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Research Article

GENETIC DIVERSITY AND BOTTLENECK ANALYSIS OF UMBLACHERY CATTLE BY MICROSATELLITE MARKERS

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ABSTRACT
Assessment of genetic variability in Umblachery breed of cattle in Tamilnadu, South India was carried out using eight bovine microsatellite markers. The mean number of allele was 10.25±1.11 per locus with a range of 6 to 15 and the allele size ranged from 95 to 382 BP. The frequency distribution of alleles in the breed was from 0.0556 to 0.2222. The estimated Heterozygosity was 1.0000 and the mean expected Heterozygosity was 0.9283±0.1060. The overall mean within population inbreeding estimate (F_{IS}) value (-0.1465±0.0197) indicated excess of heterozygotes in the population. In addition, higher Shannon Index value indicated the higher amount of variation in the population and there is more scope for conservation. Mode shift analysis revealed a normal L- shaped curve indicating that Umblachery population is non-bottlenecked. It is clear that the population has not undergone any reduction in the effective population size and remained at mutation drift equilibrium. Results obtained from this study will help to formulate conservation strategies for Umblachery cattle.

Key Words: *Cattle, Microsatellites, Umblachery, Allele, Heterozygosity, Shannon Index, Inbreeding Estimate, Mode Shift Analysis*

INTRODUCTION
Umblachery is a well known draught breed of Thanjavur, Thiruvarur and Nagapattinam districts in eastern parts of Tamilnadu state in South India. This breed is an outcome of selection for short stature, suitable for work in marshy rice fields of Cauvery delta region (Thangaraju *et al*., 2001). Umblachery is a medium sized cattle and has compact body and short legs; calves are red or brown in color at birth. Red color begins to change to grey at 3 to 4 months of age. Total grey color attained even at the age of one year. Bulls are dark grey with black patches on head, back and pelvis. Bullocks are in grey in color with dark extremities; white star at forehead, white tail twitch and white socks marks in the legs are specific characteristics of this breed (ICAR-NBAGR Network Project Survey Unit, 1999). Day by day, the population size of Umblachery breed is shrinking because of cross breeding programmes and mechanization of agriculture in the Cauvery delta region. Hence, the present study was carried out to evaluate the Heterozygosity in the population and to characterize this breed.

MATERIALS AND METHODS
Primers
A total of eight microsatellite primers were used for microsatellite analysis (Table 1). These microsatellites were originally developed by Brezinsky *et al*., (1993a,b,c) and Kemp *et al*., (1993) from the genomic library of N' Dama (*Bos Taurus*) bull. The primers were reconstituted in TE with a concentration of 40 n moles/ml as stock solution and 40 p moles/ml as working solution. All primers were tested with similar conditions on tested DNA samples.

Genomic DNA
High molecular weight template was prepared from peripheral blood mononuclear cells collected from 50 unrelated Umblachery cattle available in the farmers herd, Orathanadu taluk of Thanjavur district, Tamil Nadu. The DNA samples were dissolved in TE buffer (pH8.0) to make uniform concentration of 50 ng/ml.

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PCR Amplification

The amplification reactions were carried out in 0.2 ml microfuge tubes using a programmable thermal cycler (MJ Research). Each 20µl reaction mix comprised of primers each at 20mMoles,DNTPs each at 50mMoles, 0.5 units of Taq DNA polymerase, 200ng template genomic DNA, 10mM Tris (pH8.3), 50mM KCl, 0.001 Nonidet P40 and 1.5mM $MgCl_2$.

Table 1 Nucleotide sequence of microsatellite primers

S. No	Code	Nucleotide Sequence
1.	ILSTS 005	5' GGAAGCAATGAAATCTATACC 3' 5' TGTTCTGTGAGTTTGTAAGC3'
2.	ILSTS 006	5' TGTCTGTATTTCTGCTGTGG 3' 5' ACACGGAAGCGATCTAAACG 3'
3.	ILSTS 008	5' AGCACCTGCTGCATACTACC 3' 5' GAATCAGTGTCAGTGTTTCCC 3'
4.	ILSTS 010	5' ATGGAGAGCAAATGGTCAGC 3' 5' ACTACAATGGACATGAGTCCG 3'
5.	ILSTS 011	5' GCTTGCTACATGGAAAGTGC 3' 5' CTAAAATGCAGAGCCCTACC 3'
6.	ILSTS 012	5' TCTACCACCGATACAGATGG 3' 5' GAAGTAGGTAGTGCTGGAGG 3'
7.	ILSTS 013	5' CTTGATCCTTATAGAACCTGG 3' 5' ACACAAAATCAGATCAGT 3'
8.	ILSTS 014	5' CTGACTATGGTGATAATCCC 3' 5' TCTTTTCCCTTTCCTTCCCC 3'

Thermal Cycling

ILSTS 005 and ILSTS 006:

Amplification included an initial denaturing step of 45 seconds at 94ºC. This was followed by 19 cycles of 45 seconds at 89ºC, 1 min. at the annealing temperature of 55ºC and 20 seconds at 70ºC. Final extension was for 3 min. at 70ºC.

