



Genetic diversity and relationship among North East Hill (NEH) goats

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ABSTRACT

Genetic diversity and relationship among three goat populations i.e. Singharey, Sikkim Black goat and Long hair Nagaland goat also known as Sumi-Ne of North eastern hill region. Fifty blood samples were studied from each population and tested with a battery of 25 and satellite markers. Number of alleles observed was 116 (Sumi-Ne), 182 (Singharey) and 124 (Sikkim Black). Average numbers of alleles observed were 5.04 ± 0.38 (Sumi-Ne), 7.91 ± 0.57 (Singharey) and 5.39 ± 0.30 (Sikkim black goats) and average effective numbers were 2.57 ± 0.28 , 3.49 ± 0.32 and 2.77 ± 1.68 , respectively. Singharey had relatively more private alleles but with comparatively lower frequency. The average observed and expected heterozygosity was 0.34 ± 0.04 and 0.49 ± 0.05 in Nagaland goats, 0.50 ± 0.04 and 0.64 ± 0.03 in Singharey and 0.42 ± 0.33 and 0.57 ± 0.40 in Sikkim black goats. Average F_{IS} , F_{IT} , F_{ST} were 0.27 ± 0.04 , 0.39 ± 0.03 , 0.15 ± 0.03 , respectively. All the three populations showed absence of bottleneck (reduction in effective population size). Analysis of molecular variance revealed that 39% of the total genetic diversity existed among the individuals within populations and only 18% of the total genetic diversity accounted for differences among populations. Sumi-Ne showed similar genetic distance (0.636) from the Singharey and Sikkim Black goat, whereas, Singharey and Sikkim Black were genetically close. Compared to other Indian goat breeds, all the three NEH populations were far distanced from Berari, Black Bengal, Bundelkhandi, Ganjam, Jharkhand Black, Konkan Kanyal and Kutchi. In the neighbour joining diagram the three goat populations were plotted separately. Considering the geographical and genetic identity of NEH goats, conservation and improvement programmes may be formulated accordingly.

Key words: Genetic diversity, Heterozygosity, Microsatellites, NEH goats, Relationship

Genetic diversity is an important parameter for formulating the breeding and improvement programmes for any species. Goat is not the exception where diversity is exhibited more by their phenotypes and performance traits. Genetic polymorphism forms the basis of this diversity. Type of product and its production depends upon the climate of their habitat. Chevon production is the main utility whereas other products come as additional utility to the farmers. Goats reared in cold climate of North region yield long silky fibre apart from the meat. Goats of North Western region in India, are known for their milk production whereas those of Eastern and Southern region are good meat yielder and highly prolific. Loss/dilution of a breed due to any reason results in the loss of genetic variability from the gene pool. Genetic variability helps the population to survive and perform better under varied climatic conditions. Genetic diversity in Indian goat breeds has been studied by many workers (Dixit *et al.* 2012; Mishra *et al.* 2013, 2015;

Verma *et al.* 2015). As of today, 34 goat breeds are registered by ICAR-NBAGR (2018). This includes LHNG/Sumi-Ne from Nagaland and Assam hill goat from Assam. Goat population of NEH region of India is about 4.37 million and the population in Sikkim and Nagaland was 1,13,364 and 99,350, respectively (Livestock Census, 2012). North East hilly goats (Singharey goat and Sikkim Black goat from Sikkim and Sumi-Ne from Nagaland) have been considered for study (Fig. 1). In this study, microsatellite markers which are co-dominant in nature have been used to analyze the genetic diversity among these populations.

MATERIALS AND METHODS

Blood samples (50) were collected from genetically different animals of each population from different locations. DNA was isolated by standardized phenol-chloroform method using protocol of Sambrook *et al.* (1989). After quality check DNA was diluted appropriately ($50 \text{ ng}/\mu\text{l}$) in MiliQ water. A battery of 25 microsatellite labelled markers (Table 1) was utilized to generate allelic data. Each forward primer was tagged on the 5' end with one dye out of four dyes (FAM-Blue, VIC-Green, NED-Yellow, PET-Red) supplied by Applied Biosystems, UK.

