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Assessment of genetic variability in Hill cattle of Garhwal region of Uttarakhand by microsatellite markers

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ABSTRACT

Knowledge of livestock genetic diversity is a crucial step to respond to commercial requirements and achieve production objectives in different environments and production systems. We investigated population genetic diversity in a hitherto unexplored Hill cattle population of Garhwal region of Uttarakhand state using microsatellite markers. A total of 48 individuals belonging to the Garhwal Hill cattle population were typed with 21 microsatellites. A mean of 12.61±4.80 alleles per locus was typed, indicating high allelic diversity in the population. The mean number of effective alleles per locus was 5.66±2.64. The studied population had high gene diversity indicated by high mean observed and expected heterozygosities across the 21 loci, i.e. 0.699±0.19 and 0.721±0.10 respectively. Mean PIC across the investigated loci was 0.782±0.10. The mean F_1's value was 0.118±0.17 indicating inbreeding in the population. Genetic bottleneck evaluated by IAM, TPM and SMM methods as well as frequency distribution was found to be absent in the evaluated population.

Key words: Gene diversity, Genetic bottleneck, Genetic diversity, Microsatellite markers

The necessity to enlarge, maintain and conserve livestock genetic diversity has been well recognized worldwide (Oldenbrock *et al.* 2002). During the last about two decades, due attention has been directed to this aspect globally using various tools including phenotypic parameters, biochemical and molecular genetic techniques to assess animal genetic diversity. The job is far from being completed especially with regards to wide-ranging Indian cattle breeds and their unique characteristics and history of origin. In general higher allelic diversity (number of alleles per locus) has been identified in zebuine breeds as compared to taurine breeds (Egito *et al.* 2007). It is therefore necessary to assess diversity levels in more breeds/unexplored populations of indigenous cattle to facilitate improvement and conservation priorities. This is especially essential in consequence of the animal and agricultural husbandry systems practiced by local livestock farmers, which may possibly affect diversity levels through the breeding of relatives and high gene flow between breeds.

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Uttarakhand state of India has larger proportion of indigenous cattle vis-à-vis crossbreds as compared to other adjoining states. The state has mainly two geographically distinct regions, i.e. Kumaun and Garhwal. The cattle population generally is termed as Hill cattle as the state is largely hilly. Hill cattle contribute significantly to the economy of the state by providing draught power, milk and manure despite being maintained at zero input system. Hill cattle of Kumaun as well as Garhwal region were recently phynotypically characterized (Pundir *et al.* 2010) using the standard methodology developed by the NBAGR, Karnal. Genetic characterization of most of the recognized breeds of Indian cattle including Hill cattle from Kumaun region of Uttarakhand was accomplished (Prakash *et al.* 2010, Pandey *et al*. 2011), however, no information on genetic characterization of Hill cattle from Garhwal region is so far available. Keeping this in view, genetic variability in Garhwal hill cattle has been evaluated in this paper.

MATERIALS AND METHODS

Blood samples (48) were collected from unrelated cattle. Animals were selected from diverse regions comprising remote villages from Uttarkashi and Rudarprayag districts of Garhwal region of Uttarakhand state. DNA was extracted and purified using standard SDS/proteinase K protocol and phenol–chloroform extractions. A panel of 21 bovine-specific

microsatellite markers recommended by Food and Agriculture Organization and the International Society for Animal Geneticists (ISAG) was used to estimate the within breed genetic variability. The microsatellite markers utilized were: CSRM60, ETH10, ILSTS11, TGLA122, INRA05, INRA63, TGLA227, CSSM08, HEL05, ILSTS05, ILSTS33, INRA35, BM1824, CSSM66, ETH03, ETH225, MM12, CSSM33, HEL01, HEL09 and ILSTS34 (FAO 1998). 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 each of dATP, dCTP, dGTP, dTTP, buffer with 1.5 mM MgCl2, 50pmol primer and 0.5 U *taq* DNA polymerase. The PCR amplification was accomplished by denaturation for 1 min at 94°C, 30 cycle of 94°C for 1 min, precise annealing temperature of primer for 1 min, 72°C for 1 min, and finally extension at 72°C for 5 min. PCR amplification was checked on 1.8% agarose gel and PCR products were genotyped an ABI 3100 automated DNA sequencer. Allele size was determined using the GeneMapper software package.

