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Original Article

Evolutionary lineage and relationship of Himalayan region goats

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ABSTRACT

Goats are hardy and well-adapted to different climates at different altitudes. A combined analysis of 30 microsatellite loci and mtDNA (D-loop region) was performed to infer genetic variation and demographic history among 3 goat populations (Pashmina, Gaddi and Rampur goat) of India residing in different altitudes. The mean and effective number of alleles for Pashmina, Gaddi and Rampur goat were 6.900, 5.500, 5.655 and 3.404, 2.905, 2.979 respectively. A positive inbreeding coefficient values indicate random union of gametes, inferring local inbreeding in Pashmina and Rampur populations while out-breeding in Gaddi population. A moderate value (14%) was obtained for among population differentiation. No severe reduction in effective population size was observed in all the 3 goat populations. High values of haplotype and nucleotide diversity were observed for 3 goat populations in mitochondrial D-loop analysis. Mismatch analysis revealed Pashmina and Gaddi populations to be inpopulation expansion while Rampur population was found to be stable. The genetic distance estimates revealed close similarity between Gaddi and Rampur goats while Pashmina was found to be distinct population. Our study provides evidence that Indian Himalayan goat populations from different altitudes can be classified into distinct genetic groups based on the microsatellites as well as mtDNA information.

Keywords: Goat, Microsatellite, Mitochondrial D-loop, Genetic diversity, Bottleneck, Phylogenetic analysis.

INTRODUCTION

Goats have wide ranging adaptations for feeding behavior, body size, fleece structure and disease tolerance etc. which allow them to survive and thrive in diverse environment in which they are reared (Vanya, 2011). These characteristics help them to survive and cope up with unfavorable and harsh

environments. Genetic diversity helps the goat populations to adapt to changing environments. Increased variation increases the chances of adaptation to changing environments and thus the survivability.

Goats thrive in Himalayas ranging from high altitudes of Leh and Ladhakh, Jammu and Kashmir and Lahaul Spiti to low altitudes of Kangra, Kullu, Chamba, and Shimlain Himachal Pradesh. 3 different Himalayan goat populations were considered: Pashmina or Changthangi goat which is reared by the Changpa nomads in Changthang region of Greater Ladakh region 3524 m(11,562 ft) of India is popularly known for the value product “cashmere or pashm” a fine luxury fiber and also for its meat, Gaddi goat population, also known as the White Himalayan is reared by Gaddi community for meat and fiber and is distributed throughout Kangra, Bilaspur, Chamba, Simla and Kinnaur districts 1472m (4,829 ft) in Himachal Pradesh and a local Himachal goat population in Rampur Bushahr region 1350 m (4,429 feet) of Shimla which is also a dual purpose goat used for fibre and meat. In the present study, we compared the genetic diversity of these goats belonging to different altitudes of Himalayan region using both microsatellites as well as mitochondrial D-loop analysis.

MATERIAL AND METHOD

Blood samples (about 8ml) of goats were collected from Leh and Ladakh region of Jammu and Kashmir, Kangra and Rampur regions of Himachal Pradesh in India. The phenol-chloroform DNA isolation method was used for the isolation of the genomic DNA from the blood samples (Sambrook *et al.,* 2001). Then Agarose gel (0.6%) stained with Ethidium Bromide was used for approximate quantification of the DNA by electrophoresis.

For generation of microsatellite data, 144 samples of goats from 3 different goat populations were used. A set of 30 primers were synthesized and labelled with different fluorescent dyes as shown in Table 1. PCR amplification was done in a 10µl reaction, consisting of 1.5mM $MgCl_2$, 1X PCR buffer, 200µM dNTPs, 5pmol each of Forward (labelled) and Reverse primers, 1U *Taq* polymerase and 50ng genomic DNA. The reaction was carried out in Eppendorf ProS thermal cycler. The conditions utilized were initial denaturation at 95°C for 5min followed by 35 cycles of 45sec 94°C, 45sec at annealing temperature 55°C and 1min at 72°C. The final extension at 72°C was prolonged for 10min after the reaction; the samples were analyzed using Liz 500 as internal size standard in 3130XL Genetic Analyzer (Applied Biosystems).

Table 1: List of microsatellite primers with labelled dyes.

