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Molecular characterization of Malaimadu cattle

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Malaimadu cattle is found in villages adjoining Western Ghats area (hence locally called as Malaimadu – hill cattle) of Karur, Dindigul, Madurai, Theni, Virudhunagar and Tirunelveli districts of Tamil Nadu, maintained by communities, viz. Konar, Thevar, Naickers and Moopars. Malaimadu cattle are short and sturdy animals with their body colour varying from place to place. This genetic group is resistant to many diseases and their bullocks are useful for ploughing in the wet lands. During the monsoon season (October–December and April–June), the cattle are sent to forests (Western Ghats/ Sirumalai hills) for grazing; during off-season after the harvest of paddy or other crops, the cattle are penned in the fallow lands (300 to 400) for grazing and manuring.

The decline in population of cattle from few lakh to around 20,000 (Vivekanandan and Alagumalai 2013) is due to factors like the denial of grazing permits by forest officials, the unavailability of labour to look after the herds and mechanization, reducing the demand for draught animals. Regular animal health camps, removal of encroachments in ponds, tanks and forest grazing permits would improve the status of Malaimadu cattle and livestock keepers. Hence, Malaimadu cattle was assessed for its within population genetic diversity at molecular level through microsatellite markers.

Blood samples were collected from 50 Malaimadu cattle, unrelated by ancestry, from its breeding tract and the genomic DNA was isolated by phenol chloroform method (Sambrook *et al.* 1989). Microsatellite markers (24) were amplified with specific primer sets. The PCR mixture consisted of Master mix (containing 1.5 µL of 10× PCR buffer, 1.2 µL of 25 mM $MgCl_2$, 1 µL of 10 mM dNTPs), 1.0 U of Taq DNA polymerase, 0.5 µL of 10 pM each of the forward and reverse primers, 1 µL of the diluted genomic DNA (50–100 ng) and nuclease free water to a final volume of 15 µl. Genotyping was carried out on an automated ABI PRISM 3730XL Genetic Analyzer (Applied Biosystems, USA). Microsatellite fragment sizing was performed by the GeneMapper^{TM} version 3.7. The genotypes were scored and the number and size of alleles were calculated and further checked manually to avoid errors in scoring of alleles.

Microsatellite allele frequencies, effective number of alleles, observed and expected heterozygosities and F-statistics were calculated using microsatellite analyzer (Daniel and Christian 2003). Test for Hardy-Weinberg equilibrium (HWE) was done using POPGENE version 1.31 (Yeh *et al.* 1999). Polymorphism information content (PIC) was calculated using PIC calculator (Bolstein *et al.* 1980) and bottleneck analysis was carried out to check any recent reduction in the population (Cornuet and Luikart 1996).

All the markers were polymorphic except ETH3. Among all the 23 microsatellite markers, 208 alleles were observed with a mean of 9.04±3.28 across all loci. The parameters estimated for 23 microsatellite markers in Malaimadu cattle are presented in Table 1. Number of alleles for each locus ranged from 4 (BM1824) to 19 (CSRM60) and the effective number of alleles ranged from 1.8611 (ILSTS011) to 8.3805 (TGLA122) with a mean of 4.11±1.72. Malaimadu cattle has higher number of alleles when compared to Kangayam and Umblachery breeds, having the range from 2 to 6 alleles (Karthickeyan *et al*. 2007, 2009), Kherigarh cattle having 4 to 10 alleles (Pandey *et al*. 2006), Nimari cattle having 3 to 15 alleles (Sharma *et al*. 2010) and Malvi cattle having 4 to 18 alleles (Thakur *et al*. 2010). In this study, 95.65% (22 out of 23 loci) of the markers had more than 4 alleles indicating that the markers selected are effective in evaluating the genetic diversity of populations of *Bos indicus* cattle.

Polymorphism information content (PIC) calculated across 23 loci ranged from 0.4135 (ILSTS011) to 0.8625 (TGLA122) with an overall mean of 0.68±0.14 indicating that these markers can effectively be used for genetic diversity analysis. PIC values in the study were similar to that in Kherigarh cattle (0.669±0.097; Pandey *et al*. 2006) and Nimari cattle (0.64; Sharma *et al*. 2010). Other Tamil Nadu breeds such as Kangayam (Karthickeyan *et al*. 2009) and Umblachery (Karthickeyan *et al*. 2007) possessed

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Table 1. Genetic variations at 23 micosatellite loci in Malaimadu cattle

