

Genetic bottleneck effect and analysis of intra-population genetic diversity in Gaddi goat breed of Western Himalayas using microsatellite markers

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ABSTRACT

Genetic characterization of Gaddi goat breed was carried out with the objective to study the Bottleneck and genetic variability in the existing population. A reliable approach to measure the genetic distance between and within animal genetic resources is to estimate the difference in the frequencies of different genetic variants (alleles) at a number of marker loci. The breeds that share same alleles at similar frequencies are genetically closely related, whereas those having the same alleles at different frequencies or different allele's altogether are further apart. The variability within a breed can also be estimated by examining the number and frequency of alleles. In the present study, microsatellite markers based molecular genetic characterization of "Gaddi", a goat breed native to north temperate western Himalayan region of India, was carried out to analyze existing intra population diversity. The multi-locus genotype data was generated on 51 Gaddi goats sampled across different locations of the breeding tract in Himachal Pradesh using 15 FAO recommended goat specific microsatellite markers, which gave amplification and observed and effective number of alleles, gene frequency, observed and expected heterozygosity were estimated. The screened loci were not found to be in accordance with Hardy Weinberg equilibrium for several microsatellite frequencies except INRABERN172 and MAF065. The overall mean F_{is} (0.102 ± 0.02) ranged between -0.1406 (INRABERN-172) to 0.2527 (SPS113). The overall mean F_{is} (0.102) observed in the present study indicated a 10.2 percent shortfall of heterozygosity in the population studied. The Ewens-Watterson test for neutrality indicated that observed F value of for 11 microsatellite loci except four (MAF70, ILSTS029, P19 (DYA) and SPS113) lied within the lower and upper boundaries of the 95% confidence region for expected F. The qualitative graphical method was employed to visualize the allele frequency spectra. The mode shift analysis revealed L-shaped curve confirming that the population had not experienced genetic bottleneck effect in recent past and is in mutation-drift equilibrium.

Key Words: Gaddi, Characterization, Microsatellite, Ewens-Watterson, Genetic bottleneck

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INTRODUCTION

India is one of the world's largest mega biodiversity centre gifted with rich genetic resources in terms of its goat breeds. A breed is a genetic resource or geographically defined population. The breed can be defined and recognized as intraspecific group, the members of which share particular morphological characteristics that distinguish them from other such groups. The Genetic variation between and within the breeds also described as genetic diversity, is the raw material for animal breeders to act upon for bringing genetic improvement in livestock. The population structure and genetic variation among breeds have been studied using different markers including detection of polymorphism at DNA level including such markers like RFLP (Restriction Fragment length polymerase), RAPD (Random Amplification of Polymorphic DNA), VNTR's

(Variable number Tandem repeats), STR (Short Tandem Repeats) and SSR (Simple Sequence Repeats) or Microsatellites. The microsatellite loci could also prove highly informative markers for the construction of genetic linkage maps which could be used in search for quantitative trait loci associated with economically important traits (Georges *et al.* 1995; Womack and Kata, 1995). Hundreds of microsatellites are being characterized from the bovine, ovine and porcine genomes and these eventually led to the production of high density genetic linkage maps (Barendse and Armitage, 1994; Bishop *et al.* 1994; Eggen and Fries, 1995). The availability of a wide range of microsatellites from livestock species has also generated interest in studies of variation and evolutionary relationships among livestock populations and a number of such studies (Buchanan *et al.* 1994; Forbes *et al.* 1995; Kim *et al.* 2002) have appeared in the literature. The

present study was therefore undertaken to genetically characterize Gaddi goat germplasm using suitable goat specific microsatellite markers for analyzing the prevalent genetic diversity within existing breed population, a pre-requisite for undertaking future genetic improvement and breed conservation programme for the breed.

MATERIALS AND METHODS

Collection of Blood Samples

Seventy five venous blood samples were collected at random from genetically unrelated animals of either sex but different age groups of Gaddi goat breed from different locations of its natural breeding tract and adjoining areas of Palampur and other districts of Himachal Pradesh.

Isolation of DNA samples

Out of these, 51 samples with desired quantity and quality were further processed for DNA isolation by phenol-chloroform extraction method (Sambrook et al. 1989). The quality of DNA was assessed through 0.7 percent horizontal mini-submarine agarose gel electrophoresis. The purity of DNA was assessed by calculating ratio of optical densities at 260 nm and 280 nm. The samples with OD ratio (OD₂₆₀/OD₂₈₀) ranging from 1.7 to 1.9 was used in subsequent experiments.

Primer preparation

15 FAO (DADIS MoDAD) recommended goat specific microsatellite markers for genetic diversity analysis viz. ILSTS005, TGLA53, ETH10, OarFCB48, MAF70, ILSTS029, SRCRSP5, BM6444, INRABERN172, MAF065, DRBPP1, P19 (DYA), OarAE54, SPS113, TRBV6, which gave amplification were included in the analysis.