S.No.	Locus	Primer forward (5' to 3')	Primer Reverse (3' to 5')	Tags
1	BM875	ACCTATCTCATTTGGCTTCTGG	AAAAAACCCCAAACAACAACC	FAM
2	CA002	AGCAATGTCAAACATTGCTCC	TCCATCTTTACAACTTTTGAATC	HEX
3	RM033	GCTCATTCTCCTGGGATGCAGA	GCTCCTTTAGTTTTCTTGTGGGAG	FAM
4	BB716	GATCTTTCTACAGACTGCCAC	ACATTTGCTGATATGGTTGG	FAM
5	BM1905	GTCCATGGGTTCACAAAGAG	ACGCCTGCTGATGCTGTAG	FAM
6	BMS2270	CTGCGTTAACACCCCACC	GCAGGAAGGCTGATGCAC	FAM
7	TGLA272	GCGGTTAGAGGCTTGCACGCT	TTTCGCTGAGTAGGTCATTATTAAG	HEX
8	BMS2275	GGGGATATCGAGAGGATGTG	ATCCTCCAGCCTACACCTAGC	FAM
9	BMS1001	GAGCCAATTCCTACAATTCTCTT	AGACATGGCTGAAATGACTGA	HEX
10	BMS1909	ACTTGTTAGGAGGGCTATTGTTAA	CCACATACACCACCAACATTAA	FAM
11	BMS2295	GCTCTGGTGACCCAGGTG	CTGGCAGGAGATGAGAGGAG	HEX
12	DIK4314	GGCCCTAAAACTCATTGCAC	CCCCTGAAATCTCAAAGCAG	FAM
13	DIK5127	AATGGTGAGACATGGGATGC	TCCAAGAGAACCCAACTTTGA	HEX
14	ILSTS011	GCTTGCTACATGGAAAGTGC	CTAAAATGCAGAGCCCTACC	FAM
15	BM8215	CCAAAGAAGCTGAAGTTGACTG	CTGACTTTTGCATTTCAACCC	FAM
16	DIK5153	ACGTTTGAAGCTGGGAGATT	CATGTGGTTGCAGAGATTTGA	FAM
17	BM6425	AGTTGAACCTGGGTCTCCTG	TGCAATGGCAGTGAAAAAAG	HEX
18	BMS2321	TCACTTCACAAAATACACAATGC	CCAAACTCCATAATCACCACTT	FAM
19	CSSM066	ACACAAATCCTTTCTGCCAGCTGA	AATTTAATGCACTGAGGAGCTTGG	FAM
20	DIK1057	GGTCAACTACTCCAGTTTCCAG	TGCACTTTACTGCTTGAGTCAT	FAM
21	BMS2213	ATGGGCAGCTTAGGGATTG	CTTCAAGAGCCTTCAGTGGG	HEX
22	DIK2866	GAAACTTTTGGGCAAATGGA	GCTATCTTCCCTCCCAGCTT	FAM
23	HUJI-13	TCCTTGTATTCACACGTGGG	TTCTCAGCCAAAGTCAAGGG	FAM
24	BMS817	TGGGAAAGTTGGCAAATG	TTGTGATACCTGAAATGGTCAA	HEX
25	BMS861	ATGGTTTATTGAAGTCAAGGTAAGC	TCCTCAGATAGCCCCTTCAG	FAM
26	BM1443	AATAAAGAGACATGGTCACCGG	TCGAGGTGTGGGAGGAAG	HEX
27	BMS2055	ATGCTAAGTGAAGAACAAATCATTC	CTGGCAACTCTTCCTTAATACATT	FAM
28	BMS119	TCTGTGTTCAGGAAGCAGTTG	AGGTGTCACTTCCTTGACGC	HEX
29	BMS4037	CCCCCATAATGCTACATATTGC	TTAAGACATGAATCCTCAGGGC	HEX
30	BMS690	CATAGGGATATGTTGTGCATCC	TCAAAGAAACTTCAAGCCAGC	FAM

The sizing and allele calling was performed by using Gene Mapper v4 software. The basic genetic parameters for 30 loci were calculated – the number of alleles, effective number of alleles, expected and observed heterozygosities by utilizing the software POPGENE v1.32 (Yeh *et al.,* 1999). GENEPOP v4.2.1 software (Raymond and Rousset, 1995) was used for calculating F_{IS} values (Weir and Cockerham, 1984). Analysis of molecular variance (AMOVA) was used to estimate the variance components and their significance levels of genetic variation within and among the populations using Arlequin v3.5.1.2 software (Excoffier and Lischer, 2010). The Corresponding Analysis was carried out using GENETIX v4.05.2 software (Belkhir *et al.,* 2004). Bottleneck v1.2.02 software (Piry *et al.,* 1999) was used to study any reduction of effective population size. The three tests performed were Sign rank test, Standard differences test and Wilcoxon rank test, to find if the Himalayan goat populations had undergone any recent bottleneck as revealed by transient heterozygotic excess. For this purpose, the 3 models of microsatellite evolution Infinite alleles model (IAM), Stepwise mutation model (SMM) and Two phase model (TPM) were utilized. Populations v1.2.32 software (Langella, 1999) was utilized to estimate the genetic distances between the populations and to construct an inter-individual genetic distance based tree using FigTree v1.4.0 software (Rambaut, 2006-2012).

For mitochondrial analyses, we used 72 samples of goats belonging to higher and lower altitude regions of Himalayas. The primers were designed by using Accession no. AF533441 as reference sequence using primer3 webserver (Whitehead Institute for Biomedical Research). The sequences of forward and reverse primers used are TTCCCACTCCACAAGCCTAC and GGATGCATGATGAAATGCAA respectively. The DNA was amplified in a 25µl reaction, 1X PCR buffer, 2.0mM $MgCl_2$, 200µM dNTPs, 5pmol of each primer, 1U *Taq* polymerase and 50ng of genomic DNA. The PCR product thus amplified was checked in 2% agarose gel and was used for sequencing using Big Dye Terminator carried out on3130XL Genetic Analyzer (Applied Biosystems).

BioEdit v7.1.3 software (Hall, 1999) was used for aligning and editing the 835bp D-loop sequences. DnaSP v5.10.01 software (Rozas *et al.,* 2010) was used to identify haplotype diversity, polymorphic sites and nucleotide diversity. Neutrality tests like Tajima's D and Fu's Fs values were calculated using Arlequin v3.5.1.2 software (Excoffier and Lischer, 2010), to test for any deviation from neutral equilibrium conditions and was also used to perform AMOVA analysis. Mismatch distribution was used to analyse if any population expansion occurred.

