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Genetic Diversity Analysis of the Gohilwari Breed of Indian Goat (*Capra hircus*) Using Microsatellite Markers

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Abstract: Problem statement: Gohilwari breed of goat is a multipurpose goat mainly for milk and meat purposes and best suited in its harsh climatic condition. This breed is inadequately characterized till now at DNA level. So the present study was undertaken for population genetic analysis at molecular level to exploit the breed for planning sustainable improvement, conservation and utilization, which subsequently can improve the livelihood of its stake holders. **Approach:** The experiment was conducted on 50 genomic DNA samples of unrelated goat using 25 microsatellite markers selected from the list suggested by International Society for Animal Genetics (ISAG) and FAO's (DAD-IS). **Results:** All of the 25 microsatellites were well amplified. The observed number of alleles detected per locus ranged from 4-24 with an overall mean of 10.12±5.46. Overall mean observed heterozygosity of 0.505 was lower than the overall mean expected heterozygosity of 0.684. Most of the loci showed the heterozygote deficit as also depicted by F_{is} value. There was substantial genetic variation and polymorphism across studied loci in the Gohilwari breed of goat. And this population was not in Hardy-Weinberg equilibrium at most of the studied loci. This population was also receiving new genetic materials through introduction of immigrants. **Conclusion:** The strong inference that the Gohilwari breed of goat has not undergone bottleneck is also important for goat breeders and conservationists, as it suggests that any unique alleles present in this breed may not have been lost. Therefore, it can be recommended that within-breed diversity is actively maintained to enable these extensively unmanaged stocks to adapt to future demands and conditions and there is ample scope for further improvement in its productivity through appropriate breeding strategies. Though, microsatellites are neutral to selection with Ewens-Watterson test for neutrality some microsatellites were found not neutral or linked to some selective trait that must be further investigated for association to selective traits.

Key words: Microsatellite, Gohilwari, goat

INTRODUCTION

Gohilwari breed of goat is a multipurpose goat mainly reared by the Maldharis (Bharwar and Rabbari communities) for milk and meat purposes. The breed derived its name from the Gohilwad, which was a part of the Kathiawar region and was also the old name of Bhavnagar district of Gujarat state of India. The animals of this goat breed are mainly found in Junagarh, Amrelli and Bhavnagar districts and also to other adjacent districts of Gujarat. The goats are best fit under the harsh climate conditions of this region. In spite of their ecological and economic importance, the Gohilwari goats are inadequately characterized particularly at DNA level. Microsatellites in particular are useful in conservation genetics because the high degree of polymorphism makes them extremely informative and gives them very high discriminating

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power[12], allowing for a thorough assessment of genetic variation and structure within and among populations[6]. Genetic diversity is essential for the long-term survival of the species and populations because it provides the raw material for adoption and evolution, especially when environmental conditions have changed[10,29]. A central objective of genetic resources conservation, therefore, is to maintain genetic integrity and natural levels of genetic diversity and to enhance genetic diversity in populations and species where it has been eroded[29]. Therefore, to find out within breed genetic diversity a set of twenty five selected microsatellite s have been used. This study has been undertaken to search for the genetic variability, which could be exploited for planning sustainable improvement, conservation and utilization of the breed, which subsequently can improve the livelihood of its stake holders.

MATERIALS AND METHODS

Isolation of genomic DNA and its amplification through PCR: Genomic DNA was isolated from blood samples of 48 unrelated animals of the breed by the method described by Sambrook *et al*.[33]. A battery of 25 microsatellite markers (Table 1) was selected based on the guideline of ISAG and FAO's DADIS programme to generate data.

Table 1: Microsatellite markers, their sequences, dye labeled, type of repeat, amplified product size, location and accession numbers