Processing of DNA Samples

The microsatellite loci were amplified in programmable thermal cycler (Bio-Rad, S 1000) after optimization. The PCR programme used involved initial denaturation at 94°C for 3 minutes and 30 cycles of denaturation at 94°C for 30 seconds, annealing for 45 seconds, extension at 72°C for 45 seconds and final extension at 72°C for 10 minutes. Documentation of PCR product was done in 1.5 percent Agarose Gel Electrophoresis at 2-5 V/cm. The PCR products for different microsatellite loci

were resolved on 6 percent denaturing (urea) polyacrylamide gels along with 50 and 100 bp DNA ladders at 40-45W. Microsatellite alleles were visualized by silver staining.

Statistical Analysis of Microsatellite data

The microsatellite genotype data were analyzed using POPGENE version (1.3.1) software to calculate allele frequencies, observed and effective number of alleles, observed and effective heterozygosities and polymorphism information content in the population. It was used to compute summary statistics (e.g., allele frequency, gene diversity, genetic distance, F-statistics, multilocus structure etc.) for single-populations. The PIC was assessed using allelic frequencies evaluated according to (Botstein et al. 1980).

Hardy-Weinberg equilibrium and Linkage disequilibrium

The test for deviation from Hardy-Weinberg equilibrium was based on genotypic frequencies and Wright's fixation index (F_{IS}) statistics using F-Stat software (Goudet, 2002) and results were discussed and interpreted accordingly. The exact tests for deviations from Hardy-Weinberg equilibrium (HWE) were performed using the GENEPOP package (Raymond and Rousset, 1995).

Bottleneck analysis

The BOTTLENECK program (Cornuet and Luikart, 1996) was applied to determine if there had been past bottlenecks in population size at any locality. It tests for the departure from mutation drift equilibrium based on heterozygosity deficient or excess. The bottleneck compares heterozygosity expected at Hardy-Weinberg equilibrium to the heterozygosity expected at mutation drift equilibrium in same sample that has the same size and same number of alleles.

RESULTS AND DISCUSSION

The overall allelic diversity, a reasonable indicator of genetic variation within the population displayed high genetic variation in the breed and all 15 markers exhibited ample polymorphism for evaluating within breed genetic variability. The measures of genetic diversity estimated in Gaddi goat population under study are presented in Table 1. All 15 microsatellite

Table 1. Measures of genetic diversity in Gaddi Goat breed of Western Himalayas

S. No	Locus name	No. of allele	Size range	Observed heterozygosity	Expected heterozygosity	Nei	Average heterozygosity	PIC
1	ILSTS005	6	172-218	0.6471	0.7983	0.7905	0.7905	0.758
2	TGLA53	9	126-260	0.7843	0.8903	0.8816	0.8816	0.8694
3	ETH10	8	200-210	0.7451	0.8569	0.8485	0.8485	0.8299
4	OarFCB48	7	149-173	0.7059	0.7793	0.7716	0.7716	0.7373
5	MAF70	7	134-168	0.7347	0.8390	0.8305	0.8305	0.8077
6	ILSTS029	7	148-170	0.6471	0.8344	0.8262	0.8262	0.803
7	SRCRSP5	5	156-178	0.7451	0.7659	0.7584	0.7584	0.7148
8	BM6444	10	118-200	0.8163	0.8473	0.8386	0.8386	0.8195
9	INRABERN172	9	234-256	0.9200	0.8147	0.8066	0.8066	0.7829
10	MAF065	9	116-158	0.7200	0.8487	0.8402	0.8402	0.821
11	DRBP1	15	195-229	0.8039	0.9128	0.9039	0.9039	0.8958
12	P19(DYA)	15	160-196	0.8824	0.9247	0.9156	0.9156	0.909
13	OarAE54	14	115-138	0.7200	0.8830	0.8742	0.8742	0.8263
14	SPS113	7	134-158	0.6200	0.8380	0.8296	0.8296	0.8061
15	TRBV6	7	217-255	0.7347	0.8132	0.8049	0.8049	0.7771
	Mean	9		0.7484	0.8431	0.8347	0.8347	0.81052
	S.E	0.82		0.02	0.01	0.01	0.01	0.01

loci that have been identified to be polymorphic in domestic goats (Soranzo et al. 1999, Kim et al. 2002, Ouafi et al. 2002, Li et al. 2004, Dixit et al. 2008, Rout et al. 2008 and Ramamoorthi et al. 2009) amplified successfully in Gaddi breed also.