RESULT AND DISCUSSION

Genetic diversity analysis among high and low altitude Himalayan goats using microsatellite markers

In the present study, 30 microsatellites markers utilised were found to be highly polymorphic. The markers with their number of alleles, effective number of alleles, observed heterozygosity, expected heterozygosity and F_{IS} values have been given in Table 2. The mean of alleles for Pashmina population was 6.900 with a range of 3 (DIK5127and BM6425) to 11 (BMS2270, DIK2866, BMS817 and BMS2055). For Gaddi population the mean number of alleles was found to be 5.500 and number of alleles ranged from 1 (DIK5127 and DIK5153) to 11 (DIK6425 and DIK2866). Similar results were observed in Rampur goat population where the mean number of alleles was 5.655 and the number of alleles ranged from 1 (DIK5127) to 11 (DIK1057). The mean values of observed and expected heterozygosities are 0.623 and 0.638 in Pashmina, 0.594 and 0.557 in Gaddi and 0.529 and 0.560 in Rampur goat population. The observed heterozygosity value were found to be less than the expected in Pashmina and Rampur goat populations which points towards inbreeding or non-random mating of individuals in these two populations except in Gaddi goat population where the observed heterozygosity value was larger than the expected indicating out-breeding in the population, this may be because of the fact that Gaddi goats are reared by nomadic "Gaddi" tribe who migrate along with goat flocks to alpine pastures on high altitude in summer and remain in foothills and valleys during winter (http://agris.nic.in/22AnnGoat), thus their constant migration increases the chances of out-breeding in the population with use of bucks from different populations. Similar results were observed for Pashmina goat study by Rout *et al.*, 2008 where observed and expected heterozygosity values were 0.375 and 0.760 respectively and showed comparatively low genetic variation. This might be attributed to sampling plan as most of the samples might be from the closed herd and not from the breeding tract.

Table 2: Markers with their number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He) and F_{IS} (W&C)values for the 3 Himalayan goat populations and where * = significant values P<0.5.

	Pashmina					Gaddi					Rampur				
Locus	**Na**	**Ne**	**Ho**	**He**	**F_{IS} (W&C)**	**Na**	**Ne**	**Ho**	**He**	**F_{IS} (W&C)**	**Na**	**Ne**	**Ho**	**He**	**F_{IS} (W&C)**
BM875	5	2.402	0.489	0.59	0.172*	3	2.414	0.851	0.592	-0.444*	7	2.491	0.8	0.605	-0.327
CA002	5	1.391	0.273	0.284	0.041	2	1.022	0.021	0.021	-	3	1.139	0.077	0.124	0.380
RM033	4	1.653	0.511	0.399	-0.283	2	1.693	0.575	0.414	-0.394	4	1.503	0.356	0.339	-0.051*
BB716	7	2.097	0.511	0.529	0.034*	5	2.25	0.938	0.561	-0.682	4	2.52	0.689	0.61	-0.131
BM1905	4	3.105	0.957	0.685	-0.402	4	2.17	0.958	0.545	-0.774	2	1.837	0.702	0.461	-0.533

BMS2270	11	4.918	0.744	0.806	0.078*	9	5.519	1	0.827	-0.211	10	2.86	0.872	0.657	-0.332
TGLA272	5	3.544	0.587	0.726	0.193*	4	2.582	0.625	0.619	-0.010	4	2.333	0.458	0.577	0.208
BMS2275	6	2.05	0.438	0.518	0.156*	4	1.951	0.217	0.493	0.562*	4	1.433	0.195	0.306	0.364*
BMS1001	4	1.349	0.083	0.261	0.684*	2	1.348	0.304	0.261	-0.169	2	1.045	0	0.044	1.000*
BMS1909	9	5.192	0.83	0.816	-0.017	8	5.053	0.75	0.811	0.075*	8	3.171	0.667	0.692	0.037
BMS2295	7	3.252	0.958	0.7	-0.375	7	1.926	0.458	0.486	0.057	4	2.32	0.628	0.576	-0.092
DIK4314	7	4.204	0.809	0.77	-0.050	9	5.818	0.854	0.837	-0.021	9	6.464	0.796	0.855	0.070*
DIK5127	3	1.111	0.104	0.101	-0.031	1	1	0	0	-	1	1	0	0	-
ILSTS011	6	3.239	0.553	0.699	0.210*	6	2.945	0.523	0.668	0.219	0	0	0	0	-
BM8215	4	3.225	0.938	0.697	-0.350	4	2.564	0.75	0.616	-0.220	5	3.038	0.689	0.678	-0.016
DIK5153	7	3.931	0.702	0.754	0.069	1	1	0	0	-	3	1.18	0.07	0.155	0.552*
BM6425	3	1.163	0.021	0.142	0.851*	11	5.543	0.511	0.828	0.386*	9	4.71	0.31	0.797	0.615*
BMS2321	7	4.383	1	0.78	-0.286	7	3.208	0.872	0.696	-0.257*	5	2.722	0.553	0.641	0.140*
CSSM066	7	3.116	0.917	0.686	-0.341	8	4.064	0.75	0.762	0.016*	8	4.84	0.744	0.803	0.074*
DIK1057	9	2.781	0.771	0.647	-0.194	4	1.862	0.681	0.468	-0.463	11	4.281	0.444	0.775	0.429*
BMS2213	8	4.656	0.761	0.794	0.042*	5	2.405	0.689	0.591	-0.168	3	1.897	0.452	0.479	0.055
DIK2866	11	4.331	0.659	0.778	0.154*	11	5.035	0.818	0.811	-0.010	9	6.697	0.871	0.865	-0.008
HUJI-13	8	3.365	0.489	0.71	0.313*	6	4.027	0.304	0.76	0.602*	6	4.252	0.297	0.775	0.620*
BMS817	11	4.897	0.708	0.804	0.120	6	2.304	1	0.572	-0.763	5	2.724	0.911	0.64	-0.431
BMS861	4	1.878	0.178	0.473	0.627*	3	1.182	0.167	0.156	-0.070	4	1.477	0.255	0.327	0.220
BM1443	10	4.452	0.979	0.784	-0.253	7	3.211	0.958	0.696	-0.383	6	3.196	0.978	0.695	-0.415
BMS2055	11	6.48	0.756	0.855	0.118	10	4.845	0.563	0.802	0.301*	10	5.508	0.705	0.828	0.150*
BMS119	7	3.383	0.75	0.712	-0.054	6	2.11	0.532	0.532	0.000	6	1.887	0.575	0.475	-0.212
BMS4037	9	5.056	0.578	0.811	0.290*	3	2.039	0.5	0.515	0.029	5	2.92	0.702	0.665	-0.057
BMS690	8	5.51	0.644	0.828	0.223*	7	4.053	0.646	0.761	0.153*	7	4.95	0.544	0.807	0.329*
Mean	**6.900**	**3.404**	**0.623**	**0.638**	-	**5.500**	**2.905**	**0.594**	**0.557**	-	**5.655**	**2.979**	**0.529**	**0.560**	-