Locus	Primer sequence	Dye	Type of repeat	Size range	*Ch. No	Gen bank accession No.
ILST008	gaatcatggattttctgggg tagcagtgagtgaggttggc	FAM	$(CA)_{12}$	167-195	14	L23483
ILSTS059	gctgaacaatgtgatatgttcagg gggacaatactgtcttagatgctgc	FAM	$(CA)_4(GT)_2$	105-135	13	L37266
ETH225	gatcaccttgccactatttcct acatgacagccaagctgctact	VIC	$(CA)_{18}$	146-160	14	Z14043
ILST044	agtcacccaaaagtaactgg acatgttgtattccaagtgc	NED	$(GT)_{20}$	145-177	Ann	L37259
ILSTS002	tctatacacatgtgctgtgc cttaggggtgtattccaagtgc	VIC	$(CA)_{17}$	113-135	Ann	L23479
OarFCB304	ccctaggagctttcaataaagaatcgg cgctgctgtcaactgggtcaggg	FAM	$(CT)_{11}$	119-169	Ann	L01535
OarFCB48	gagttagtacaaggatgacaagaggcac gactctagaggatcgcaaagaaccag	VIC	$(CT)_{15}$ $(CT)_{10}$	149-181	17	M82875
OarHH64	cgttccctcactatggaaagttatatatgc cactctattgtaagaatttgaatgagagc	PET	-	120-138	4	212[a]
OarJMP29	gtatacacgtggacaccgctttgtac gaagtggcaagattcagaggggaag	NED	$(CA)_{21}$	120-140	Ann	U30893
ILSTS005	ggaagcaatgaaatctatagcc tgttctgtgagtttgtaagc	VIC	$(nn)_{39}$	174-190	10	L23481
ILSTS019	aagggacctcatgtagaagc acttttggaccctgtagtgc	FAM	$(TG)_{10}$	142-162	Ann	L23492
OMHC1	atctggtgggctacagtccatg gcaatgctttctaaattctgaggaa	NED	-	179-209	Not reported	228[a]
ILSTS087	agcagacatgatgactcagc ctgcctcttttcttgagagc	NED	$(CA)_{14}$	142-164	Ann	L37279
ILSTS30	ctgcagttctgcatatgtgg cttagacaacaggggtttgg	FAM	$(CA)_{13}$	159-179	2	L37212
ILSTS34	aagggtctaagtccactggc gacctggtttagcagagagc	VIC	$(GT)_{29}$	153-185	5	L37254
ILSTS033	tattagagtggctcagtgcc atgcagacagttttagaggg	PET	$(CA)_{12}$	151-187	12	L37213
ILSTS049	caattttcttgtctctcccc gctgaatcttgtcaaacagg	NED	$(CA)_{26}$	160-184	11	L37261
ILSTS065	gctgcaaagagttgaacacc aactattacaggaggctccc	PET	$(CA)_{22}$	105-135	24	L37269
ILSTSO58	gccttactaccatttccagc catcctgactttggctgtgg	PET	$(GT)_{15}$	136-188	17	L37225
ILSTSO29	tgttttgatggaacacagcc tggatttagaccagggttgg	PET	$(CA)_{19}$	148-191	3	L37252
RM088	gatcctcttctgggaaaaagagac cctgttgaagtgaaccttcagaa	FAM	$(CA)_{14}$	109-147	4	U10392
ILSTS022	agtctgaaggcctgagaacc cttacagtccttggggttgc	PET	$(GT)_{21}$	186-202	Ann	L37208
OARE129	aatccagtgtgtgaaagactaatccag gtagatcaagatatagaatattttcaacacc	FAM	$(CA)_{14}$	130-175	7	L11051
ILSTS082	ttcgttcctcatagtgctgg agaggattacaccaatcacc	PET	$(GT)_{17}$	100-136	2	L37236
RM4	cagcaaaatatcagcaaacct ccacctgggaaggccttta	NED	$(CA)_{13}$	104-127	15	U32910

*: Chromosome number; [a]: Accession number of Arkdb data base (http://www.thearkdb.org)

Only forward primers at 5' end of each pair were labeled with one of the four fluorophore i.e., FAM (Blue), VIC (Green), NED (Yellow) and PET (red). Most of the microsatellite primers used was independent and belonged to different chromosome except (ILSTS30 and ILSTS082 on Chromosome 2, RM088 and Oar HH64 on chromosome 4, ILSTS008 and ETH225 on chromosome 14, OarFCB48 and ILSTS058 on chromosome 17). Polymerase Chain Reaction (PCR) was carried out on about 50-100 ng genomic DNA in a 25 µL reaction volume. The reaction mixture consisted of 200 µM of each dNTP, 50 nM KCL, 10 mM Tris-HCL (pH 9.0), 0.1% Triton X-100, 2.0 mM $MgCl_2$, 0.75 unit Taq DNA polymerase and 4 ng μL^{-1} of each primer using PTC-200 PCR machine (MJ Research). The 'touchdown' PCR protocol used with initial denaturation of 95°C for 3 min, 3 cycles of 95°C for 45 sec and 60°C for 1 min, 3 cycles of 95°C for 45 sec and 57°C for 1 min, 3 cycles of 95°C for 45 sec and 54°C for 1 min and 20 cycles of 95°C for 45 sec and 51°C for 1 min with final extension at 72°C for 5 min. PCR products were loaded on to a 2% agarose gel, electrophoresed and visualized over UV light after ethidium bromide staining to detect the amplification.

