionate to body. Nose is straight. The horns are strong, broader at base, pointed tip, grey in colour, curving backward. Muzzle is black, hooves are grey. Underbelly is also black. Beard is seen in few animals of both sexes. These goats are slightly shorter than Singharey goats but their face, horn and tail are comparatively longer. The mean estimates for height at withers, body length, Chest/heart girth, paunch girth, face length, horn length, ear length and tail length in adult (>18 months) female goats were 50.06±0.85, 58.75±0.73, 70.65±0.73, 79.57±1.17, 17.11±0.20, 10.17±0.53, 12.70±0.23, 10.35±0.24 cm, respectively whereas for males the average measurements were 54.70±1.19, 61.80±1.39, 75.97±1.48, 82.30±1.76, 17.90±0.29, 15.070±.77, 13.50±0.36 cm. The average body weight for adult females was 28.48±0.72 kg and males 34.87±1.56 kg. Observed number of alleles varied from 2 to 9 with mean value 5.391±0.411, effective number varied from 1.145 to 6.400 with mean 2.771±0.261. Observed heterozygosity (Ho) was less than the expected (He) at most of the loci leaving ILSTS008, ILSTS005, ILSTS0087, ILSTS0029 and ILSTS034. The values ranged from 0.125 to 0.882 with mean 0.420±0.038 for observed and 0.127 to 0.844 with mean 0.575±0.035 for expected heterozygosity. Shannon's information index value varied from 0.291 (ILSTS029) to 1.950 (ILSTS030) with mean value 1.168±0.085. F estimates varied from -0.175 (ILSTS087) to 0.835 (ILSTS058) with mean value 0.239±0.058. The population also exhibited HW equilibrium w.r.t. ten loci where chi square values were non-significant. A normal 'L' shaped distribution of mode-shift test, non-significant heterozygote excess suggested absence of bottleneck in the existing Sikkim Black goat population. The study concluded that there was a reduction in genetic variability in Sikkim Black goat population. Looking at the distinct phenotype but sharing the breeding tract with Singharey goats, there is a need to conserve and propagate the population through appropriate scientific management.

Keywords : microsatellite, genetic diversity, goat

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INTRODUCTION

The goat population of Sikkim state of India is 1,13,364 (19th livestock census, 2012) spread in all the four districts. The male and females are almost in equal proportion (53103 and 60261 respectively). The study was planned to know the phenotype and biometry of Sikkim goats. The visits were made to North, East and West districts of Sikkim and information on phenotypic and biometric traits of its native goats were collected and analyzed. The flocks seen during the survey consisted of black, white,

brown and mixture of these colours. The goats, Singharey, with stripes on face extending from base of horn to the muzzle mainly constituted the flocks for which the phenotypic and biometric profile has also been studied (Verma et al. 2015a). Apart from this, goats with jet black uniform colour were also seen. These goats are distinct from Singharey goats in respect of coat colour, type of horns and their size. They look like Black Bengal but are bigger in their body size and have longer horn. The phenotypic traits of these goats were recorded by visual



Figure 1. Sikkim Black goats exhibiting the different morphological features

observation. Blood samples were collected from possibly genetically different animals for estimating the genetic diversity.

MATERIAL AND METHODS

The measurements of different body traits viz. height at withers, body length, Chest/heart girth, paunch girth, face length, horn length, ear length and tail length and body weights were recorded on 93 (63 females and 30 males) animals. The body weights were taken with the help of spring balance. Means with standard errors were estimated to know the average profile of studied traits.

Genomic DNA was extracted from blood samples using a standard phenol: chloroform extraction method (Sambrook et al., 1989). A battery of 23 microsatellite markers based on the guidelines of ISAG & FAO's DAD-IS program and used by us (Aggarwal et al., 2006, Verma et al., 2007, Dixit et al. 2010, Verma et al., 2015) for other goat populations was utilized to generate allelic data. Microsatellites amplification was carried out using fluorescent-labeled primers. The amplified products were analyzed with a DNA capillary sequencer ABI Prism® 310 Genetic Analyzer (Applied Biosystems). Gene ALEX software package (Peakall and Smouse, 2012) was used to calculate allele frequencies, observed number of alleles, effective number of alleles (Kimura and Crow, 1964), observed (H_o) and expected (H_e) heterozygosity at each locus. Polymorphism information content (PIC) value for each locus was calculated by using the method described by Bostein et al. (1980). Finally the bottleneck hypothesis was investigated using BOTTLENECK 1.2.01 (Conuet and Luikart, 1996).

RESULTS AND DISCUSSION

Morphological features of Sikkim Black goats are shown in fig. 1. They are of medium size. Head is proportionate to body. Nose is straight. The horns are strong, broader at base, pointed tip, grey in colour, curving backward. Muzzle is black, hooves are grey. Underbelly is also black. Beard is seen in few animals of both sexes. These goats are slightly shorter than Singharey goats but their face, horn and tail are comparatively longer.