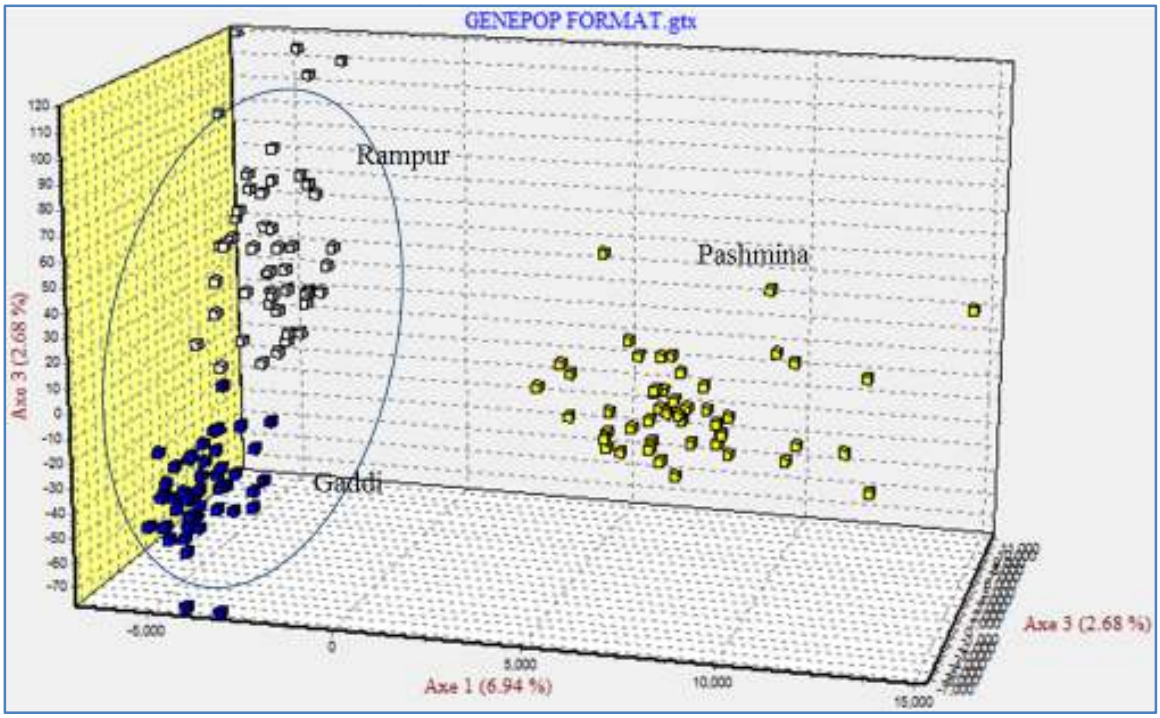
The Hardy-Weinberg equilibrium of loci was tested against null hypothesis of random union of gametes. GENEPOP v4.2.1 software was used to calculate estimate of F_{IS} (Weir and Cockerham, 1984) given in Table 2. The test revealed that out of 30 loci analysed 14 loci showed significant deviation from Hardy-Weinberg equilibrium in Pashmina goat population, 9 loci deviated in Gaddi goat and 12 loci in Rampur goat population.

Out of 30 microsatellites loci, 12 loci had negative F_{IS} values in Pashmina and Rampur goat population which indicate excess of heterozygotes (indicating out-breeding) and remaining 18 markers have positive F_{IS} values which indicate excess of homozygotes (inbreeding which is result of mating between related individuals), while in Gaddi 17 loci showed negative F_{IS} values and 13 showed F_{IS} positive values indicating large out-breeding in the Gaddi goat population. The overall inbreeding coefficients (F_{IS}) per locus revealed more positive than negative values, indicating a larger number of homozygotes in the population which might be representing local inbreeding effect.

AMOVA was performed to partition the total variation using Arlequin v3.5.1.2 software. Among population component contributed 14% of the total variation and the remaining 86% of the variation was within populations. The high within-population genetic diversity and fairly high among-population differentiation observed may be due to local inbreeding in different populations between the populations, such results were also observed in Indian goat study by Rout *et al.,* 2008.

The Correspondence Analysis was utilized to infer the relationship among 3 Himalayan goat populations (Figure 1) and exhibited three main clusters showing Gaddi goat and Rampur goat population clusters close to one another and distant from Pashmina goat population.

Figure 1: Clustering of individuals using Corresponding Analysis.