Genotyping and allele detection: After determining the optimal pooling ratio and dilution ratio for a set of primers, the PCR products were mixed in ratio of 1:1.5:2:2 of FAM (blue), VIC (green), NED (yellow) and PET (red) labeled respectively. 0.5 µL of this mixture was combined with 0.3 µL of Liz 500 as internal lane standard (Applied Biosystems) and 9.20 µL of Hi-Di Formamide per sample. The resulting mixture was denatured by incubation for 5 min at 95°C. These denatured samples were run on automated DNA sequencer of Applied Biosystems (ABI 3100 Avant). The electropherograms drawn through Gene Scan were used to extract DNA fragment sizing details using Gene Mapper software (version 3.0) (Applied Biosystems).

Statistical analysis: Genetic diversity within population was determined as the observed and expected number of alleles[17] and Shanon's Information Index[22] using Popgene software[39]. Observed and expected heterozygosity were calculated as per Levene[21] as implemented in Arlequin software (version 3.11)[11]. A Monte Carlo method[14], with forecasted chain length 1000000 was used to compute unbiased estimate of the exact probability (p-value) also implemented in the Arlequin. Wright's F-statistics[37] were estimated in accordance with the procedures described by Weir and Cokerhan[35] using the F-stat 2.9.3[13]. A more appropriate measure of genetic variation within a population is gene diversity (average expected heterozygosity)[27] at each locus was calculated by the same software. Polymorphic Information Content (PIC) value was calculated according to Botstein *et al*.[5] implemented in Cervus 3.0.3 software package[17]. Hardy-Weinberg Equilibrium (HWE) at each locus was tested by Chi Squire (χ^2) goodness-of-fit test with Yat's Correction and significant test was done with Bonferroni corrections[30] to reduce the type I error, implemented in Cervus 3.0.3 software package[40]. Ewens-Watterson test was performed to test the neutrality for microsatellite markers; the statistics F (sum of square of allelic frequency) and limit (upper and lower) at 95% confidence region for the test were calculated using the algorithm by Manly[25] using 1000 simulated samples and implemented in Popgene software package[39]. Bottleneck events were tested by three methods. The first method consisted of three excess heterozygosity tests developed by Cornuet and Luikart[9]; (i) sign test (ii) standardized difference test and (iii) wilcoxon sign-rank test. The probability distribution was established using 1000 simulations under three models; Infinite Allele Model (IAM), Step wise Mutation Model (SMM) and Two Phase Model of mutation (TPM).

The second method was the graphical representation of mode-shift indicator originally proposed by Luikart *et al*.[23]. Loss of rare alleles in bottlenecked populations is detected when one allele class have a higher number of alleles than the rare allele class[23]. This test was rescaled so that frequency distribution of the allele frequency class would be based on equal 0.05 increments. These two methods were conducted using Bottleneck (version 1.2.03)[9].

RESULTS

Various measures of genetic variation in terms of allele number, information index, PIC value and gene diversity are presented in Table 2. The observed number of alleles detected per locus ranged between 4 (ILST008, ETH225, OarJMP29 and RM088) to 24 (OarFCB304) with an overall mean of 10.12±5.46.

Shannon's Information Index[22], which measures the level of diversity, was sufficiently high with an overall mean of 1.603. Most of the studied loci showed the Polymorphic Information Content (PIC) values greater than 0.5 except a very few loci with an overall mean 0.647.

The average expected heterozygosity was with an over all mean of 0.686 (Table 2). In Gohilwari goat breed, the mean effective number of alleles (4.78) was less than the half of the observed number of alleles (9.04) (Table 2).