Amplifications were conducted in a $25 \mu\text{l}$ reaction

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Fig. 1. Singharey, Sikkim Black and Long Hair Nagaland goat.

volume consisting of 2.5 μ l of 10 $\times$ PCR buffer, 0.5 μ l dNTP (200 μ M), 50 nM KCl, 10 nM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.0 mM MgCl₂, 5 μ l of each primer (10 pmol) and 0.25 μ l of *Taq* polymerase (Sigma) using touchdown protocol and standard PCR conditions (Verma *et al.* 2019). 6.0 μ l of PCR product was electrophoresed on 2% agarose gel and visualized over UV light after ethidium bromide staining to detect the amplification. The PCR products were mixed in the ratio of 1:1.5:2:2 of FAM, VIC, NED and PET labeled primers respectively. 0.5 μ l of this mixture (PCR multiplex) was combined with 0.3 μ l LIZ 500 as internal lane standard and 9.20 μ l formamide per sample. The mixture was denatured by incubating at 95°C for 5 min. The denatured samples were run on automatic DNA sequencer (ABI 3100 Avant). The electropherograms drawn through Gene Scan were used to extract DNA fragment sizing details using Gene Mapper software version 3.0.

Observed and expected allele numbers, heterozygosity, were calculated using Pop Gene software, version 1.32 (Yeh *et al.* 1999). F statistics were determined using F-stat (Goudet 2002) with jackknifing procedure. Tests for deviation from Hardy Weinberg equilibrium were conducted. Genetic relationship between breeds was measured by genetic distance (Nei *et al.* 1983).

RESULTS AND DISCUSSION

Sumi-Ne, Singharey and Sikkim Black goat are phenotypically different from each other as well as from other goat breeds of India. Morphological traits and average

measurements for different traits (height at withers, body length, Chest/heart girth, paunch girth, face length, horn length, ear length and tail length) and body weights for Sumi-Ne, Singharey and Sikkim Black goat have been reported by Verma *et al.* 2018, 2015a and 2015b, respectively.

Genetic diversity in terms of allelic number, heterozygosity, for these three populations are shown in Table 1. Out of the 25 loci investigated, 23 were polymorphic in nature exhibiting two or more alleles. The total number of alleles observed across these microsatellite loci was 116 (Sumi-Ne), 182 (Singharey) and 124 (Sikkim Black). Observed number of alleles varied from 2 (OarJMP29) to 9 (ILSTS058) with an overall mean of 5.04 $\pm$ 0.38 in Nagaland goats; 3 (RM4) to 12 (ILSTS033, ILSTS029, ILSTS030 and ILSTS082) with mean 7.91 $\pm$ 0.57 in Singharey and 2 (RM4) to 9 (OarAE129) with mean 5.39 $\pm$ 0.30 in Sikkim black goats. Average effective numbers of alleles were found to be 2.57 $\pm$ 0.28, 3.49 $\pm$ 0.32 and 2.77 $\pm$ 1.68, respectively. Interestingly, private alleles were also found in these goat populations and the frequency of private alleles was up to 89.80% in Sumi-Ne Goat. Singharey had relatively more private alleles but with comparatively lower frequency. Low frequency private alleles in Long Hair Nagaland goat were at the ILSTS019 locus (16.7%), ILSTS058 (20.9%), and locus ILSTS082 allele 120 (17.0%) whereas, high frequency alleles were at ILSTS034 (89.8%), RM088 allele (89.50%). An allelic pattern exhibited by Sikkim Black (SB), Singharey (SS)

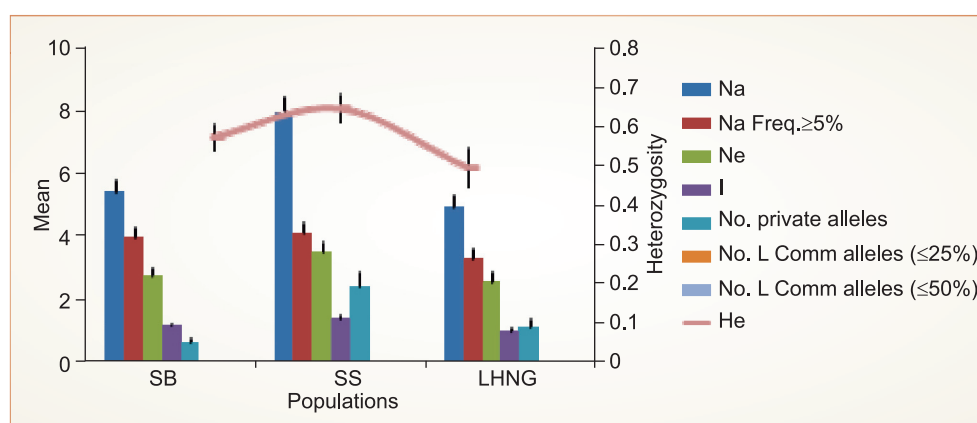


Fig. 2. Allelic patterns across Sikkim Black (SB), Singharey (SS) and Long Hair Nagaland goat (LHNG).

