Genetic characterization of hill cattle of Himachal Pradesh using molecular markers

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ABSTRACT

DNA from 50 randomly selected blood samples of hill cattle of Himachal Pradesh (H.P) was extracted as per Johns' method with modification. The Hill cattle contribute about 43.53% of the total Livestock population of H.P. The animals are small sized, tough, fit for hilly terrain, adapted to cold and harsh climate, disease resistant and thrive on poor pastures and nutrients. Diversity analysis was carried out using fourteen microsatellite markers. The microsatellite loci were amplified from genomic DNA samples by PCR. The PCR products for different microsatellite loci were resolved on 6% denaturing (urea) polyacrylamide gels along with 10 bp DNA ladder at 80 W (1600-2500 V). Microsatellite alleles were visualized by silver staining. The diversity parameters were estimated through PopGene Programme. The mean observed and effective numbers of alleles were 4.71±1.64 and 2.93±1.16, respectively across all loci. A total of 66 alleles were observed in hill cattle with maximum alleles (8) contributed by locus HAUT-27 and the least alleles (2) by ETH-3. The average heterozygosity was 0.43±0.26 across all loci. The observed and expected heterozygosity (0.96 and 0.84 respectively) were highest for ETH-185 and BM-1824 locus and least (0.06 and 0.17, respectively) for ETH-225 and ETH-152 locus. Except ETH-03, all other microsatellites showed high levels of variability. It appears that lesser degree of genetic diversity and reduced heterozygosity in Hill cattle of HP is probably due to high level of inbreeding. The increased level of inbreeding could be due to lesser number of breeding bulls. The exchange of the males from adjacent places may assist in resolving the problem.

Keywords: Hill cattle, microsatellite markers, diversity, conservation

INTRODUCTION

The Himalayan economy is basically agro-pastoral and dependent on livestock. Out of the 449 million domestic animals in the country nearly 9% are in the Himalayan region. The Hill cattle contribute about 43.53% of the total Livestock of Himachal Pradesh and still they are considered as non-descript type due to lack of systematic study. The hill cattle, although, have low productivity, but these animals are tough, fit for hilly terrains, adapted to cold and harsh climate, disease resistant and thrive well on poor pastures and nutrients. To explore the genetic diversity in the hill cattle, there is a need to take up a programme using molecular methodology for characterization of these animals as per Bjornstad and Roed, 2001. In this study, microsatellite markers have been used for the characterization of hill cattle and to study the genetic diversity of hill cattle sampled from different locations of Himachal Pradesh.

MATERIAL AND METHODS

Blood samples were collected from 50 randomly selected unrelated animals from the breeding tracts in Shimla, Kinnaur, Solan and Sirmaur districts of Himachal Pradesh as per the guidelines of MoDAD (Measurement of domestic animal diversity) programme (FAO, 1995). DNA was extracted from blood as per John's method (John *et al.*, 1991) with some modifications. Fourteen microsatellite markers were included in the analysis (Table -1). The microsatellite loci were amplified from genomic DNA by PCR. Polymerase chain reaction (PCR) was carried out on about 50-100 ng genomic DNA in a 25 µl reaction volume. The PCR program comprised of initial denaturation at 94°C for 10 minutes, denaturation at 94°C for 15 seconds, annealing at 55-58°C for 20 seconds, extension at 72° C for 20 sec, 39 cycles, final extension at 72°C for 10 minutes and cooling at 4°C. The PCR products for different microsatellite loci were resolved on 6% denaturing (urea) polyacrylamide gels along with 10 bp DNA ladder at 80 W (1600-2500 V). Microsatellite alleles were visualized by silver staining (Bassam *et al.* 1991). Genotyping of each sample was done manually and allelic size was determined.

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Table 1: Primer sequences for different microsatellites used for estimation of genetic diversity in Hill cattle of Himachal Pradesh.