Table 2: Number of alleles (Observed: n_a and effective: n_e), Shannon's Information index (I) and Polymorphic Information Content (PIC) for Gohilwari goats

Locus	n_a	n_e	I	PIC	Gene diversity
ILST008	4.0000	1.4122	0.5890	0.273	0.295
ILSTS059	6.0000	2.5860	1.1348	0.541	0.628
ETH225	4.0000	2.0306	0.9488	0.464	0.526
ILSTS044	11.0000	2.0507	1.2654	0.498	0.520
ILSTS002	12.0000	8.1337	2.2678	0.866	0.893
OarFCB304	24.0000	9.0865	2.6726	0.883	0.902
OarFCB48	11.0000	5.3629	1.9294	0.791	0.823
OarHH64	12.0000	8.9476	2.2986	0.878	0.900
OarJMP29	4.0000	1.0937	0.2293	0.084	0.087
ILSTS005	7.0000	2.6523	1.2652	0.574	0.635
ILSTS019	9.0000	5.2158	1.8465	0.784	0.818
OMHC1	17.0000	10.6420	2.5340	0.899	0.916
ILSTS087	13.0000	8.0222	2.2606	0.863	0.893
ILSTS30	9.0000	5.9606	1.9362	0.811	0.842
ILSTS34	6.0000	1.6329	0.8202	0.366	0.393
ILSTS033	12.0000	3.7921	1.6797	0.701	0.747
ILSTS049	9.0000	3.4047	1.5594	0.671	0.717
ILSTS065	6.0000	3.1625	1.3018	0.628	0.697
ILSTS058	23.0000	12.7735	2.8052	0.917	0.939
ILSTS029	14.0000	5.5954	2.0448	0.801	0.830
RM088	4.0000	1.8398	0.7801	0.388	0.464
ILSTS022	6.0000	1.9523	0.9137	0.431	0.494
OarAE129	9.0000	3.6736	1.6306	0.699	0.735
ILSTS082	15.0000	5.9767	2.1839	0.818	0.840
RM4	6.0000	2.6197	1.1667	0.544	0.628
Mean	10.1200	4.7848	1.6026	0.647	0.686
SD	5.4568	3.2091	0.6913	0.223	0.219

n_a: Observed number of alleles; n_e: Effective number of alleles[17]; I: Shannon's Information index[22]; PIC: Polymorphic Information Content

Observed heterozygosity was lowest (0.074) at ETH225 locus and highest (0.979) at ILSTS082 locus with overall mean of 0.505 (Table 3). Expected heterozygosity ranged from 0.0869 (OarJMP29) to 0.935 (ILSTS058) with an over all mean of 0.684. The observed heterozygosity was lower than that of the expected heterozygosity at most of the loci except OarJMP29, ILSTS029, OarAE129 and ILSTS058.

This breed of Goat also deviated from HWE at 15 loci out of 25.

Ewens-Watterson test for neutrality of microsatellite markers: As the microsatellite markers have the specific property, as they are neutral to selection even the neutrality of each microsatellite marker was tested by Ewens-Watterson test for neutrality. In Gohilwari goat, F value (sum of square of allelic frequency) lied outside the lower and upper limit of 95% confidence region of expected F value at 6 loci (ILSTS044, ILSTS002, OarHH64, OarJMP29, OMHC1 and ILSTS030) (Table 4).

Table 3: Observed and expected heterozygosity with p-value, F_{is} value for each microsatellite locus and mean estimate of different parameters for Gohilwari goats