ILSTS 008:

Amplification reactions were carried out as same as that of ILSTS 005 and ILSTS 006 but the annealing temperature was 58ºC.

ILSTS 010, ILSTS 011, ILSTS012, ILSTS 013 and ILSTS 014:

Amplification reactions were carried out as same as that of ILSTS 008 but the temperature cycling after initial denaturation was 24 times instead of 19 times.

Analysis of PCR Products

The PCR products were made to run on a 6 per cent Agar gel electrophoresis together with100 bp DNA ladder at 100 volts for 90 min. The gel was stained by ethidium bromide for 10 min. and viewed by UV illumination. The alleles were photographed and were analyzed by software aided gel documentation system (comprised of Ultra-lum scanner, Scion image capturing system and Sigma gel package)

Statistical Analyses

Microsatellite allele frequencies, observed number of alleles, effective number of alleles, and test of Hardy-Weinberg equilibrium, observed and expected Heterozygosity and F-statistics were calculated using the POPGENE 1.31 version (Yeh *et al*. 1999). Shannon Index was calculated for determining diversity index (Shannon and Weaver, 1949). Bottleneck analysis was performed using the software BOTTLENECK 1.2.02 Version (Botstein *et al.,* 1980).

RESULTS AND DISCUSSIONS

The parameters such as number of alleles, effective number of alleles, range of allele sizes, range of allele frequency, chi-square value with respect to Hardy- Weinberg equilibrium testing, Shannon Index, expected Heterozygosity and F_{IS} are presented in the Table 2.

Table 2: Microsatellite allele frequency, size range, HWE Chi-square values, and Shannon Index and Heterozygosity values of Umblachery

S. No	Micro-Satellite Loci	N_o	N_e	Allele Size (bp)	Allele Frequency	HWE Chi-Square Value	Shannon Index	H_e	F_{IS}
1	ILSTS 005	15	13.50	177-242	0.0556 - 0.1111	93.00^{NS}	2.6593	0.9804	- 0.0800
2	ILSTS 006	12	10.89	170-347	0.0714 - 0.1429	61.25^{NS}	2.4410	0.9780	- 0.1011
3	ILSTS 008	8	5.33	112-131	0.0625 – 0.3125	28.00^{NS}	1.8577	0.8667	- 0.2308
4	ILSTS 010	14	11.57	246 - 382	0.0556 – 0.1667	76.00^{NS}	2.5532	0.9673	- 0.0946
5	ILSTS 011	10	8.10	289 - 320	0.0556 – 0.1667	40.11^{NS}	2.1870	0.9281	- 0.1408
6	ILSTS 012	9	8.10	95 - 114	0.0556 – 0.1667	26.89^{NS}	2.1391	0.9281	- 0.1408
7	ILSTS 013	8	7.04	140 - 162	0.0556 – 0.1667	19.80^{NS}	2.0039	0.9085	- 0.1655
8	ILSTS 014	6	5.58	148 - 185	0.1111 – 0.2222	7.41^{NS}	1.7540	0.8693	- 0.2180
Mean ± S.E		10.25± 1.11	8.77± 1.04				2.1994± 0.1159	0.9283± 0.0160	0.1465±0 .0197

N_o: Observed No. of alleles, N_e: Expected No. of alleles.

DISCUSSION

Alleles at Microsatellite Loci

The number of alleles present in eight microsatellite loci were ranged from 6 (ILSTS 014) to 15 (ILSTS 005) with a mean of 10.25 ± 1.11 alleles per locus. The effective number of alleles were ranged from 5.33 (ILSTS 008) to 13.50 (ILSTS 005) with a mean of 8.7654 ± 1.0397. The mean effective number of alleles observed in this study is similar to that reported in Tharparkar (9) and Rathi (9.6) zebu cattle by Sodhi *et al.*, (2008). In contrast to this findings, less effective number of alleles were reported in Umblachery 4.00 ± 0.11 per locus (Karthickeyan *et al.,* 2007), Sahiwal (5.2) and Deoni (5.9) breeds of cattle of India (Mukesh *et al.,* 2004).