To find out whether the 3 goat populations are in mutation-drift equilibrium, Bottleneck v1.2.02 software (Piry *et al.,* 1999) was used. Three different tests viz., Sign test, Standardised Differences test and Wilcoxon test were performed to detect the microsatellite loci with significant transient heterozygosity excess under the assumption of three different mutation models viz. Infinite alleles model (IAM), Stepwise mutation model (SMM) and Two phase model (TPM).

The expected number of loci with heterozygosity excess in Pashmina goat were 17.41 (IAM), 17.76 (TPM) and 17.76 (SMM) while the corresponding observed values were 22, 13 and 6 respectively (Table 3). For Gaddi goat, the expected number of loci with heterozygosity excess were observed to be 15.6, 15.99 and 16.2 for IAM, SMM and TPM respectively while observed number of loci with heterozygotic excess 23, 16 and 10. While for Rampur goat population the expected number of loci with heterozygosity excess were 15.87, 16.29 and 16.35 for IAM, TPM and SMM respectively, and number of loci actually observed with heterozygosity excess were19, 17 and 9 respectively. The sign test revealed all the 3 Himalayan goat populations to be under mutation-drift equilibrium under IAM and TPM while the null hypothesis of mutation drift is significantly rejected for SMM but depicting heterozygotic deficiency.

For Standardized difference test the statistic T_2 values for IAM in all the 3 populations were greater than 1.645 (value from table of normal distribution) thus the null hypothesis of mutation-drift equilibrium was rejected in all the 3 goat populations for IAM (Table 3). While the null hypothesis of mutation-drift equilibrium was accepted for TPM and SMM models in all the 3 goat populations, as the T_2 values were less than 1.645, but there was significant heterozygotic deficiency instead of heterozygotic excess. Wilcoxon Rank test which is a nonparametric test gave probability values less than 0.05 for IAM and SMM models thus rejecting the null hypothesis of mutation-drift equilibrium, whereas for TPM the probability values were greater than 0.05 in the 3 goat populations thereby accepting the null hypothesis of mutation-drift equilibrium.

Mode shift test was also carried out to check if the population had gone a recent reduction in effective population size. The L-shaped curves were obtained for all the 3 Himalayan goat populations (Figure 2) indicating that these populations have not undergone any recent bottleneck. Mishra *et al.,* 2010 also showed L-shaped curve for Changthangi goat population, indicating no recent population bottleneck.

Figure 2: Mode shift test for 3 Himalayan populations.

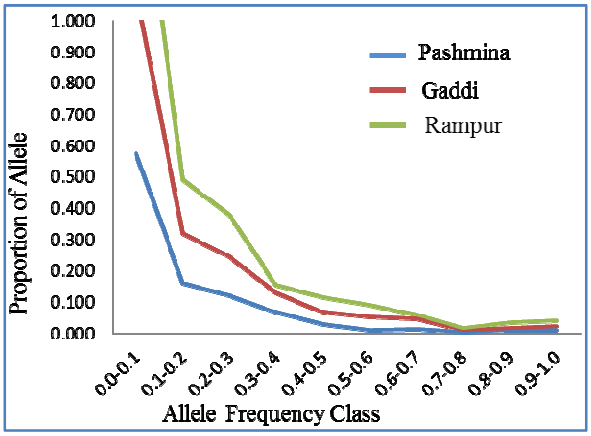


Table 3:Different tests values for mutation-drift equilibrium in the 3 Himalayan goat populations (where, IAM-Infinite alleles model, TPM-Two Phase Model, SMM-Stepwise mutation model and * = significant values (P<0.5).

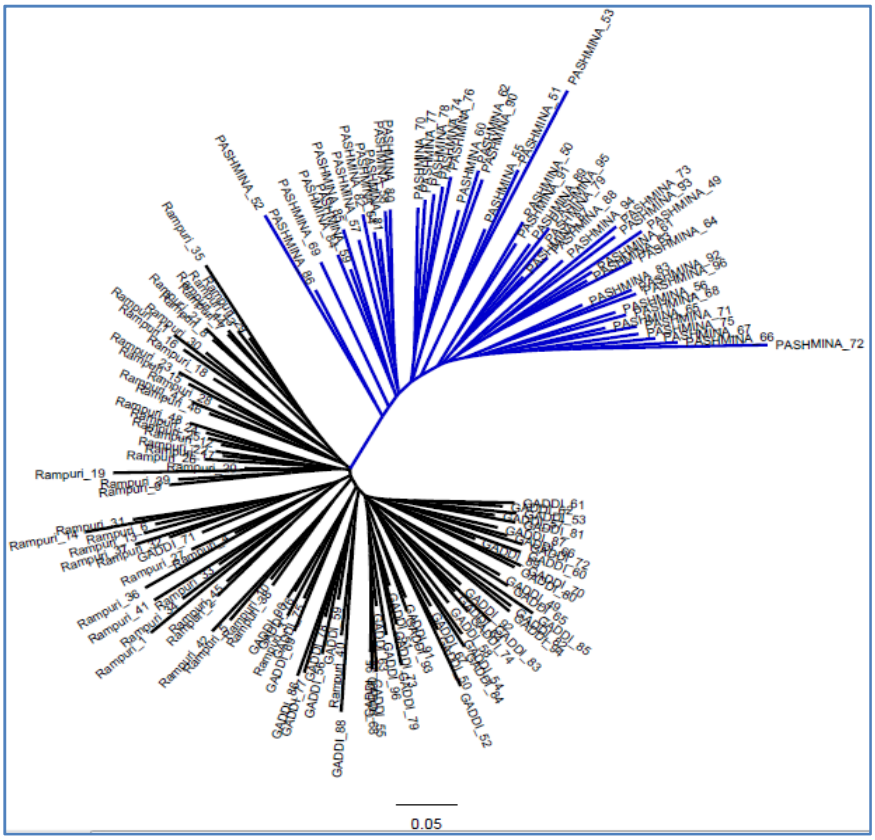
Sign's Test									
	IAM			TPM			SMM		
Populations	Expected No. of loci with heterozygotic excess	Observed No. of loci with heterozygotic Excess	Probability	Expected No. of loci with heterozygotic excess	Observed No. of loci with heterozygotic Excess	Probability	Expected No. of loci with heterozygotic excess	Observed No. of loci with heterozygotic Excess	Probability
Pashmina	17.410	22	0.063	17.760	13	0.058	17.760	6	0.000*
Gaddi	15.600	23	0.003*	15.990	16	0.577	16.200	10	0.015*
Rampuri	15.870	19	0.156	16.290	17	0.472	16.350	9	0.004*