Different measures of within–breed genetic variations, namely observed number of alleles (*no*), effective number of alleles (*ne*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), and the within–population inbreeding estimate also known as Wright's fixation index (F_{IS}) at each microsatellite locus were estimated to evaluate variability at DNA level using the POPGENE software (Yeh *et al.*1999). Polymorphic information content (PIC) for each locus was calculated according to Botstein *et al.* (1980). Departure from Hardy–Weinberg proportions was determined using exact probability tests provided in GENEPOP version 3.1 a (Raymond and Russet 1995).

Existence of genetic bottleneck in the Hill cattle population was evaluated using three heterozygosity excess tests developed by Cornuet and Luikart (1996), namely, Sign test, Standardized differences test and a Wilcoxon sign-rank test. The probability distribution was established using 1 000 simulations under the three models – Infinite allele (IAM), Stepwise mutation (SMM) and Two-phase model of mutation (TPM). The second approach employed was the graphical representation of the mode shift indicator originally proposed by Luikart *et al*. (1998). These 2 approaches were conducted using bottleneck v1.2.02 software (Cristescu *et al.* 2010).

RESULTS AND DISCUSSION

All the 21 loci were found to be polymorphic in the investigated Hill cattle population. The 21 microsatellites generated 265 alleles, with an average number of 12.61±4.80 alleles per locus (Table 1), which, in general, is higher to values found in other local and selected cattle breeds reared in India (Prakash *et al*. 2010). The number of alleles per locus ranged from 6 (CSSM08) to 22 (CSRM60).

The average observed heterozygosity was 0.699±0.19 which ranged from 0.271 (CSSMO8) to 0.938 (CSSM33).

Table 1. Genetic variability parameters in Hill cattle of Garhwal region of Uttarakhand

Locus	Tm	Na	Ne	Ho	He	PIC	F_{IS}	HWE (χ^2)
CSRM60	58	22	7.76	0.833	0.871	0.860	0.043	412.339***
ETH10	58	10	4.82	0.854	0.793	0.767	–0.078	88.524***
ILSTS11	58	7	2.73	0.458	0.634	0.575	0.277	46.620***
TGLA122	58	18	8.60	0.875	0.884	0.873	0.010	227.847***
INRA05	54	8	6.05	1.000	0.835	0.813	–0.198	34.833 NS
INRA63	54	15	4.94	0.595	0.798	0.777	0.254	318.671***
TGLA227	55	15	3.22	0.583	0.689	0.662	0.154	188.637***
CSSM08	55	6	2.41	0.271	0.585	0.540	0.537	47.109***
HEL05	55	10	6.59	0.864	0.848	0.825	–0.018	139.675***
ILSTS05	55	16	4.17	0.625	0.760	0.735	0.178	58.213 NS
ILSTS33	55	21	11.39	0.766	0.912	0.902	0.160	330.341***
INRA35	55	14	8.66	0.833	0.885	0.874	0.058	138.988***
BM1824	58	7	2.52	0.479	0.602	0.530	0.205	106.683***
CSSM66	60	9	4.21	0.531	0.763	0.759	0.303	83.956 NS
ETH03	64	8	3.69	0.646	0.729	0.688	0.114	122.778***
ETH225	57	10	2.95	0.479	0.661	0.615	0.275	35.850NS
MM12	52	20	9.64	0.609	0.896	0.888	0.321	287.431***
CSSM33	58	15	9.22	0.938	0.891	0.880	–0.052	62.451 NS
HEL01	59	12	3.99	0.771	0.749	0.713	–0.029	27.918 NS
HEL09	59	11	6.46	0.917	0.845	0.830	–0.084	45.915 NS
ILSTS34	58	11	4.91	0.750	0.796	0.782	0.130	113.238 NS
Mean±SD		12.61±4.80	5.66±2.64	0.699±0.19	0.782±0.10	0.757	0.118±0.17	

Na, Observed number of alleles; Ne, effective number of alleles; Ho, observed heterozygosity; He, expected heterozygosity; PIC, polymorphism information content and F_{IS},heterozygote deficiency.