Locus	Obs. Het.	Exp. Het.	p-value	SD	F_{is}	HWE
ILST008	0.25000	0.29496	0.22591	0.00043	0.154	NS
ILSTS059	0.17391	0.62327	0.00000	0.00000	0.723	***
ETH225	0.07407	0.51712	0.00000	0.00000	0.859	***
ILSTS044	0.35417	0.51776	0.00049	0.00002	0.318	ND
ILSTS002	0.56410	0.88844	0.00008	0.00001	0.368	***
OarFCB304	0.85366	0.90093	0.11483	0.00014	0.053	NS
OarFCB48	0.79545	0.82288	0.51338	0.00049	0.034	NS
OarHH64	0.66667	0.89759	0.00000	0.00000	0.259	***
OarJMP29	0.08824	0.08692	1.00000	0.00000	-0.015	NS
ILSTS005	0.20000	0.62996	0.00000	0.00000	0.685	***
ILSTS019	0.76744	0.81778	0.95550	0.00020	0.062	NS
OMHC1	0.83333	0.91557	0.02936	0.00014	0.091	NS
ILSTS087	0.42105	0.88702	0.00000	0.00000	0.529	***
ILSTS30	0.78261	0.84138	0.05658	0.00019	0.071	***
ILSTS34	0.25000	0.39167	0.00000	0.00000	0.364	***
ILSTS033	0.48889	0.74457	0.00000	0.00000	0.346	NS
ILSTS049	0.43478	0.71405	0.00004	0.00001	0.394	***
ILSTS065	0.19149	0.69115	0.00000	0.00000	0.725	***
ILSTS058	0.70588	0.93547	0.00031	0.00001	0.248	***
ILSTS029	0.86364	0.83072	0.00000	0.00000	-0.040	***
RM088	0.27907	0.46183	0.00012	0.00001	0.399	***
ILSTS022	0.38298	0.49302	0.01885	0.00012	0.225	***
OarAE129	0.82609	0.73579	0.53051	0.00041	-0.124	NS
ILSTS082	0.97917	0.84145	0.00067	0.00002	-0.166	NS
RM4	0.40000	0.62522	0.00016	0.00001	0.363	***
Mean	0.50507	0.68426			0.264	
SD	0.28051	0.21917				

p-value for F_{is} within samples based on: 500 randomizations; Indicative adjusted nominal level (5%) is: 0.00200; NS: Not Significant; ***: Significant at the 0.1% level

Table 4: The Ewens-Watterson test for Neutrality at 25 microsatellite loci in Gohilwari goat breed

Locus	k	Obs. F	SE	L95	U95
ILST008	4	0.7081	0.0285	0.3099	0.8997
ILSTS059	6	0.3867	0.0192	0.2255	0.7469
ETH225	4	0.4925	0.0246	0.2929	0.8594
ILSTS044	11*	0.4876	0.0067	0.1419	0.4505
ILSTS002	12*	0.1229	0.0047	0.1239	0.3892
OarFCB304	24	0.1101	0.0005	0.0634	0.1478
OarFCB48	11	0.1865	0.0061	0.1369	0.4282
OarHH64	12*	0.1118	0.0053	0.1309	0.4240
OarJMP29	4*	0.9144	0.0253	0.3058	0.8607
ILSTS005	7	0.3770	0.0165	0.1975	0.6835
ILSTS019	9	0.1917	0.0106	0.1650	0.5654
OMHC1	17*	0.0940	0.0022	0.0972	0.2776
ILSTS087	13	0.1247	0.0035	0.1170	0.3431
ILSTS30	9*	0.1678	0.0100	0.1694	0.5603
ILSTS34	6	0.6124	0.0199	0.2307	0.7706
ILSTS033	12	0.2637	0.0056	0.1269	0.4042
ILSTS049	9	0.2937	0.0100	0.1626	0.5385
ILSTS065	6	0.3162	0.0206	0.2275	0.7836
ILSTS058	23	0.0783	0.0005	0.0631	0.1440
ILSTS029	14	0.1787	0.0036	0.1103	0.3474
RM088	4	0.5435	0.0277	0.3102	0.8886
ILSTS022	6	0.5122	0.0198	0.2259	0.7648
OarAE129	9	0.2722	0.0107	0.1638	0.5735
ILSTS082	15	0.1673	0.0033	0.1068	0.3220
RM4	6	0.3817	0.0205	0.2264	0.7560

k: No. of alleles; Obs. F: Observed sum of the squared of allelic frequency; L95, U95: The 95% confidence interval upper and lower limit; SE: Standard error for observed F were calculated using 1000 simulated sample; *: F-value that outside the limit (lower and upper) of 95% confidence region

Table 5: Test for null hypothesis under three microsatellite evolution models, (genetic bottleneck analysis)

IAM		TPM		SMM	
Expected	Observed	Expected	Observed	Expected	Observed
Sign test: Number of loci with heterozygosity excess (probability)					
15.02	15	14.81	8	14.79	3
(0.57288)		(0.00543)		(0.00000)	
Standard differences test: T_2 values (probability)					
0.643		-4.435		-11.841	
(0.26025)		(0.00000)		(0.00000)	
Wilcoxon-rank test (probability of heterozygosity excess)					
0.16270		0.99201		1.00000	