Standardized Differences Test						
	IAM		TPM		SMM	
	T_2	Probability	T_2	Probability	T_2	Probability
Pashmina	2.090	0.018*	-1.244	0.107	-7.636	0.000*
Gaddi	2.862	0.002*	0.328	0.371	-4.416	0.000*
Rampuri	2.066	0.019*	-0.671	0.251	-5.662	0.000*

Wilcoxon Test			
	IAM	TPM	SMM
	Probability (two tails for H excess and deficiency)	Probability (two tails for H excess and deficiency)	Probability (two tails for H excess and deficiency)
Pashmina	0.022*	0.452	0.000*
Gaddi	0.000*	0.522	0.001*
Rampuri	0.019*	0.552	0.007*

The Neighbor joining tree was constructed based on inter-individual genetic distance from the Nei's standard D_S (Figure 3) which clearly revealed two clusters, one cluster of goats belonging to higher altitude region i.e. Pashmina goat and other cluster of goats belonging to lower altitude region i.e. Gaddi and Rampur goat populations. This may be due to the reason that Pashmina goats were quite isolated and has different genetic structure as they thrive in high altitude region where the temperature drops to-40°C. Similar results were reported by Dixit *et al.,* 2012, where high altitude region goats (Chegu and Changthangi goat) differed from that of the lower altitude region goats (Gaddi goat) on the basis of microsatellite markers.

Figure 3: Inter-individual radiation tree of 3 Himalayan goat populations based on Nei's standard genetic distance.



Mitochondrial D-loop analysis of high and low altitude Himalayan goats

D-loop sequence analysis of 72 goat sequences (24 samples from each population) of 835bp mtDNA D-loop region was done and a very high number of haplotypes were identified, 52 mtDNA haplotypes were identified. These haplotypes were submitted in NCBI (Accession numbers: Pashmina: KC818039-KC818062, Gaddi: KC817883-KC817906 and Rampur goats taken as Local Himachali population: KC817948-KC817953). 99 polymorphic sites were found out of which 51 were singleton and 48 were

Parsimony informative sites. The highest number of polymorphic sites (57) was found in high altitude goats (Pashmina goat). The haplotype diversity value was found to be highest 1.000 in both Pashmina and Gaddi goat breeds although the nucleotide diversity was found to be low in all the 3 populations, being highest for Pashmina population (0.012) are given in Table 4. Haplotype diversity reflects a difference in variability. This pattern of high haplotype diversity and low nucleotide diversity may be an indication of recent expansion (Bras *et al.,* 2013) under the infinite site model. High genetic diversity also reduces extinction risk in the species as it provides the evolutionary potential to adapt to the rapidly changing environmental conditions (Hobbs *et al.,* 2012).

Table 4: Sample size, number of haplotypes, number of polymorphic sites, haplotype diversity and nucleotide diversity of 3 Himalayan goat populations of India.

Populations	Sample Size	No. of haplotypes	No. of polymorphic sites	Haplotype Diversity	Nucleotide Diversity
Pashmina	24	24	57	1.000	0.012
Gaddi	24	24	53	1.000	0.0114
Rampur	24	6	24	0.826	0.008

Analysis of molecular variance (AMOVA) calculated revealed that 4.18 % of the total genetic variation was among populations while the remaining 95.82% was within populations which indicated that the individuals within the populations contribute more to the variability compared to between population component (Oliveira *et al.,* 2007).

The demographic history was examined by Tajima's D test of neutrality (Tajima, 1989) and Fu's Fs statistics test (Fu, 1997), to test for deviation of sequence variation from evolutionary neutrality (Table 5). Fu's Fs statistic (Fu, 1997), is based on the probability of having a number of alleles greater or equal to the observed number in a sample drawn from a stationary population, this is a sensitive test for population expansion. Table 5 gives the values of Tajima's D and Fu's Fs statistics test values for the 3 Himalayan goat populations. Neutrality tests of Tajima's D revealed non-significant negative values in all populations indicating an excess of rare nucleotide site variants compared to what would be expected under a neutral model of evolution, except Rampur goats which revealed positive but non-significant values. However, large negative Fu's Fs values were observed for Pashmina and Gaddi goat populations which were highly significant and indicated excess of rare mutations, an evidence for historical population expansion (Fu, 1997) but were exceptionally positive and non-significant for Rampur