Table 1. Genetic variability in Nagaland and Sikkim goats

Locus	Long Hair Nagaland Goat (Sumi-Ne)				Singharey				Sikkim Black			
	Allele Number		Heterozygosity		Allele number		Heterozygosity		Allele number		Heterozygosity	
	Na	Ne	Ho	He	Na	Ne	Ho	He	Na	Ne	Ho	He
ETH225	3.00	1.24	0.04	0.19	5.00	2.60	0.19	0.61	3.00	1.68	0.33	0.40
ILSTS044	7.00	1.62	0.16	0.38	5.00	1.94	0.44	0.48	6.00	2.37	0.20	0.57
ILSTS008	3.00	2.34	0.17	0.57	5.00	1.81	0.28	0.44	5.00	1.89	0.50	0.47
OarHH64	8.00	4.62	0.52	0.78	7.00	3.24	0.60	0.69	6.00	3.70	0.57	0.73
ILSTS059	4.00	2.06	0.53	0.51	8.00	3.94	0.66	0.74	4.00	2.01	0.25	0.50
ILSTS065	4.00	2.04	0.18	0.51	5.00	1.80	0.21	0.44	7.00	2.61	0.31	0.61
OarJMP29	2.00	1.04	0.04	0.04	5.00	1.56	0.21	0.36	4.00	1.65	0.30	0.39
ILSTS033	6.00	1.40	0.27	0.29	12.0	4.62	0.71	0.78	7.00	2.16	0.43	0.53
OarFCB48	5.00	4.06	0.37	0.75	8.00	4.28	0.50	0.76	4.00	3.52	0.55	0.71
OMHC1	7.00	5.01	0.78	0.80	11.0	4.64	0.56	0.78	7.00	5.12	0.62	0.80
ILSTS005	6.00	2.21	0.47	0.54	7.00	2.46	0.47	0.59	3.00	1.48	0.38	0.32
ILSTS019	6.00	4.03	0.39	0.75	6.00	4.32	0.45	0.76	3.00	2.50	0.22	0.60
ILSTS058	9.00	5.41	0.51	0.81	11.0	3.93	0.27	0.74	6.00	4.12	0.12	0.75
ILSTS087	6.00	3.73	0.52	0.73	10.0	6.69	0.74	0.85	8.00	4.01	0.88	0.75
ILSTS029	6.00	4.08	0.65	0.75	12.0	1.94	0.48	0.48	3.00	1.14	0.13	0.12
ILSTS049	6.00	2.23	0.43	0.55	8.00	2.49	0.57	0.59	6.00	2.91	0.36	0.65
ILSTS30	3.00	1.28	0.13	0.22	12.0	6.96	0.51	0.85	8.00	6.40	0.58	0.84
ILSTS34	3.00	1.23	0.20	0.18	6.00	1.44	0.25	0.31	5.00	2.11	0.61	0.52
ILSTS022	4.00	1.21	0.25	0.17	9.00	3.54	0.73	0.71	4.00	1.86	0.42	0.46
RM088	3.00	1.23	0.20	0.19	7.00	4.33	0.92	0.76	8.00	3.13	0.61	0.68
RM4	4.00	2.25	0.22	0.55	3.00	2.03	0.46	0.508	2.00	1.90	0.45	0.47
ILSTS082	5.00	2.91	0.45	0.65	12.0	5.02	0.75	0.80	6.00	2.67	0.42	0.62
OarAE129	6.00	1.91	0.390	0.47	8.00	4.71	0.61	0.78	9.00	2.71	0.35	0.63
Mean	5.04	2.57	0.34	0.49	7.91	3.49	0.50	0.64	5.39	2.77	0.42	0.57
S.E.	0.38	0.28	0.04	0.05	0.57	0.32	0.04	0.03	0.30	1.68	0.33	0.40

Na, observed no. of alleles; Ne, effective no. of alleles; Ho, observed heterozygosity; He, expected heterozygosity.