The average measurements of different body traits are presented in table 1. The mean estimates for height at withers, body length, Chest/heart girth, paunch girth, face length, horn length, ear length and tail length in adult (>18 months) female goats were 50.06 ± 0.85 , 58.75 ± 0.73 , 70.65 ± 0.73 , 79.57 ± 1.17 , 17.11 ± 0.20 , 10.17 ± 0.53 , 12.70 ± 0.23 , 10.35 ± 0.24 cm, respectively whereas for males the average measurements were 54.70 ± 1.19 , 61.80 ± 1.39 , 75.97 ± 1.48 , 82.30 ± 1.76 , 17.90 ± 0.29 , 15.070 ± 0.77 , 13.50 ± 0.36 cm. The average body weight for adult females was 28.48 ± 0.72 kg and males 34.87 ± 1.56 kg. The averages for the same biometric traits of adult Singharey goats were 52.52 ± 0.71 , 60.29 ± 0.66 , 67.98 ± 0.59 , 74.52 ± 1.25 , 16.16 ± 0.19 , 8.84 ± 0.33 , 13.48 ± 0.18 & 10.88 ± 0.24 cm respectively in females and 55.67 ± 0.93 , 61.48 ± 0.86 , 71.66 ± 0.85 , 76.47 ± 1.16 , 17.20 ± 0.22 , 14.58 ± 0.57 , 12.92 ± 0.24 & 11.89 ± 0.25 cm respectively in males. The body weights were 27.33 ± 0.65 kg in adult females and 31.03 ± 0.92 in males (Verma et al. 2015b). On comparing the biometry of two populations Sikkim Black goats were found to be heavier due to more



Figure 2. A flock of Sikkim Black goats



Figure 3. Housing and stall feeding

average values of Chest girth, paunch girth, face length and horn length.

Management: The flocks varying from 2 to more than 20 animals were observed in the field (fig.2). These goats like other goats of Sikkim are also maintained under semi extensive production system where the animals are left for pasture grazing in the morning and brought back in the evening. Animal when stay at home are kept on stall feeding. They are fed with available local grass, leaves and sometimes supplemented with crushed maize. At night, animals are sheltered in temporary houses made of wooden logs, bamboos and planks. The floor of such houses is made 3-4 ft above the ground. This type of housing helps in maintaining cleanliness and proper ventilation. Some houses have inbuilt mangers. The metallic utensils are also used to serve the feed. A special type of feeding manger is also made out of wooden logs (fig.3). Breeding is through natural

mating. Kidding season is March-April and October –November. Since these goats are reared for meat purpose, milk is not drawn but left for suckling of kids. The male goats mature and becomes available to serve at the age of 9-10 months but the males reared for meat purpose are castrated at the age of 3 months. The age of maturity in female goats also varies between 9-12 months. The average gestation period is about five months. Twinning is very common in these goats.

Genetic Diversity: The allelic number, heterozygosities, Shannon's information index, *f* values were estimated for Sikkim Black goats and are given in table 2. Observed number of alleles varied from 2 to 9 with mean value 5.391 ± 0.411 whereas effective number varied from 1.145 to 6.400 with mean 2.771 ± 0.261 . Observed number of alleles is more than the expected number across the loci. Observed heterozygosity (H_o) was less than the

Table 1. Mean body measurement (cm) and body weights (kg) of adult Sikkim Black goats

TRAITS	Females (n=63)	Male (n=30)
Body Height	50.06±0.85	54.70±1.19
Body Length	58.75±0.73	61.80±1.39
Chest Girth	70.65±0.73	75.97±1.48
Paunch Girth	79.57±1.17	82.30±1.76
Face Length	17.11±0.20	17.90±0.29
Horn Length	10.17±0.53	15.070±.77
Ear Length	12.70±0.23	13.50±0.36
Tail Length	10.35±0.24	12.20±0.40
Body Weight	28.48±0.72	34.87±1.56

Table 2. Allele frequency and Genetic diversity of microsatellite loci in Sikkim Black goats