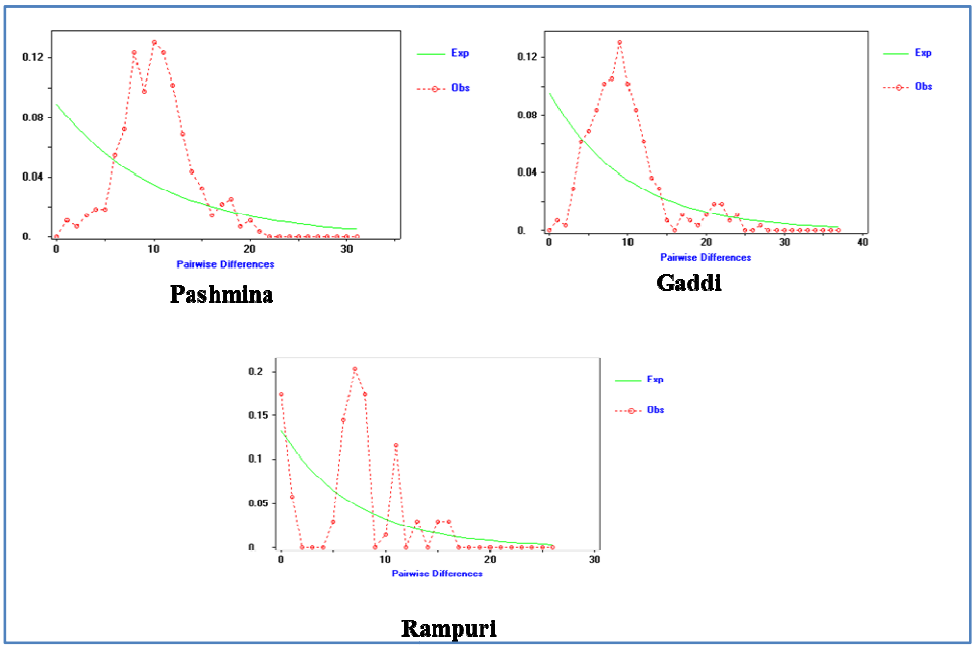
population indicating stationary population. Fu's Fs is considered to be more sensitive in detecting deviations from neutrality than Tajima's D and thereby suggesting possible population expansion (Makhawi *et al.,* 2013) or purifying selection in Pashmina and Gaddi population while Rampur goat population may have undergone genetic bottleneck or it may be the effect of balancing selection in the population. The expansion may have been restricted to separate local areas that resulted in the non-significant negative Tajima's D values (Makhawi, 2013). The results seem to be indicative as the generation interval in goats is small and have a very high replacement rate due to high kidding rate and usage for meat purpose.

Table 5: Tajima's D and Fu's Fs values of 3 Himalayan goat populations of India, where * = significant values (P<0.5).

Populations	Tajima's D (p)	Fu's Fs(p)
Pashmina	-1.271	-16.667*
Gaddi	-1.292	-17.608*
Rampur	0.088	4.746
Mean	**-0.825**	**-9.843**

Mismatch distribution has been extensively used for estimating the demographic parameters of past population expansion or contraction. It is the distribution of the observed number of differences between pairs of haplotypes. Populations at demographic equilibrium show a multimodal distribution while unimodal mismatch distributions have been interpreted as being due to past demographic expansions (Slatkin and Hudson 1991, Rogers and Harpending, 1992). In the present study, unimodal mismatch distribution curves were observed for Pashmina (vaguely bimodal)and Gaddi goat populations, clearly supporting population expansion unlike Rampur goat population which showed multimodal mismatch distribution curve (Figure 4) pointing towards a population at demographic equilibrium or stable population.

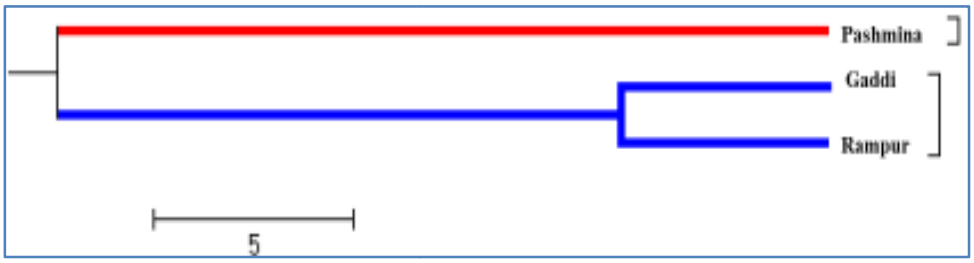
Figure4: Mismatch distribution graphs for 3 Himalayan goat populations of India.



Thus, both the Tajima and Fu's F_S tests as well as the mismatch distribution models revealed similar results showing population expansion in Pashmina and Gaddi goat populations while slight reduction or stability in the Rampur goat population.

A Neighbor-joining tree based on mtDNA D-loop sequences was also constructed and clear differentiation between the high altitude region and low altitude region goats was seen as Gaddi and Rampur goats belonging to lower Himalayan region clustered together while Pashmina goats which belongs to higher altitudes of Himalayas formed a separate group (Figure 5). The microsatellite data also revealed similar result.

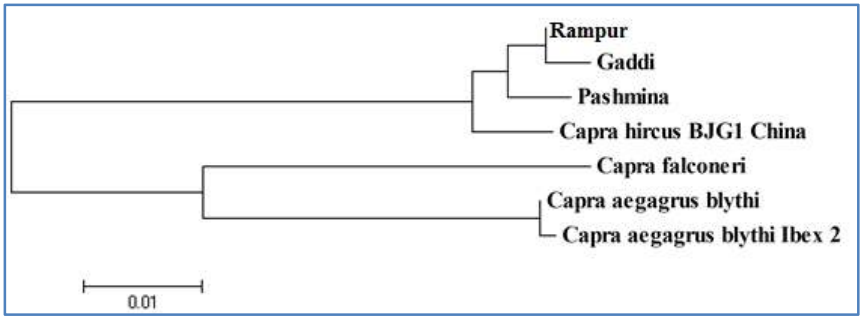
Figure5: Neighbor-joining tree of 3 Himalayan goat populations of India based on the mitochondrial D-loop sequences.