The overall mean F_{IS} (0.102) observed in the present study indicated a 10.2 percent shortfall of heterozygosity in population studied, which was comparatively lower than the heterozygote deficiency reported in Ganjam goat (21.7 percent; Sharma et al. 2009), Gohilwari goat (26.4 percent; Kumar et al. 2009), Kutchi goat (26 percent; Dixit et al. 2008) and Mehsana goat (14 percent; Aggarwal et al. 2007). The mildly positive intra population inbreeding ($F_{IS}= 0.102\pm0.02$) estimated in the present study indicated heterozygosity deficit or mild level of inbreeding. It generally happens when population size is small and less gene exchange occurs between it and other populations. The F_{IS} estimates ranged between -0.1406 (INRABERN-172) to 0.2527 (SPS113). This level of inbreeding may be a result of mild to moderate levels of mating between closely related individuals under migratory

conditions.

The positive deviation from Hardy-Weinberg equilibrium indicated deviations from random mating causing some studied loci to be homozygous in these populations. This heterozygote deficiency with mild inbreeding may be related to flock management conditions like few does maintained with continuous use of one or two breeding bucks over longer duration. The mild F_{IS} value indicated that some loci became homozygous presumably by mating between relatives and consequent genetic drift. Therefore, homozygous loci, relatedness and population structure of the breeds, segregation of non-amplifying (null) alleles, Wahlund effects (presence of population substructure), locus under selection (genetic hitchhiking), scoring biases (heterozygotes scored incorrectly as homozygotes) or inbreeding could be the most reasonable causes of mild heterozygote deficit as observed in present study. The mild inbreeding observed in the studied population was in agreement with inbreeding values reported in some Europe and Middle east breeds ($F_{IS} = 0.10$), Jamunapari ($F_{IS}=0.19$), Mehsana ($F_{IS}=0.16$),

Mehsana ($F_{is}=0.14$) and overall F_{is} value of 0.183 reported from all Indian goat breeds (Dixit et al. 2012) including Gaddi goat (F_{is} value 0.22), which were comparable to present findings. 14 out of 15 microsatellite loci studied, contributed to this deficit of heterozygosity.

Similarly low inbreeding values were also reported in 45 rare breeds of 15 European and Middle Eastern countries (Canon et al. 2006). On the other hand, some of the Indian breeds showed significant inbreeding such as Marwari ($F_{is}=0.26$) and Kutchi ($F_{is}=0.23$) breeds. Similarly Fatima et al. (2008) depicted comparative low rate of inbreeding (-0.058, 0.057 and 0.070) of within population inbreeding coefficient (F_{is}) for Zalawadi, Gohilwadi and in Surti respectively. Among the three breeds, the F_{is} values were highest for Surti breeds, possibly because of small population size. These values reflected low rate of inbreeding within the populations as compared to 45 rare breeds of 15 European and Middle Eastern countries reported by Canon et al. (2006) ($F_{is} = 0.10$) which were comparable with our findings in present study. Significant heterozygote deficiency has also been reported in Marwari ($F_{is} = 0.264$) and Mehsani ($F_{is} = 0.16$) goat breeds. Similarly Verma et al. (2010) reported significant positive F_{is} values from 0.118 (OarFCB304) to 1.0 (ETH225) with overall mean of 0.245.

The Ewens- Watterson test for neutrality indicated that observed F values for 11 of microsatellite loci studied except for 4 loci (MAF70, ILSTS029, P19 (DYA), and SPS113) lied within the lower and upper boundaries of 95% confidence interval region for expected F (Table 2).

The Chi-square (χ^2) test for HW equilibrium revealed positive deviation from Hardy-Weinberg equilibrium at 13 microsatellite loci indicating that samples were not drawn from large random mating population. The presence of low frequency null alleles segregating at these loci, Linkage between loci, sampling procedure, physical linkage, epistatic selection and genetic hitchhiking could be the possible expected sources of the disequilibrium. The mode shift analysis revealed L-shaped curve for distribution of allelic frequencies indicating no mode-shift in the frequency distribution. This confirms that the population had not experienced genetic bottleneck effect in the recent past and is in mutation-drift equilibrium. (Figure 1).

Genetic bottleneck effect

Microsatellite data were also subjected to statistical analysis to test whether the populations have undergone recent genetic bottleneck. Any population that experienced a recent bottleneck will