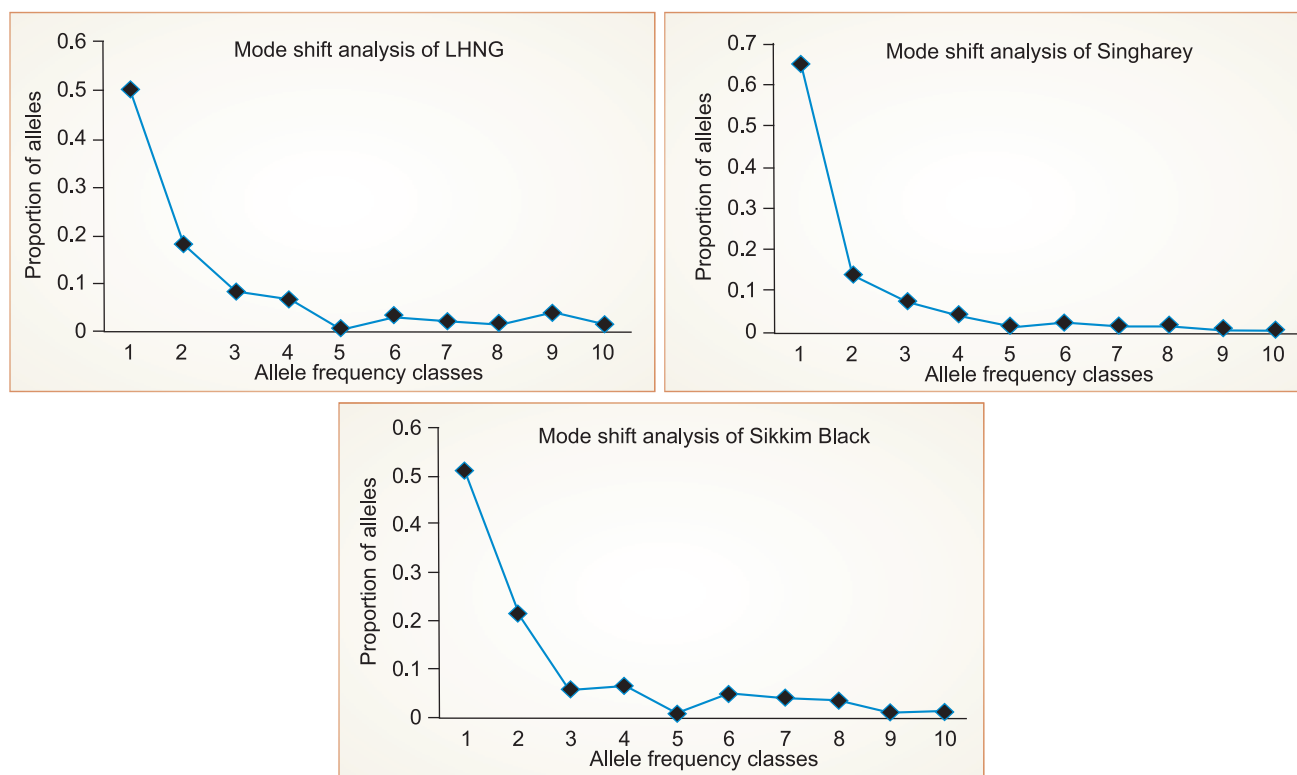


Fig. 3. L-shaped curve exhibited by all three populations.

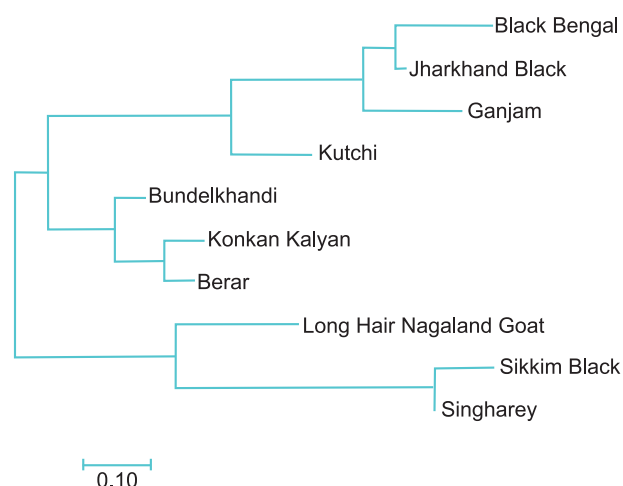


Fig. 4. Neighbour joining consensus tree.

and Long Hair Nagaland Goat (LHNG) is shown in Fig 2. The number of samples, loci analyzed and their heterozygosity determines the precision of genetic diversity (Barker 1994) where the loci with minimum 4 alleles reduce the error of estimation of genetic relationship. The average number of observed and effective alleles noted in the presented study matches with the values (N_a - 5.0; N_e - 2.93) reported by Zaman *et al.* (2013) in Nagaland goats. The allelic variations in LHNG were lower than Singharey goat and Sikkim Black goats (Verma *et al.* 2015b, Shivhare *et al.* 2017). Most of the Indian goat breeds exhibited higher number (observed and effective) of alleles (Dixit *et al.* 2012) for the same microsatellites.