S.NO	Locus Name	Sequence	No. of bp
1	MM-8	cccaaggacagaaaagact	19
		ctcaagataagaccacacc	19
2	ETH-152	tactcgtagggcaggctgcctg	22
		gagacctcagggttggtgatcag	23
3	INRA-005	caatctgcatgaagtataaatat	23
		cttcaggcataccctacacc	20
4	INRA-23	gagtagagctacaagataaacttc	24
		taactacagggtgttagatgaactca	26
5	BM-1824	gagcaaggtgtttttccaatc	21
		cattctccaactgcttccttg	21
6	ETH-3	gaacctgcctctcctgcattgg	22
		actctgcctgtggccaagtagg	22
7	HAUT-27	ttttatgttcatttttgactgg	23
		aactgctgaaatctccatctta	22
8	ETH-225	gatcaccttgccactatttcct	22
		acatgacagccagctgctact	21
9	CSRM-60	aagatgtgatccaagagagaggca	24
		aggaccagatcgtgaaaggcatag	24
10	ETH-185	tgcatggacagagcagcctggc	22
		gcaccccaacgaaagctcccag	22
11	HEL-13	taaggacttgagataaggag	20
		ccatctacctccatcttaac	20
12	ILST-030	ctgcagttctgcatatgtgg	20
		cttagacaacaggggtttgg	20
13	CSSM-66	acacaaatcctttctgccagctga	24
		aatttaatgcactgaggagcttgg	24
14	ILST-033	tattagagtggctcagtgcc	20
		atgcagacagttttagaggg	20

POPGENE, a Microsoft window-based computer package for the analysis of genetic variation among and within natural populations using co-dominant and dominant markers and quantitative traits was used for the analysis of data. It was used to compute summary statistics for single-population. The parameters calculated included- allele frequency, gene diversity, heterozygosity- observed and expected computed after Nei (1973) as executed in the software (Yeh et. al. 1999), fixation index and neutrality of markers.

RESULTS AND DISCUSSION

The FAO/DADIS listed 14 microsatellite loci, which have been documented to be polymorphic in various bovine breeds (Mukesh et. al., 2004), amplified effectively and generated specific banding patterns from which individual genotypes were assessed. The observed number of alleles varied from 2.0 (ETH-3) to 8.0 (HAUT-27) with an average value of 4.71±1.64. The effective number of alleles per locus varied from 1.21 (ETH-3) to 5.85 (BM-1824), with an average number of 2.93±1.16 (Table-2). The level of variation depicted by number of alleles at each locus, in the present study, was similar to earlier reports in other cattle breeds (Glowtzki *et al.* 1995). The microsatellite loci showed high level of genetic variability as exhibited by wide range of alleles, which varied from 2 (ETH-3) to 8 (HAUT-27) across different loci. A total of 210 alleles at 17 microsatellites were observed across 10 cattle breeds (Moazami-Goudarzi *et al.* 1997). The lowest frequency of H allele was 0.0213 at HAUT-27 locus, and the highest (0.3830) at C allele. Allele frequency distribution, at the 14 investigated loci, varied between 0.0128 (INRA-23) and 0.9125 (ETH-152). Considerable variation in the distribution of allele frequencies between loci observed in Hill cattle is similar to that reported by Sodhi *et al.* (2005). The lowest frequency of 0.012 (INRA-023) was not due to any chance factor but it does exist in the random breeding population. Majority of the alleles at different loci are informative for genetic diversity analysis.

Table 2: Measures of genetic variation of hill cattle of Himachal Pradesh at investigated microsatellite loci.

Locus	Observed no. of alleles	Effective no. of alleles	Shannon's Information index	Observed heterozygosity	Expected heterozygosity	Nei's	F_{IS}
MM8	4.0000	3.0600	1.1683	0.5435	0.68	0.67	0.19
ETH152	3.0000	1.1945	0.3491	0.1250	0.17	0.16	0.23
1NRA05	4.0000	2.6328	1.0938	0.2381	0.63	0.62	0.62
INRA23	5.0000	2.8725	1.2376	0.4359	0.66	0.65	0.33
BM1824	7.0000	5.8534	1.8382	0.3913	0.84	0.83	0.53
ETH3	2.0000	1.2094	0.3156	0.1915	0.18	0.17	-0.11
HAUT27	8.0000	4.3356	1.7046	0.4894	0.78	0.77	0.36
ETH225	3.0000	2.1234	0.8297	0.0652	0.53	0.53	0.88
CSRM60	6.0000	2.6124	1.2147	0.3529	0.63	0.62	0.43
ETH185	4.0000	3.0556	1.2224	0.9565	0.68	0.67	-0.42
HEL13	5.0000	3.2240	1.3386	0.5682	0.70	0.69	0.18
ILST30	6.0000	2.9605	1.3679	0.6667	0.67	0.66	-0.01
CSSM66	4.0000	2.9229	1.1921	0.7857	0.66	0.66	-0.19
ILST33	5.0000	2.9385	1.2869	0.2250	0.67	0.66	0.66
Mean	4.7143	2.9283	1.1543	0.4311	0.61	0.60	0.26
St. Dev.	1.6375	1.1588	0.4248	0.2579	0.20	0.19	0.36