The high altitude region of Leh and Ladhakh is also the bordering region of China and share topographically similar environment and altitude regions thus we compared the goat sequences of this present study with the China goat sequence (DQ121491) and the wild goat sequences (AB044306.1,

AB110590.1 and AB110591.1) taken as out-group. We found that the lower altitude region goats Rampur goat and Gaddi goat clustered together while the Pashmina goat population was found to be closer to that the China goat sequence (Figure 6), this may be because of their similar breeding tract and similar altitude regions and probable exchange of germplasm, while the wild goat sequences differed completely.

Figure 6: Neighbor-joining tree of 3 Indian goat populations, China goat and wild goat mtDNA D-loop sequences.



CONCLUSION

In the present study, both microsatellite and mitochondrial D-loop analysis were employed for comparing the genetic diversity of goats of higher altitudes (Pashmina goat) with goats of lower altitudes (Gaddi and Rampur local goat of Himachal Pradesh) of Himalayan region of India. The microsatellite data analysis revealed that there is good amount of heterozygosity in the Himalayan goat populations of India. Inbreeding was observed in Pashmina and Rampur population while out-breeding was observed for Gaddi population. All the populations were found to be in mutation-drift equilibrium. AMOVA results revealed high within population variation. The mtDNA analysis revealed high haplotype diversity in all the 3 goat populations. Demographic study, using mitochondrial D-loop revealed slight population expansion in Pashmina and Gaddi while largely stable population size in Rampur goat population. The phylogenetic analysis for both microsatellite as well as mitochondrial D-loop analysis revealed similar results where Pashmina being a higher Himalayan altitude region goat population was different from Gaddi and Rampur goat populations both of which belong to lower Himalayan altitude regions and probably have different evolutionary origin than Pashmina goat.

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REFRENCES

1. A. Rambaut, Institute of Evolutionary Biology, University of Edinburgh, UK (2006–2012).
2. A.M. Makhawi, X.B.Liu, S.R. Yangand Q.Y. Liu. *Parasites & Vectors,* 2013, 6:290.
3. A.R. Rogers and H. Harpending. *Mol. Biol. Evol.,* 1992, 9: 552–569.
4. B. S. Weir and C. C. Cockerham. *Evolution,* 1984, 38: 1358-1370.
5. C.L.Vanya.(adaptation-of-goats-by-charles-langton-vanya) 2011.
6. F.Tajima. *Genetics*, 1989, 123 (3): 585–95.
7. F.C. Yeh, R.C. Yang, T.B.J. Boyle, Z.H. Ye and J.X. Mao. POPGENE Version 1.32, Molecular Biology and Biotechnology Centre (University of Alberta, Canada, 2000).
8. J. Rozas, P. Librado, J. C. Sánchez-DelBarrio, X. Messeguer and R. Rozas. Universitat de Barcelona. Current Released Version: 5.10.1 (March 4, 2010).
9. J.D. Oliveira, M.L.S. Paiva Igarashi, T.M.M. Machado,M.M. Miretti,J.A. Ferro and E.P.B. Contel. *Genet. Mol. Biol.,* 2007, 30, 2, 356-363.
10. J.P.A. Hobbs, L. Herwerden, D.R. Jerry, G.P. Jones and P.L. Munday. *Diversity*, 2013, 5, 39-50.
11. K. Belkhir, P. Borsa, L. Chikhi, J. Goudet and F. Bonhomme. GENETIX 4.05. University of Montpellier, France, Universite Montpellier II//www.univmontp2. fr/∼genetix/genetix/genetix.htm//December 2004 (1996-2004).
12. L.Excoffier and H.Lischer.Arlequin suite ver3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol.Resour.*, 2010, 10: 564–567.
13. M. Raymond and F. Rousset. *Evolution,* 1995, 49:1280-1283.
14. M. Slatkin and R.R. Hudson. *Genetics*, 1991, 129: 555–562.
15. O. Langella. Populations 1.2.28. Available from [Email] Olivier.langella@moulon.inra.fr. (Last accessed 3 September 2013) 1999.
16. P. K. Rout, M.B. Joshi, A. Mandal, D. Laloe, L. Singh and K. Thangaraj. *BMC Genet.*, 2008, 9(11):1-11.
17. P. Mishra, N.K. Verma, R.A.K. Aggarwal, and S.P. Dixit. *Indian. J. Anim. Sci.,* 2010, 80(12), 43-48.
18. P.G. Bras, R. Martins, J. Martins, M.T. Rebelo, J.C. Franco, C. Mateus, O.S. Paulo, E. Figueiredo and S.G. Seabra. *XIV Congress ofEuropean Society for Evolutionary Biology*, 2013, 19-24 Ago, Lisboa, Portugal.
19. S. Piry, G. Luikart and J.M. Cornuet. *J. Hered.,* 1999, 90:502–503.
20. S.P. Dixit, N.K. Verma, R.A.K. Aggarwal, M.K. Vyas, J. Rana, and A. Sharma. *Small Rumin. Res.*, 2012, 105:38– 45.
21. J. Sambrook and D.W. Russell. Molecular Cloning: A Laboratory Manual. 3rd ed. Cold Spring HarborLaboratory Press, Cold Spring Harbor (New York,2001).

22. T.A. Hall. *Nucleic Acids Symp.,* 1999, 41:95–98.

23. Y.X. Fu. *Genetics*, 1997, 147: 915-925.