The average observed and expected heterozygosity were 0.34 ± 0.04 and 0.49 ± 0.05 in Nagaland goats, 0.50 ± 0.04 and 0.64 ± 0.03 in Singharey and 0.42 ± 0.33 and 0.57 ± 0.40 in Sikkim black goats. The expected heterozygosity was significantly higher than observed heterozygosity at most of loci in these three populations. The observed heterozygosity lower than expected values at few loci indicated the heterozygotic deficiency.

The lower heterozygosity in Nagaland goats than the other two populations may be due to availability of less breeding bucks and thus more inbreeding in the population. Among the Indian goat breeds, highest average observed and expected heterozygosity was reported for Kanniadu goat (0.53, 0.73) and lowest for Osmanabadi (0.42, 0.61) by Dixit *et al.* 2012. Higher heterozygosity than LHNG population was reported in Singharey goat (Shivhare *et al.* 2017), Sikkim Black goat (Verma *et al.* 2015b), Berari (Mishra *et al.* 2013), South Indian goats (Dixit *et al.* 2010). In another study conducted in Nagaland goats by Zaman *et al.* 2013 the overall means for observed and expected heterozygosity were 0.56 ± 0.29 and 0.48 ± 0.24 , respectively. The moderate level of gene diversity observed in Nagaland goats by us and Zaman *et al.* (2013) may be again due to the small population size and limited number of good bucks available for breeding. On the contrary, high genetic diversity was observed in many of the Indian goat breeds (Dixit *et al.* 2012), Chinese goats (Li *et al.* 2008), Spanish goats (Serrano *et al.* 2009), and Egyptian and Italian goat breeds (Agha *et al.* 2008).

Locus wise F-statistics (F_{IS} , F_{IT} and F_{ST}) across the three populations have been given in Table 2. F_{IS} ranged from -0.06 (RM088) to 0.66 (ILSTS058) with mean 0.27 ± 0.42 . F_{IT} ranged from 0.14 (ILSTS87) to 0.69 (ILSTS058) with mean 0.39 ± 0.37 . F_{IT} ranged from 0.03 (ILSTS082) to 0.55 (OarJMP29) with mean 0.15 ± 0.30 . Pairwise F_{ST} values were 0.04 (Sikkim Black and Singharey) to 0.26 (Sikkim Black and LHNG) and 0.21 (Singharey and LHNG). F_{ST} values were significantly different from zero for all pairwise combinations representing the significant amount of genetic differentiation between populations.

A genetic differentiation (F_{ST} , 0.156) between the investigated NEH goat populations was significantly different from zero ($P > 0.01$), which means that 15.6% of the total genetic variation was explained by population differences and 84.4% corresponds to the differences among individuals within each breed. The overall F_{IS} value (0.274) was higher and significantly different from zero indicating

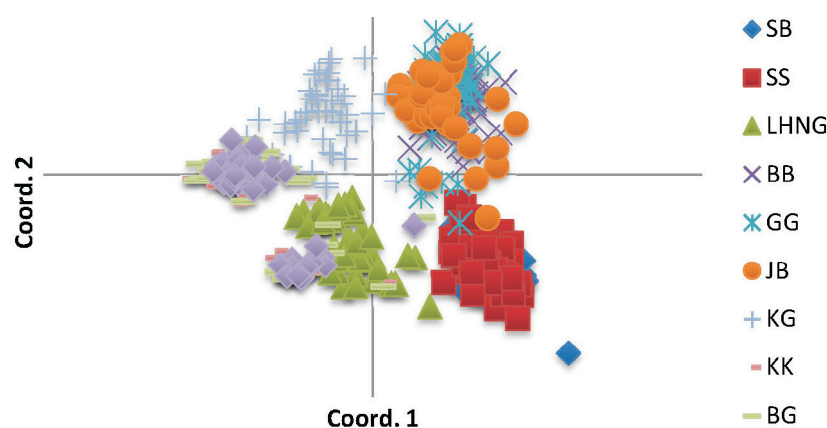


Fig. 5. Principal Coordinate Analysis (PCA) - SB (Sikkim Black), SS (Singharey), LHNG (Long hair Nagaland Goat), BB (Black Bengal), GG (Ganjam goat), JB (Jharkhand Black), KG (Kutchi goat), KK (Konkan Kanyal), BG (Berari goat), BK (Bundelkhandi).