Effective number of alleles (Kimura and Crow, 1964); Shannon's Information index (Lewontin, 1972); Expected heterozygosity were computed using Levene (1949) and Nei's (1973) expected heterozygosity.

The average observed heterozygosity was 0.43±0.26 across the 14 loci. The highest level of observed heterozygosity was 0.96 (ETH-185) and lowest as 0.06 (ETH-225). The expected heterozygosity was highest (0.84) at BM-1824 locus and lowest (0.16) at ETH-3 locus. The difference in average observed and expected heterozygosity was markedly higher. The Wright's fixation index was highest 0.616 at INRA- 05 and lowest -0.007 (ILST-30). On an average, the heterozygous deficiency was higher at different loci, which indicates that the heterozygosity is being lost at all loci. These results are in accordance with that of previously reported for Greek population by Maule *et al.* (1990). From the patterns of within population genetic variation at marker loci it was possible to deduce demographic factors important for conservation of domestic animal diversity.

Majority of the loci had U95 values in the range of 0.81 (ILST-33) to 0.98 (ETH-03), except HAUT-27 (0.63), BM-1824 (0.69), ILST30 (0.76) and CSRM-60 (0.76). Their values at L95 were in the lower range, from 0.18 (HAUT-27) to 0.31 (CSSM-66) except ETH-3 having L95 of 0.50. Therefore, these microsatellite markers supported their usefulness to establish genetic structure of Hill cattle population of Himachal Pradesh, similar to other analysis of genetic diversity parameters among Indian cattle breeds as reported by Sodhi *et al.* (2005).

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REFERENCES

Bassam BJ, Coetano-Anolles G and Gresshoff PM. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. *Analytical Biochemistry* 196: 80-83.

Bjornstad G and Roed KH. 2001. Breed demarcation and potential for breed allocation of horses assessed by microsatellite markers. *Animal Genetics* 32: 59-65.

FAO 1995. Global project for the maintenance of domestic animal genetic diversity (MoDAD)- Draft project formulation report. FAO, Rome Italy.

Glowatzki-Mullis ML, Gaillard C, Wigger G and Fries R. 1995. Microsatellite based parentage control in cattle. *Animal Genetics* 28: 274-290.

John AJ, Weitzner G, Rozen R and Scriver CR. (1991). A rapid procedure for extracting genomic DNA from leucocytes. *Nucleic Acid Research* 19: 408.

Maule JP. 1990. The Cattle of the Tropics. (1st ed.) Edinburgh: University of Edinburgh

Moazami-Goudarzi K, Laloe D, Furet JP and Grosclaude F. 1997. Analysis of genetic relationships between 10 cattle breeds with 17 microsatellites. *Animal Genetics* 28: 338–45.

Mukesh M, Sodhi M, Bhatia S and Mishra BP. 2004. Genetic diversity of Indian native cattle breeds as analyzed with 20 microsatellite loci. *Journal of Animal Breeding and Genetics* 121: 416-431.]

Nei M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of National Academy of Sciences* (USA) 70: 3321-23.

Sodhi M, Mukesh M, Mishra BP, Mitkari KR, Prakash B and Ahlawat, S.P.S. 2005. Evaluation of genetic differentiation in Bos indicus cattle breeds from Marathwada region of India using microsatellite polymorphism. *Animal Biotechnology* 16:127-37.

Yeh FC, Boyle T, Rongcai Y, Ye Z and Xian JM. 1999. POPGENE version 1.32, the user-friendly software for the population genetic analysis. Molecular biology and biotechnology center, University of Alberta, Canada. (http://www.ualberta.ca/-fyeh/fyeh.).