The size of the microsatellite alleles in Umblachery cattle ranged from 95 (ILSTS 012) to 382 (ILSTS 010) bp. The allele sizes observed are in accordance with the sizes of some highly informative markers (ILSTS 005, ILSTS 006, ILSTS 011 and ILSTS 033) described for *Bos Taurus* cattle (Kemp *et al.,* 1995). These microsatellite alleles are present in the population of Umblachery breed at a minimum frequency of 0.0556 (289 bp allele in ILSTS 011) to a maximum of 0.3125 (131 bp allele in ILSTS 008).

Hardy-Weinberg Equilibrium

In all the eight microsatellite loci screened, the Umblachery population was in Hardy-Weinberg equilibrium as revealed by chi-square test for Hardy-Weinberg equilibrium. This may be due to large population of Umblachery cattle available in Orathanadu Taluk of Thanjavur Disrict and the natural insemination practice prevalent in this area. In contrast to this report, Karthickeyan *et al.,* (2007) found Hardy-Weinberg disequilibrium with respect to 17 out of 25 loci screened in Umblachery breed.

Shannon Index

The Shannon Index is a parameter for determining diversity index. Shannon Index values ranges from 1 to 7. The mean value for Shannon Index is 2.1994 which clearly indicate high gene diversity within Umblachery population. In accordance with this report Thakur *et al.,* (2010) reported the mean Shannon Index value of 1.67 in Malvi population.

Heterozygosity

The overall mean observed and expected heterozygosities were 1.0000 and 0.9283 ± 0.1060 respectively and the expected heterozygosities ranged from 0.8667 (ILSTS 008) to 0.9804(ILSTS 005). The mean expected heterozygosity value is more than that of Umblachery (0.61) cattle (Karthickeyan *et al.,* 2007), Sahiwal (0.61), Hariana (0.66) and Deoni (0.70) cattle (Mukesh *et al.,* 2004); as well as Krishnavalley (Karthickeyan *et al.,* 2006). The high heterozygosity values may be due to more number of polymorphic loci in this Umblachery breed of cattle.

Within Breed Diversity

The inbreeding estimates were calculated using the F_{IS} values (Wright's Fixation Index). This revealed that Umblachery breed is having wider genetic variability. In the present study, in all the microsatellite loci negative F_{IS} values are exhibited ranging from – 0.0800 to – 0.2308 with the mean value of -0.1441. This negative mean value of 0.1441 suggests that 14 per cent of heterozygous excess individuals available in the population and the samples were collected from highly heterozygous population. This high heterozygosity values are comparable with Umblachery (-0.0487) cattle (Karthickeyan *et al.,* 2007). In contrast to this report, Metta (2004) reported high F_{IS} values (0.36) which resulted from small sample size (n=17) in Ongole breed of cattle, Sharma *et al.,* (2006) in Bachaur breed (F_{IS}=0.22) and Sharma *et al.,* (2007) in Gangatiri (FIS=0.31) breed of cattle.

Table 3: Bottleneck Analysis in three models of mutations

Model		IAM	TPM	SMM
Sign Rank Test (Number of Loci with Heterozygosity Excess)	Expected	4.83	4.75	4.70
	Observed	0.01747	0.01549	0.01407
Standardized Differences Test (T2 Values)		4.467	4.556	3.750
Wilcoxon Test (Probability of Heterozygosity Excess)		0.00195	0.00195	0.00195

The results of bottleneck analysis using three tests viz., Sign rank test, Standardzed differences test and Wilcoxon test in each of three models of mutations namely, infinite allele model (IAM), two phase model (TPM) and stepwise mutation model (SMM) are depicted in Table 3.

The results of bottleneck analysis revealed that the Umblachery population is non-bottlenecked. It is clear that the population has not undergone any reduction in the effective population size and remained at mutation drift equilibrium. Under all the three models, the heterozygosity excess values obtained in the

present study are not significant which indicates the population at mutation drifts equilibrium. In the present study, no mode-shift was detected in the frequency distribution of alleles and a normal L-shaped curve was observed.

Conclusion

This study demonstrated the polymorphic nature of microsatellite loci analysed in Umblachery breed of cattle. The negative F_{IS} values at all loci are indicative of highly heterozygous population of Umblachery cattle considered in this study. The data derived from this study will be useful for further characterization of Umblachery cattle. Comparative analysis with other zebu breeds of cattle in India will lead to determination of genetic divergence among breeds of India.

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