Locus	No. of alleles	Effective no. of alleles	PIC	Heterozygosity		F_{IS}	HW equilibrium	
				Observed	Expected		X^2 value	df
CSRM60	19	6.0027	0.8026	0.9787	0.8424	–0.1692	221.99**	171
CSSM66	8	4.1829	0.723	0.6122	0.7688	0.2003	26.68^{NS}	28
TGLA122	14	8.3805	0.8625	0.9184	0.8898	–0.0377	100.91 ^{NS}	91
INRA063	10	2.3651	0.5443	0.5106	0.5834	0.1206	118.79**	45
INRA035	10	3.4328	0.6653	0.8298	0.7163	–0.1657	114.34**	45
ILSTS005	8	4.7350	0.7582	0.6047	0.7981	0.2390	62.47**	28
HEL1	7	3.3496	0.6585	0.7442	0.7097	–0.0551	9.75 ^{NS}	21
HAUT024	6	3.1582	0.6427	0.5455	0.6912	0.2073	43.69**	15
TGLA53	11	4.0179	0.717	0.5778	0.7596	0.2361	157.74**	55
ILSTS034	12	5.5404	0.7969	0.6889	0.8287	0.1648	171.06**	66
INRA005	9	4.9847	0.7705	0.8261	0.8082	–0.0279	31.44 ^{NS}	36
MM8	7	3.3168	0.6423	0.5957	0.706	0.1523	15.88 ^{NS}	21
MM12	7	3.2554	0.6578	0.5	0.7004	0.2833	148.09**	21
TGLA277	7	2.0326	0.4654	0.4375	0.5134	0.1440	37.77*	21
ILSTS006	8	3.5956	0.6945	0.3529	0.7327	0.5165	124.12**	28
BM1824	4	2.6498	0.5563	0.375	0.6292	0.4021	118.87**	6
ILSTS033	7	4.1490	0.7207	0.7826	0.7673	–0.0257	17.08 ^{NS}	21
CSSM008	7	2.8448	0.5894	0.4043	0.6555	0.3812	54.14**	21
CSSM033	13	3.3721	0.6811	0.7391	0.7112	–0.0453	51.49 ^{NS}	78
HEL9	8	5.8626	0.8082	0.8125	0.8382	0.0256	34.54 ^{NS}	28
ILSTSO11	6	1.8611	0.4135	0.375	0.4675	0.1945	68.27**	15
ETH10	8	3.3828	0.6522	0.7447	0.712	–0.0519	13.14 ^{NS}	28
ETH225	12	8.1115	0.8622	0.9592	0.8858	–0.0890	52.83 ^{NS}	66
Mean	9.04±3.28	4.11±1.72	0.68±0.11	0.65±0.19	0.73±0.11	0.1130±0.19		

comparatively lower PIC values of 0.5628±0.03 and 0.5625±0.03 respectively. Similarly, higher PIC values of 0.71±0.009 and 0.78±0.024 were noted by Ganapathi *et al*. (2012) in Bargur, another South Indian hill cattle breed and Deepika and Salar (2012) in 10 Indian grey cattle breeds. Observed heterozygosity ranged from 0.3529 (ILSTS006) to 0.9787 (CSRM60) with a mean value of 0.65±0.19. The expected heterozygosity ranged from 0.4675 (ILSTS011) to 0.8898 (TGLA122) with a mean value of 0.73±0.11. The observed and expected mean heterozygosity values were similar to those in Bargur (0.808±0.023 and 0.762±0.008; Ganapathi *et al*. 2012) and in Indian grey cattle breeds (6.093±0.56 and 0.722±0.026; Deepika and Salar 2012). Lower observed heterozygosity than expected indicates existence of a breed structure in Malaimadu cattle.

F_{IS} is the proportion of the variance in the subpopulation contained in an individual. High F_{IS} implies a considerable degree of inbreeding (Daniel and Clark 2007). The within population inbreeding estimates (F_{IS}) in this study was positive in 14 out of 23 loci, with the F_{IS} values ranged from –0.1692 (CSRM60) to 0.5165 (ILSTS006) with a mean value of 0.1130±0.19. This value indicates a moderate deficit of heterozygotes in the population. Comparatively, Kherigarh population had higher heterozygote deficit (0.188; Pandey *et al*. 2006). On the contrary, only 3% of the Indian grey cattle breeds were heterozygote deficit (Deepika and Salar 2012) and the F_{IS} values were close to 0 in few endangered Spanish breeds, for all loci analysed (Martin-Burriel *et al*. 2007).

Bottleneck analysis revealed no mode shift in the frequency distribution of alleles and a normal L-shaped form was observed suggesting Malaimadu population is non-bottlenecked, i.e. it has not undergone any recent reduction in the effective population size and remained at mutation-drift equilibrium. While Bargur, another hill cattle breed, revealed a mode-shift suggesting the loss of alleles in low frequency from the population.

SUMMARY

Malaimadu cattle, a draught cattle found in villages adjoining Western Ghats of Tamil Nadu, is known for their workability. The population has possessed high number of allelic variability with few alleles at low frequencies and was non-bottlenecked proving no recent reduction in effective population size. This study revealed that Malaimadu cattle possess high within-breed genetic diversity with many polymorphic loci and hence could be recognised as a separate breed.

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