Table 2. Test for null hypothesis under 3 microsatellite evolution models

Test	Infinite alleles model (IAM)			Two-phase model (TPM)			Step-wise mutation model (SMM)		
Sign test	Expected	Observed	P-value	Expected	Observed	P-value	Expected	Observed	P-value
	12.69	12	0.459	12.64	7	0.012	12.37	2	0.000
Standardized differences test	Infinite alleles model (IAM)		Two-phase model (TPM)		Step-wise mutation model (SMM)				
	T2	P -Value	T2	P- Value	T2	P- Value			
	0.678	0.249	–4.394	0.000	–12.43	0.000			
Wilcoxon-rank test	IAM	TPM	SMM						
	0.178	0.982	1.000						

The expected heterozygocity ranged from 0.585 (CSSMO8) to 0.912 (ILSTS33) with the overall mean of 0.782±0.10. Heterozygosities of similar magnitude have been reported in other Indian cattle breeds, i.e. Tharparker cattle of India (Ho, 0.643; He, 0.72; Sodhi *et al.* 2008); Kumauni Hill cattle of Uttarakhand (Ho, 0.64; He, 0.73; Pandey *et al.* 2011); Gir (Ho, 0.679), Deoni (Ho, 0.674) and Kankrej (Ho, 0.674) (Kale *et al*.2010); Kenkatha (Ho, 0.470; He, 0.62) and Gaolao (Ho, 0.535; He, 0.689) (Chaudhari *et al*.2009) and Hariana (Ho, 0.768; He, 0.777) and Mewati (Ho, 0.740; He, 0.724) (Prakash *et al*. 2010), exotic Burlina cattle (Ho, 0.670) by Dalvit *et al.* (2008). Lower observed heterozygosity than the present study was observed in Kenkatha (Ho, 0.540; Pandey *et al.* 2006), Bachaur (Ho, 0.534; Sharma *et al.* (2007) and in exotic Brown Swiss cattle (0.563; Schmid *et al.* 1999).

The test for Hardy-Weinberg equilibrium (HWE) showed that 13 of the 21 loci investigated deviated significantly (P<0.01) from HWE (Table 1). Departure from HWE was primarily due to heterozygote deficiency. Deviations from HWE measured by the inbreeding coefficient (f) can be ascribed to a variety of causes. Outbreeding or over-dominant selection may lead to heterozygote excess, whereas inbreeding results in heterozygote deficiency (Hartl and Clark 1997), which may results from one or more of the following reasons: presence of null alleles, small sample size and Wahlund effect, i.e. presence of fewer heterozygotes in a population than predicted on account of population sub-division. The estimate of heterozygote deficiency varied from –0.198 (INRA05) to 0.537 (CSSMO8) with a mean of 0.118±0.17 indicating that there was around 12% deficiency of heterozygotes in this population. Higher heterozygote deficiency than the present study was observed in some other Indian native cattle–Sahiwal (32.6%), Hariana (21.1%) and Deoni (17.2%) (Mukesh *et al*. 2004); Red Kandhari (27.8%) (Sodhi *et al*. 2005) Bachaur (22.8%; Sharma *et al.* 2006); Ponwar (25.9%, Kerigarh (17.7%), Gangatiri (28.8%) and Kenkatha (17.7%) (Sharma *et al.* (2009); Kenkatha (21.21%) and Gaolao (22.48%) (Chaudhari *et al*.2009); and Kumauni Hill cattle (Pandey *et al.* 2011).

