

Estimation of genetic variability parameters in Kumaun hill cattle (Kumauni cattle) by STR markers

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Kumaun hill cattle (Kumauni cattle) have been named after their habitat i.e. Kumaun hills of Uttarakhand state of northern India. Kumauni cattle are poor milkers and are used for the field work and production of manure. The present work investigates the existing diversity in Kumauni cattle of India by utilizing bovine-specific microsatellite markers.

Blood samples from 50 unrelated animals of Kumauni cattle were collected randomly from the Almora and Nainital districts of Kumaun region of Uttarakhand state. Genomic DNA was isolated as per Sambrook *et al.* (1989) with minor modifications.

A panel of 23 bovine-specific microsatellite markers selected from the recommended list of the Food and Agricultural Organization (FAO 1998) and the International Society for Animal Geneticists (ISAG) was used in this study for estimating within breed genetic variability in Kumauni cattle.

Polymerase chain reaction (PCR) was carried out on about 50–100ng genomic DNA in a 25 µl reaction volume using i-cycler. The reaction mixture consisted of 200µM 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.

To quantify the genetic variation in Kumauni cattle, number of observed alleles (No) and number of effective

alleles (N_e), observed heterozygosity (H_o) and the unbiased expected heterozygosity or gene diversity (H_e) were estimated using the GenAEx 6.2 software (Peakall and Smouse 2008). All loci were tested for deviation from Hardy-Weinberg equilibrium as implemented in GenAEx 6.2 software.

All the 23 microsatellite loci were polymorphic and a total of 222 alleles were observed. Sufficient allelic diversity was found in Kumauni cattle with observed number of alleles varying from 5 (ETH3, ILSTS11) to 18 (ILSTS34) with overall mean number of alleles per locus as 9.652 ± 3.406 . The effective number of alleles ranged between 1.755 (TGLA227) to 8.363 (ILSTS34). The mean effective number of alleles (4.344 ± 1.789) was lower than mean observed numbers of alleles (9.652 ± 3.406). The mean observed number of alleles in this study for Kumauni cattle population was similar to that reported in Tharparker (9) and Rath (9.6) Zebu cattle by Sodhi *et al.* (2008). However, lower mean number of alleles per locus was reported in other Indian cattle breed like Kherigarh (6.24) by Pandey *et al.* (2006), and Bachaur (6.30) by Sharma *et al.* (2007).

The observed and expected heterozygosity values ranged from 0.452 (TGLA227) to 1.0 (INRA35) with the overall mean of 0.664 ± 0.133 and 0.436 (TGLA227) to 0.877 (HEL9) with the overall mean 0.736 ± 0.120 , respectively. Heterozygosity of similar magnitude was also reported in Tharparker cattle of India (H_o , 0.643; H_e , 0.72) by Sodhi *et al.* (2008) and exotic Burlina cattle (H_o , 0.670) by Dalvit *et al.* (2008). Lower observed heterozygosity than Kumauni cattle was reported in Kenkatha (H_o , 0.540) by Pandey *et al.* (2006), Bachaur (H_o , 0.534) by Sharma *et al.* (2007) and in exotic Brown Swiss cattle (0.563) by Schmid *et al.* (1999).

The test for Hardy-Weinberg equilibrium (HWE) showed that 12 loci deviated significantly ($P < 0.05$) from HWE. Departure from HWE is mostly due to heterozygote deficiency, 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-

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Table 1. Estimates of genetic variability parameters in Kumauni cattle

Locus	N _o	N _e	Heterozygosity		F _{IS}
			H _o	H _e	
BM1824	6	2.519	0.532	0.610	0.118
CSSM08	9	2.511	0.682	0.633	0.094
CSSM33	13	7.127	0.775	0.871	0.099
CSSM45	12	5.898	0.767	0.840	0.076
CSSM60	14	5.072	0.702	0.812	0.126
CSSM66	9	4.059	0.773	0.762	-0.025
ETH10	8	3.926	0.761	0.754	-0.021
ETH225	10	2.674	0.682	0.633	-0.089
ETH3	5	2.672	0.546	0.633	0.128
HEL 9	9	7.518	0.682	0.877	0.214
HEL 5	9	3.893	0.468	0.751	0.370
ILSTS06	7	4.246	0.595	0.774	0.221
ILSTS11	5	1.925	0.477	0.486	0.007
ILSTS34	18	8.363	0.477	0.486	0.200
ILSTS33	9	4.477	0.721	0.786	0.072
INRA05	6	4.534	0.841	0.788	-0.079
INRA35	11	6.348	1.000	0.852	-0.187
INRA63	7	3.592	0.596	0.729	0.174
MM12	12	4.451	0.568	0.784	0.267
MM8	9	3.191	0.628	0.695	0.086
TGLA122	15	5.595	0.750	0.831	0.087
TGLA227	6	1.755	0.452	0.436	-0.051
TGLA53	13	3.560	0.714	0.728	0.007
Mean	9.652	4.344	0.664	0.736	0.082
St. Dev	3.406	1.789	0.133	0.120	0.129

