



Microsatellite based genetic characterization of Motu cattle

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Motu cattle are found in Southern part of Malkangiri district and adjoining area extended to Konta block of Dantewada (Chhattisgarh) and border areas of Andhra Pradesh. These cattle are mostly reared by Koya tribe for ploughing and carting. No information is available on molecular genetic diversity of Motu cattle. So this study was carried out to estimate the genetic variability in Motu cattle population by microsatellite markers.

Unrelated blood samples (50) of 5–6 ml conc, were collected from jugular vein of the animals in vacutainers having 15% EDTA (ethylene diamine tetra acetic acid). Sampling was done from different villages of Malkangiri district of Orissa as per guidelines of FAO's, Measurement of domestic animal diversity (MODAD) programme (FAO 1995) to make them representative of population. Blood samples were processed for the isolation of genomic DNA by the method of Sambrook *et al.* (1989) with slight modifications. A set of 23 microsatellite marker were selected according to guidelines of ISAG and FAO's DADIS (domestic animal diversity information system) programme. 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, 1.5 mM MgCl₂, 50pmol primer, 0.5 U Taq polymerase and Taq buffer. The PCR reaction cycle 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. The PCR product was checked on 1.8% agarose gel and analyzed by automated DNA sequencer and genotype were assigned using GeneMapper Software Package.

To quantify the genetic variation in Motu cattle population, number of observed alleles (N_o) 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 microsatellite loci, which have been identified as polymorphic in different cattle breeds, were also polymorphic in Motu cattle. Genetic variability, viz. observed and effective number of allele, observed and expected heterozygosity and heterozygote deficiency at each of the microsatellite loci in Motu cattle are presented in the Table 1.

Across the amplified microsatellite loci, a total of 231 alleles were identified in Motu cattle. The number of observed alleles varied from five (BM1824, INRA63) to 20 (ILSTS34) with an overall mean number of alleles (MNA) per locus of 10.04 ± 3.843 . The observed number of alleles for all the loci exceeded the effective number of alleles which varied from 1.704 (TGLA227) to 9.404 (ILSTS34) with a mean of 4.725 ± 2.235 . The mean observed number of alleles in this study for Motu cattle population was similar in magnitude as in Tharparker (9) and Rathi (9.6) cattle (Sodhi *et al.* 2008) but higher than Kherigarh cattle (6.24) as reported by Pandey *et al.* (2006b). Lower mean number of alleles were observed in Gangatiri (6.27) and Sahiwal cattle (5.2) by Sharma *et al.* (2006) and Mukesh *et al.* (2004), respectively. The mean expected number of alleles in Motu cattle was higher than that reported in other Indian cattle breeds like Kenkatha (3.37) by Pandey *et al.* (2006a) and Bachaur cattle (3.65) by Sharma *et al.* (2007).

The observed heterozygosity was 0.660 ± 0.168 which was slightly lower than the expected heterozygosity. The average expected heterozygosity within Motu cattle population diverged from 0.418 (TGLA227) to 0.904 (ILSTS33) with overall mean of 0.749 ± 0.119 . Motu cattle thus encompassed considerable measure of genetic variation derived from its gene diversity as estimated against the genetic variation described in several breeds scrutinized worldwide. The average heterozygosity is similar with other Indian cattle breeds, viz. Tharparker (0.643), Rathi (0.694) by Sodhi *et al.* (2008) and exotic Burlina cattle (0.677) by Dalvit *et al.*

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Table 1. Estimates of genetic variability parameters in Motu cattle

Locus	N _o	N _e	Heterozygosity		F _{IS}
			H _o	H _e	
BM1824	5	2.853	0.667	0.656	-0.026
CSSM08	6	2.657	0.348	0.630	0.442
CSSM33	12	7.127	0.675	0.871	0.215
CSSM45	12	6.275	0.745	0.850	0.114
CSSM60	10	3.475	0.708	0.720	0.006
CSSM66	12	4.311	0.729	0.776	0.051
ETH10	7	3.952	0.745	0.755	0.003
ETH225	9	2.833	0.674	0.654	-0.042
ETH3	7	3.047	0.717	0.679	-0.068
HEL09	11	7.584	0.652	0.878	0.249
HEL 5	11	3.802	0.375	0.745	0.491
ILSTS06	8	4.228	0.556	0.772	0.272
ILSTS11	7	2.373	0.396	0.585	0.316
ILSTS34	20	9.404	0.938	0.903	-0.049
ILSTS33	17	9.443	0.833	0.904	0.068
INRA05	7	4.111	0.708	0.765	0.064
INRA35	11	6.509	1.000	0.855	-0.182
INRA63	5	3.247	0.583	0.699	0.157
MM12	11	6.630	0.771	0.858	0.092
MM8	8	2.806	0.630	0.651	0.021
TGLA122	12	6.592	0.688	0.857	0.190
TGLA227	7	1.704	0.354	0.418	0.143
TGLA53	16	3.935	0.688	0.754	0.078
Mean	10.04	4.735	0.660	0.749	0.113
St. Dev	3.843	2.235	0.168	0.119	0.163

No, Observed no. of alleles; Ne, effective no. of alleles; Ho, observed heterozygosity; He, expected heterozygosity; F_{IS}, heterozygote deficiency

(2008). Whereas it is higher than some other Indian cattle breeds, viz. Sahiwal (0.40) by Mukesh *et al.* (2004), Gangatiri (0.464) by Sharma *et al.* (2006) and Kenkatha (0.540) by Pandey *et al.* (2006a).

Out of 23 loci, 10 loci deviated significantly ($P < 0.05$) from HWE. Within population inbreeding estimate f (F_{IS}) was positive (0.113) ranging from -0.182 (INRA35) to 0.491 (HEL5). Thus on an average heterozygote deficiency of 11.3% exists in Motu cattle population. It is much lower as compared to Sahiwal (32%) by Mukesh *et al.* (2004), Gangatiri (31%) and Bachaur cattle (22.8%) by Sharma *et al.* (2007). This obviously signifies that, this breed retains considerable genetic variability and negligible amount of heterozygote deficiency.

SUMMARY

The study was conducted to estimate the genetic variability in Motu cattle population by microsatellite markers. Significant level of variability in Motu cattle is indicative of

a valuable reservoir of genetic diversity in this breed. High mean number of alleles (10.04) per locus showed sufficient allelic diversity in the population. The mean observed heterozygosity in the population was 0.660, reflecting reasonable within breed genetic diversity. Population showed heterozygote deficiency (F_{IS} = 0.113).

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