Genetic bottleneck: For microsatellite loci, allele number and frequency distribution in the natural populations result from an equilibrium between mutations and genetic drift. The mutation drift equilibrium depends on the mutation rate and the effective population size. Whenever there is bottleneck in a population the effective population size is significantly reduced. Any population that experienced a recent bottleneck will show higher than expected (equilibrium) heterozgosity for a large number of loci. To determine whether Hill cattle population exhibits a significant number of loci with gene diversity excess, three tests, namely a "sign test", a "standardized differences test" (Cornuet and Luikart 1996), and a "Wilcoxon sign-rank test" (Luikart *et al.* 1997) were employed. The test for null hypothesis under the three models is given in Table 2.

Under the infinite alleles model, sign test revealed significant deviations from mutation drift equilibrium in the Hill cattle population investigated. The expected number of loci with heterozygote excess under the sign rank test was 12.69 (IAM), 12.64 (TPM) and 12.37 (SMM), respectively, while the corresponding observed values were 12, 7 and 2. Across the loci, the value for He was less than the Heq for most of the loci. Similarly, standardized differences test and Wilcoxon signed-rank test also revealed significant heterozygosity excess. The T_2 statistics values for standardized difference test were 0.678, –4.394 and –12.430 for IAM, TPM and SMM, respectively. The sign rank test accepted the hypothesis of mutation drift equilibrium under IAM (p= 0.459) but rejected under TPM (0.012) and SMM (0.000) respectively. In the standardized difference test the null hypothesis was accepted under IAM but rejected under SMM and TPM. In Wilcoxon test it was accepted in IAM, TPM and SMM.

Additionally, the qualitative test for mode shift utilizing the frequency distribution of different alleles was performed. Genetic bottleneck is expected to cause alleles with low frequency, i.e. rare alleles, to become less abundant in the population than alleles with intermediate frequencies. In such cases, the plotting of proportion of different alleles against allele frequency classes will cause mode shift from the normal L-shaped distribution. In the present study, a normal L-shaped distribution of allele frequencies (Fig. 1) was observed in the investigated cattle population, indicating the absence of genetic bottleneck in the population in near past. Absence of genetic bottleneck has also been reported in other Indian

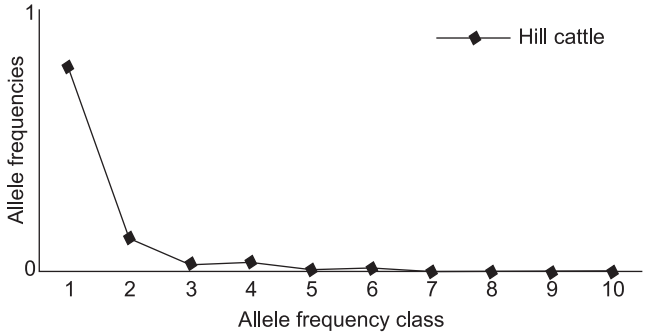

Fig. 1. Mode-shift analysis for test for genetic bottleneck in Hill cattle of Garhwal region.

cattle breeds (Sodhi *et al*. 2006, 2007, Thakur *et al*. 2010, Sharma *et al*. 2010).

In summary, we used microsatellite markers to analyze genetic variation within the hitherto unexplored population of Hill cattle of Garhwal region of Uttarakhand state. The chosen set of 21 microsatellites proved informative since most of the markers showed high levels of expected heterozygosity and PIC values. The employed set of markers can be applied in more detailed studies in the future by analyzing more breeds/unexplored populations, larger numbers of animals per breed. This should allow detection of possible sub-population structures, improve our knowledge of origin and phylogenetic relationships to other breeds and provide a basis for preservation of the breed in the original phenotype favoured by the traditional selection schemes and breeding programmes.

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