N_o, Observed no. of alleles; N_e, effective no. of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; F_{IS}, heterozygote deficiency.

division. The estimate of heterozygote deficiency varied from -0.187 (INRA35) to 0.370 (HEL5) with a mean positive values of 0.082. Thus it was observed that 8% deficiency of heterozygotes exists in Kumauni cattle population. However, higher heterozygote deficiency than the Kumauni cattle (0.082) has been observed in several Indian cattle like Sahiwal (F_{IS} = 0.32) by Mukesh *et al.* (2004), Bachaur (F_{IS} = 0.22) by Sharma *et al.* (2006) and Gangatiri (F_{IS} = 0.31) by Sharma *et al.* (2007).

SUMMARY

Microsatellite data on 23 loci was generated and utilized to evaluate the genetic variability of Kumaun cattle. Sufficient allelic diversity was observed with a mean number of 9.652 alleles per locus. The mean observed heterozygosity in the population was 0.664, reflecting reasonable within breed genetic diversity. Population showed little heterozygote

deficiency (F_{IS}, 0.082) and deviated from HWE at 12 investigated loci.

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REFERENCES

- Dalvit C, De Marchi M, Dal Zotto, Zanetti E, Meuwissen T and Cassandro M. 2008. Genetic characterization of Burlina cattle breed using microsatellite markers. *Journal of Animal Breeding and Genetics* **125**: 137–44.
- FAO (Food and Agricultural Organization of the United Nations) 1998. Food and Agriculture Organization, Rome, repost. <http://dad.fao.org/en/refer/library/guideline/workgrp.pdf>
- Mukesh M, Sodhi M, Bhatia S and Mishra B P. 2004. Genetic diversity of Indian native cattle breeds as analyzed with 20 microsatellite loci. *Journal of Animal Breeding and Genetics* **121**(6): 416–24.
- Pandey A K, Sharma Rekha, Singh Y, Prakash B, and Ahlawat S P S. 2006a. Evaluation of Genetic Variation in Kenkatha cattle by microsatellite markers. *Asian-Australasian Journal of Animal Science* **19**(12): 1685–90.
- Pandey A K, Sharma Rekha, Singh Y, Prakash B and Ahlawat S P S. 2006b. Genetic diversity studies of Kherigarh cattle based on microsatellite markers. *Journal of Genetics* **85**(2): 117–22.
- Peakall R and Smouse P E. 2008. A heterogeneity test for fine-scale genetic structure. *Molecular Ecology Notes* **17**: 3389–3400.
- Sambrook J, Fritsch E F and Maniatis T. 1989. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbour Lab. Press, Cold Spring Harbour, NY.
- Schmid M, Saipveaova N, Gaillard, C and Dolf G. 1999. Genetic diversity in Swiss cattle breeds. *Journal of Animal Breeding and Genetics* **116**: 1–8.
- Sharma Rekha, Pandey A K, Singh S C P, Singh Y, Mishra B P, Singh S R, Mandal G K, Singh P K, Singh G and Ahlawat S P S. 2007. Diversity assessment of Bachaur cattle from India: A draft breed still relevant in mechanized Era. *Korean Journal of Genetics* **29**(3): 369–78.
- Sharma Rekha, Pandey A K, Singh Y, Prakash B, and Ahlawat S P S. 2006. Assessment of genetic variability in Ganagtiri cattle by microsatellite marker. *Korean Journal of Genetics* **28**(1): 35–42.
- Sodhi M, Mukesh M, Ahlawat S P S, Sobti R C, Gahlot G C, Mehta S C, Prakash B and Mishra B P. 2008. Genetic diversity and structure of two prominent zebu cattle breeds adapted to the arid region of India inferred from microsatellite polymorphism. *Biochemical Genetics* **46**: 124–36.