Locus	Allelic Number		Hetrozygosity		Shannon's Information Index	F estimate	HWE ChiSq	Sign.
	Na	Ne	Ho	He				
ETH225	3.000	1.686	0.333	0.407	0.699	0.181	21.000	***
ILSTS044	6.000	2.374	0.200	0.579	1.248	0.654	59.710	***
ILSTS008	5.000	1.896	0.500	0.473	0.933	-0.058	18.503	*
OarHH64	6.000	3.703	0.579	0.730	1.507	0.207	31.711	**
ILSTS059	4.000	2.014	0.250	0.503	0.937	0.503	17.108	**
ILSTS065	7.000	2.616	0.316	0.618	1.240	0.489	87.801	***
OarJMP29	4.000	1.656	0.304	0.396	0.769	0.232	19.454	**
ILSTS033	7.000	2.160	0.438	0.537	1.173	0.185	27.878	ns
OarFCB48	4.000	3.522	0.556	0.716	1.320	0.224	6.407	ns
OMHC1	7.000	5.120	0.625	0.805	1.771	0.223	20.836	ns
ILSTS005	3.000	1.489	0.385	0.328	0.619	-0.171	0.737	ns
ILSTS019	3.000	2.502	0.222	0.600	0.998	0.630	13.835	**
ILSTS058	6.000	4.129	0.125	0.758	1.581	0.835	40.000	***
ILSTS087	8.000	4.014	0.882	0.751	1.630	-0.175	50.711	**
ILSTS029	3.000	1.145	0.133	0.127	0.291	-0.053	0.077	ns
ILSTS049	6.000	2.916	0.364	0.657	1.397	0.447	35.750	**
ILSTS030	8.000	6.400	0.583	0.844	1.950	0.309	32.333	ns
ILSTS034	5.000	2.113	0.615	0.527	1.043	-0.169	6.181	ns
ILSTS022	4.000	1.866	0.421	0.464	0.902	0.093	8.252	ns
RM088	8.000	3.130	0.611	0.681	1.461	0.102	47.739	*
RM4	2.000	1.902	0.455	0.474	0.667	0.041	0.414	ns
ILSTS082	6.000	2.674	0.421	0.626	1.215	0.327	23.697	ns
OARE129	9.000	2.714	0.353	0.631	1.520	0.441	85.170	***
Mean	5.391	2.771	0.420	0.575	1.168	0.239		
SE	0.411	0.261	0.038	0.035	0.085	0.058		

expected (He) at most of the loci leaving ILSTS008, ILSTS005, ILSTS0087, ILSTS0029 and ILSTS034. The values ranged from 0.125 to 0.882 with mean 0.420 ± 0.038 for observed and from 0.127 to 0.844 with mean 0.575 ± 0.035 for expected heterozygosity. Shannon's information index value varied from 0.291 (ILSTS029) to 1.950 (ILSTS030) with mean value 1.168 ± 0.085. F estimates varied from -0.175 (ILSTS087) to 0.835 (ILSTS058) with mean value 0.239 ± 0.058. Some loci (ILSTS008, ILSTS005, ILSTS0087,



Table 3. Population bottle neck analysis in Sikkim Black goat

Model used		I.A.M.	T.P.M.	S.M.M.
Sign test (No. of loci with hetrozygosity excess)	Exp	13.27	13.43	13.80
	Obs	9	7*	5*
	P- value	0.05588	0.00622	0.00021
Standardized differences test	T2 value	-0.838	-3.830*	-7.909*
	P- value	0.20104	0.00006	0.0000
Wilcoxon test (one tail for H excess)	P- value	0.77740	0.99106	0.99985

ILSTS0029 and ILSTS034) having higher observed heterozygosities than the expected showed negative F values. These loci indicated inbreeding depression as a result of which there was increase in heterozygosity. The population also exhibited HW equilibrium w.r.t. ten loci where chi square values were non-significant, however, the reason for HW disequilibrium at other loci is difficult to mark in the small population of Sikkim Black which is also likely to be subjected to in and out movement of animals from the population.

Possibility of differentiation between Black Sikkim and Singharey goats of Sikkim was also explored. The multi-locus F_{ST} values of breed differentiation for different populations of Sikkim goats indicated that only 3.6% of the total genetic variation was due to unique allelic differences between the populations, with the remaining 96.4% corresponding to differences among individuals within the populations across the 23 markers (Verma *et al.*, 2015b). Dixit *et al.*, (2012) observed moderate level (16.5%) of genetic differentiation among Indian goat breeds. Pairwise population Nei's genetic distance and genetic identity values between Black Sikkim and Singharey goat populations were 0.122 and 0.885 respectively. The assignment test based on likelihood method with the leave one out procedure assigned 57% of the Black Sikkim individuals to Singharey population indicating the intermixing of two populations. Principle Component Analysis performed also did not indicate the distinctiveness of these populations.

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. Significant deviations from the mutation-drift equilibrium are simply assessed by the probability given by each test (Standardized differences test or Wilcoxon test). A probability less than 0.05 would allow accepting the hypothesis of genetic bottleneck within the analyzed data. To estimate the 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. A normal 'L' shaped distribution of mode-shift test (fig. 4), non-significant heterozygote excess on the basis of different models, as revealed from Sign, Standardized differences and Wilcoxon sign rank tests (table 3) suggested that there was no recent bottleneck in the existing Sikkim Black goat population.

The study concluded that there was genetic diversity among the studied population but because of small population size, and its intermixing with Singharey goats, there is a reduction in genetic variability in Sikkim Black goat population. Principal Component analysis also showed the mixing of Sikkim Black and Singharey goats in the same cluster. About 40% of the loci were not in HW equilibrium and were heterozygotic deficit. Looking at the distinct phenotype but sharing the breeding tract with Singharey goats, there is a need to conserve and propagate the population through appropriate scientific management.

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