Table 2. Ewens-Watterson test for neutrality in Gaddi goat population

Locus	n	k	Obs. F	Min F	Max F	Mean*	SE*	L95*	U95*
ILSTS005	102	6	0.2395	0.1667	0.9068	0.4286	0.0209	0.2336	0.7662
TGLA53	102	9	0.1684	0.1111	0.8554	0.3071	0.0114	0.1676	0.5865
ETH10	102	8	0.1915	0.1250	0.8722	0.3462	0.0155	0.1820	0.6703
OarFCB48	102	7	0.2284	0.1429	0.8893	0.3789	0.0171	0.2051	0.7015
MAF70	98	7	0.1695	0.1429	0.8850	0.3672	0.0158	0.2041	0.6899
ILSTS029	102	7	0.1738	0.1429	0.8893	0.3774	0.0172	0.2084	0.7030
SRCRSP5	102	5	0.2716	0.2000	0.9246	0.4897	0.0265	0.2605	0.8687
BM6444	98	10	0.1614	0.1000	0.8332	0.2699	0.0085	0.1499	0.5010
INRABERN 172	100	9	0.1934	0.1111	0.8528	0.3022	0.0112	0.1656	0.5772
MAF065	100	9	0.1698	0.1111	0.8528	0.3067	0.0120	0.1654	0.5754
DRBP1	102	15	0.1158	0.0667	0.7632	0.1793	0.0030	0.1092	0.3212
P19(DYA)	102	15	0.0844	0.0667	0.7632	0.1774	0.0034	0.1061	0.3258
OarAE54	100	14	0.1258	0.0714	0.7738	0.1876	0.0036	0.1132	0.3392
SPS113	100	7	0.1704	0.1429	0.8872	0.3715	0.0168	0.2060	0.6990
TRBV6	98	7	0.2155	0.1429	0.8850	0.3784	0.0175	0.2099	0.7074

show higher than expected (equilibrium) heterozygosity for a large number of loci.

When a population goes through a bottleneck, rare alleles tend to be lost and average number of alleles

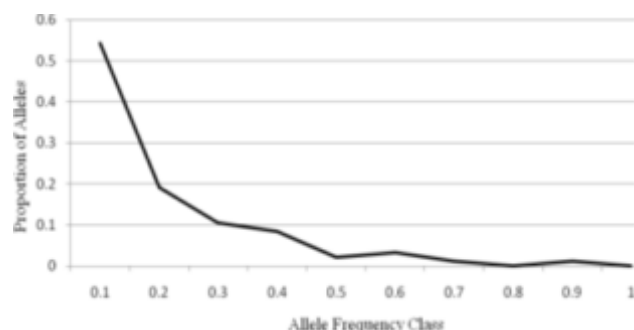


Figure 1. Mode shift analysis depicting absence of genetic bottleneck in Ardi goat, suggesting no bottleneck in Gaddi goat.

per locus, or allelic diversity is reduced. Heterozygosity, however, is not reduced proportionally, because rare alleles contribute little to heterozygosity. The qualitative graphical method (Cornuet and Leukart, 1996) was employed to visualize the allele frequency spectra. The microsatellite alleles were categorized into 10 frequency classes, which permits checking whether the scattering followed the normal L-shaped form, where alleles with low frequency (0.01 – 0.1) were the most abundant. The mode shift analysis exhibited no distortion of allelic frequency and L-shaped curve with the L-shaped distribution of allelic frequencies indicating no mode-shift in the frequency distribution confirming that the Gaddi goat breed had not experienced genetic bottleneck in the recent past and is in mutation-drift equilibrium as shown in Fig. 1.

The findings of present study were consistent with (Fatima et al. 2008) who reported non-significant heterozygote excess on the basis of IAM (Infinite allele model), TPM (Two phase model) and SMM (Stepwise mutation model) as revealed from Wilcoxon sign-rank tests, along with a normal 'L'-shaped distribution of mode-shift test which indicated no bottleneck in Zalawadi and Gohilwadi goat populations, whereas mild bottleneck in the recent past for Surti breed. Similarly (Mahmoudi et

al. 2013) reported that the Najdi population of goat has not undergone bottleneck, as it suggests that any unique alleles present in this breed may not have been lost and further Zaman et al. (2013) who analyzed bottleneck effect in Assam Hill Goat also revealed that the breed is non-bottle-necked where the mode-shift for the frequency distribution of alleles had a normal L-shaped curve stating that there was no recent and sudden reduction in the population. The present study revealed that the use of microsatellite loci can be effectively used for genetic characterization and diversity studies in Gaddi goats also.

CONCLUSIONS

Overall there is mild to moderate heterozygous deficiency at various loci indicating mild to moderate level of inbreeding in studied population. The analysis of studied microsatellite revealed high level of polymorphism and informativeness of markers for genetic diversity analysis studies in Gaddi goats. The significant variability reflects that the existing Gaddi goat population possess substantial genetic diversity with is good scope for bringing effective genetic improvement, conservation and designing future breeding policies for these goats. . Present study may therefore be useful in characterization of local gaddi goat population and to study the genetic diversity from the other recognized breeds which are mainly used for breeding in the adjacent area, and hence it will be helpful to develop strategies for conservation and utilization of to develop strategies for conservation and utilization of Gaddi goat breed but the study needs to be extended to include more microsatellites in a large sample size to further validate the results.

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