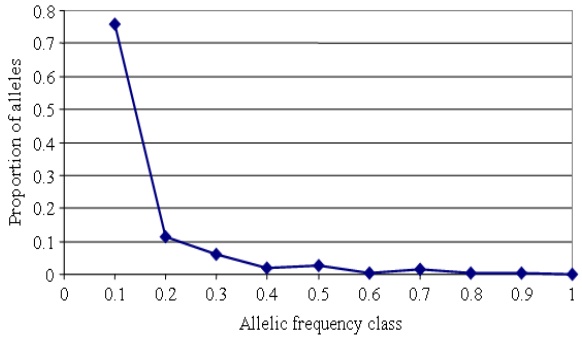

Fig. 1: Graphical representation of proportions of alleles and their distribution in Gohilwari goat breed

Genetic bottleneck: In Gohilwari goat, under Sign test, the expected numbers of loci with heterozygosity excess were 14.81 (TPM) and 14.79 (SMM) which were substantially higher than the observed numbers of loci 8 (TPM) and 3 (SMM) with heterozygosity excess (Table 5). So the null hypothesis that as the population is under Mutation-drift equilibrium was accepted. The expected number of loci (15.02) with heterozygosity excess was not significantly ($p>0.05$) higher than the observed numbers of loci (15) with heterozygosity excess under IAM. So, the null hypothesis was again accepted under IAM for the sign test. Standard difference test (T_2 statistics) in this population provided the significant ($p<0.05$) gene diversity deficit under TPM (-4.435) and SMM (-11.841) (Table 5). In IAM there was heterozygosity excess (0.643) but not significant ($p>0.05$). Positive values of the Bottleneck statistic T_2 are indicative of gene diversity excess caused by a recent reduction in effective population size, while negative value are consistent with a recent population expansion without immigration or immigration of some private (unique) alleles in population. Under Wilcoxon rank test, probability values of 0.1627 (IAM), 0.99201 (TPM) and 1.0 (SMM) were non-significant ($p<0.05$). So, null hypothesis of mutation drift equilibrium was accepted under all the tests under all the three models.

The mode shift indicator i.e. qualitative method of estimation of bottleneck showed the normal L-shaped curve[23] (Fig. 1) in graphical representation of proportion of alleles verses class of frequency distribution.

DISCUSSION

All measures of genetic variation: observed number of alleles, effective number of alleles, Shannon's Information Index and PIC values showed that most of the studied loci were highly informative, indicating high polymorphism across the loci, thus suggesting suitability of these markers for genetic diversity studies in goats. Suitability of these studied markers was further strengthened as the number of alleles for each marker was higher, than the minimum number of four alleles recommended for microsatellite markers to be used in the estimation of genetic distance[41] in order to reduce the standard error.

The average expected heterozygosity i.e., gene diversity[27] was in the range of 0.3 to 0.8 as determined by Takezaki and Nei[34] for markers to be useful in measuring genetic variation in a population.

Overall mean observed heterozygosity was lower than the overall mean expected heterozygosity. Most of the loci showed the heterozygote deficit as also depicted by F_{is} value (Table 3).

Mean number of alleles observed over a range of loci in different populations is considered to be a reasonable indicator of genetic variation within the populations[31]. This breed of goat showed the drastic low number of the effective number of alleles (even lower than half) than the observed number of alleles. This is 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.

Even these revealed the high level of allelic diversity; a more appropriate measure of genetic variation within a population is gene diversity (average expected heterozygosity)[27]. Overall mean of 0.686 (Table 2) of gene diversity was higher to the value reported in Swiss goat breeds (0.51 to 0.58) for 20 microsatellite loci[32] and 11 indigenous south east Asian goats (0.43-0.60)[3] but is slightly lower than those reported in Chinese goat breeds (0.777-0.823) for 6 microsatellite loci[38].

Another measure of genetic variation is observed heterozygosity. This population had higher mean observed heterozygosity than what was observed in

Jakhrana and Marwari[19], Attapady[1] and many other Asian goats[3] but lower in Chegu breed of goat[4]. Higher genetic variation in this studied breed may be due to its large effective population size, immigration of new gene due to intermixing of different population and low selection pressure. Breeding policies and different crossbreeding programmes might have contributed to higher genetic variation in Gohilwari goat population.