Table 2. F-statistics across NEH goat populations

Locus	F _{IS}	F _{IT}	F _{ST}	Locus	F _{IS}	F _{IT}	F _{ST}
ETH225	0.53	0.57	0.09	ILSTS058	0.66	0.69	0.08
ILSTS044	0.43	0.61	0.32	ILSTS087	0.08	0.14	0.06
ILSTS008	0.35	0.45	0.14	ILSTS029	0.12	0.31	0.22
OarHH64	0.22	0.25	0.03	ILSTS049	0.24	0.26	0.02
ILSTS059	0.17	0.34	0.19	ILSTS30	0.35	0.47	0.19
ILSTS065	0.56	0.63	0.16	ILSTS34	-0.04	0.43	0.46
OarJMP29	0.48	0.77	0.55	ILSTS022	0.01	0.30	0.29
ILSTS033	0.10	0.18	0.08	RM088	-0.06	0.25	0.30
OarFCB48	0.36	0.38	0.03	RM4	0.39	0.42	0.03
OMHC1	0.19	0.23	0.05	ILSTS082	0.21	0.24	0.03
ILSTS005	0.09	0.14	0.06	OarAE129	0.28	0.32	0.06
ILSTS019	0.49	0.52	0.04				

Mean 0.27± 0.04 (F_{IS}), 0.39±0.03, (F_{IT}), 0.15±0.03 (F_{ST}).

departure from random mating and suggested that some of the studied loci were homozygous in the populations.

To detect any reduction in effective population size, Sign test, Standardized difference test and Wilcoxon sign-rank test were employed under different mutation models of microsatellite evolution i.e. infinite allele model, step-wise mutation model and two-phase model. The L-shaped mode-shift curve (Fig. 3) indicates that all these populations are non-bottlenecked and have not shown any reduction in the effective population size in the recent past and thus remained at mutation drift equilibrium.

Analysis of molecular variance revealed that 39% of the total genetic diversity existed among the individuals within populations and only 18% of the total genetic diversity accounted for differences among populations, which is exactly in accordance with the F_{ST} value. Pairwise Nei's genetic distances for the breeds were calculated and are presented in Table 3.

LHNG showed similar distance (0.636) from the Singharey and Sikkim Black goat, whereas, Singharey and Sikkim Black showed very less distance (0.122). Singharey, Sikkim Black and LHNG were far distanced from other seven Indian breeds/population (Berari, Black Bengal, Bundelkhandi, Ganjam, Jharkhand Black, KonkanKanyal, Kutchi) taken for comparative study. Sikkim Black and Ganjam have the highest genetic distance (1.458) from the others. In the neighbour joining diagram (Fig. 4), the Sikkim Black, Singharey and Long Hair Nagaland goat populations plots separately from the other breeds/populations (Black Bengal, Ganjam, Jharkhand Black and Kutchi).

The Principal component analysis of allelic distribution for these studied goat populations has been shown in Fig. 5. It is obvious that Singharey and Sikkim Black goats are intermixed population whereas Long Hair Nagaland Goat is distant from these populations. It is also clear that these populations are clustered separately from other goat population inhabiting different regions of India.

The microsatellite markers used in the study worked suitably for the genetic diversity analysis of NEH goats. The allelic variations in LHNG were lower than Singharey

Table 4. Nei's Genetic Distance

Breed/Population	Singharey	Sikkim Black	LHNG/Sumi-Ne
Singharey	0.000		
Sikkim Black	0.122	0.000	
LHNG/Sumi-Ne	0.636	0.636	0.000

goat and Sikkim Black goats. The heterozygosity observed in Nagaland goats was also lesser than the other two populations. Most of the loci exhibited heterozygotic deficiency. A significant genetic differentiation (F_{ST}0.156) between the investigated NEH goat populations indicated that about 15.6% of the total genetic variation was explained by population differences. LHNG was genetically distant showing similar genetic distance (0.636) from the Singharey and Sikkim Black goat but Sikkim goats showed genetic mixing among them. Sikkim Black, Singharey and Long Hair Nagaland goat populations plotted separately from the other Indian goat breeds/populations as shown by PCA indicating their genetic identity in their natural habitat. Therefore, conservation and improvement programmes for NEH goats may be formulated considering their geographical and genetic identity.

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