Majority of loci in this breed exhibited deficiency of heterozygosity at majority of loci. Overall mean F_{is} value of 0.264 was significantly different from zero. Significant heterozygote deficiency has been also reported in other studies of goat[3,42]. Heterozygote deficiency in this breed of goat could be due to one or more of the following reasons: segregation of non-amplifying (null) allele, Wahlund effect or inbreeding. However distinguishing among these was generally difficult[7]. Null alleles arise more in case of heterologous primer (Microsatellite of different species) leads to underestimation of heterozygosity but Callen *et al*.[8] identified null alleles using homologous microsatellite primers. This may be due to Wahlund effect or the fact that few bucks were used for the whole and nearby villages in the breeding region for breeding.

Deviation from HWE had also been reported in many other studies. Kim *et al*.[43] reported HWE deviations in Korean, Chinese and Saanen goats. The main reasons for the deviation from HWE are most likely the genetic drift; non-random mating, non-amplifying alleles or the population might be divided into a series of closely related or inbred family groups.

In Ewens-Watterson test for neutrality for markers the observed loci, which lied outside the limit of 95% confidence region, were not neutral and may be linked with some selection traits. If a neutral allele statistically associated with a selected allele at another locus or genes where selection is operating significantly may be carried along and alleles cannot be separated from their genetic background. This phenomenon is known as hitchhiking. Genetic hitchhiking can be potent force in changing allelic frequency and heterozygosity.

Maynard-Smith and Haigh[26] first suggested that molecular polymorphism may be modified by hitchhiking of neutral alleles adjacent to loci undergoing allelic substitution. Potentially one of the most important effects of hitchhiking is the reduction of heterozygosity of such molecular variation in area of low recombination due to selective sweeps at some of these loci substantially low level of heterozygosity has been observed (Table 3). In another specific study, Haiguo *et al*.[15] found that the some alleles of Microsatellite markers (ETH10 and IDVGA46) was linked to beef performance of cattle and showed positive or negative correlation with the different beef performance of cattle. Microsatellite ETH10 was also found linked to milk production performance in cattle[18]. In this study, microsatellite that were found not neutral or linked to some selective trait must be further investigated for association to selective traits. This may help in MAS (marker assisted selection) in breeding programmes if the association to selective traits is established.

Genetic bottleneck: Genetic bottleneck occurs when population experiences some temporary reduction in size. This may influence distribution of genetic variation within and among populations. Loss of genetic diversity may reduce the potential of small populations to respond to selective pressure[2] and increased inbreeding may reduce population viability[20,28,36].

The three tests (sign test, standard difference test and wilcoxon rank test) under these three model (IAM, TPM and SMM) for heterozygosity excess can detect the bottleneck for only a short duration of time after a bottleneck has been initiated. These are the quantitative test[9] that can detect bottleneck up to 50-250 generations. As discussed above, the null hypothesis of mutation drift equilibrium was accepted overall, there was no serious recent genetic bottleneck in Gohilwari goat breed.

In case of existence of bottleneck event the rare alleles are lost more often than the commonly occurring alleles and consequently there is a reduction in population size. Allele loss does not occur at the extreme of allele size distribution so the range in allele size remains constant. The non-bottleneck populations that are near mutation drift equilibrium are expected to have a large proportion of alleles in the range of low frequency and proportion of alleles decreasing or even nil at higher frequency class so normal L shaped curve. It can detect the recent bottleneck up to 40-80 generations only.

CONCLUSION

In conclusion, there was substantial genetic variation and polymorphism across studied loci in the Gohilwari breed of goat. And this population was not in Hardy-Weinberg equilibrium at most of the studied loci. This population was also receiving new genetic materials through introduction of immigrants. The

strong inference that the Gohilwari breed of goat has not undergone bottleneck is also important for goat breeders and conservationists, as it suggests that any unique alleles present in this breed may not have been lost. Therefore, it can be recommended that within-breed diversity is actively maintained to enable these extensively unmanaged stocks to adapt to future demands and conditions and there is ample scope for further improvement in its productivity through appropriate breeding strategies.

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