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Umblachery Breed of Cattle in South India: Genetic Assessment Through Microsatellite Markers

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Abstract: Umblachery cattle was assessed genetically using 25 microsatellite markers, as recommended by FAO. The number of alleles was ranging from 2 to 6 with a mean of 4.0±0.11. The mean number of effective alleles were 2.91±0.09. The allele sizes were ranging from 94 to 300 bp with the frequency distribution of 0.0111 to 0.9375. The estimated heterozygosity value was high 0.6139±0.02 and the PIC was 0.5625±0.03 and the loci screened were polymorphic and overall mean F_{IS} value (-0.0487) suggested the excess of heterozygosity in the population.

Key words: Cattle, microsatellites, PIC, umblachery

INTRODUCTION

Umblachery is a reputed draught breed of Thanjavur, Thiruvarur and Nagapattinam districts in eastern parts of Tamilnadu state in south India. This breed is the outcome of selection for short stature, suitable for work in marshy rice fields of Cauvery deltaic region (Thangaraju *et al*., 2001). As per the 1998 estimate, a total of 2.83 lakhs of Umblachery cattle were available in the breeding tract. However, the breeding tract of this breed has shrunken over the years due to mechanization. Introduction of crossbreeding and lack of concerted efforts for improvement and conservation have deteriorated the status of this breed. Umblachery bullocks are used for ploughing, carting, thrashing and paddling. The bullocks are capable of doing work continuously for 6 to 7 h under hot sun. A pair of bullocks can haul a load of 2 to 2.2 tonnes (including the cart weight) over a distance of 20 km in about 7 h.

The phenotypic, biochemical and cytogenetic characteristics have been studied and documented already (Sivaselvam *et al*., 2003; Kumarasamy *et al*., 2003). Hence, the present study was carried out with a view to characterize the breed in respect of microsatellite markers as microsatellites are the most powerful genetic markers for biodiversity evaluation. In addition, this study would bring out the variability at DNA level and genetic structure of the breed.

MATERIALS AND METHODS

Genomic DNA for Analysis

Blood samples were collected from 48 unrelated Umblachery cattle in its breeding tract. Genomic DNA was extracted from the samples using a routine high salt method as described by Miller *et al*. (1988). Quantity and quality assessment of isolated DNA samples were done by spectrophotometer.

Microsatellite Analysis

As per the suggestions of FAO (2004) in the Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans for global management of cattle genetic resources

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using reference microsatellites, a panel of 25 sets of microsatellite markers (Table 1) were selected to screen the population of Umblachery cattle during the year 2006. These markers were amplified using thermal cycler (MJ Peltier Thermal Cycler 200) in a PCR reaction mixture volume of 20 µL, containing 50-100 ng of template DNA; 1.5 mM $MgCl_2$; 5 picomoles each of forward and reverse primers; 0.75 units of Taq DNA polymerase and 100 mM dNTPs. Amplification was carried out with different annealing temperature (51 to 58°C for 45 sec) for various primers for 30 cycles.

Amplified PCR products were checked on one per cent agarose gel and visualized through UV illumination after staining with ethidium bromide. The samples which showed amplification were resolved through 6% denaturing polyacrylamide gel at a voltage of 1200 to 1400 for a period of 2-3 h (depending upon the size of PCR products) with 10 bp DNA ladder (Invitrogen, USA) as a molecular weight marker. The gel was subjected to silver-staining procedure (Cominicini *et al*., 1995) for genotyping.

Statistical Analysis

The basic statistics such as mean and standard error were calculated as per Snedecor and Cochran (1989). Scoring of alleles and sizing of fragments were done using Diversity Database (BioRad, USA) software followed by manual verification. Allele frequencies were estimated by direct counting. Polymorphism Information Content (PIC) was estimated using the formula developed by Bostein *et al*. (1980). The observed heterozygosity, Hardy-Weinberg equilibrium proportion, expected heterozygosity and Wright's fixation index were calculated by using the software POPGENE 32 (http://www.ualberta.ca/~fyeh/).

RESULTS AND DISCUSSION

The parameters estimated out of microsatellite analysis in Umblachery cattle such as number, size and frequency of microsatellite alleles, Chi-square value, polymorphism information content and heterozygosity for different microsatellite loci are furnished in Table 1.

Number, Size and Frequency of Microsatellite Alleles

The number and size of alleles were ranging from 2 to 6 and 94 to 300 bp, respectively in 25 microsatellite loci in Umblachery cattle. The mean number of alleles was found to be 4.00±0.11 per locus. A total of 100 numbers of alleles were found distributed in these polymorphic loci in the breed. The loci, ETH152 and INRA063 exhibited lowest number of two alleles each and ETH225 and INRA005 loci, each possessed 6 number of alleles. However, the effective number of alleles (ne) was ranging from 1.13 to 4.89 with a mean of 2.91±0.09. The mean number of alleles observed in the study is lesser than the number reported in Sahiwal (5.2) and Deoni (5.9) breeds of cattle of India (Mukesh *et al*., 2004). Prabhu (2004) and Kumar (2006) studied the microsatellite pattern, respectively in Amritmahal and Hallikar cattle breeds in Karnataka, a neighbouring state of Tamilnadu, using the same sets of microsatellites. The respective number and sizes of alleles were ranging from 2 to 8 and 89 to 302 bp and 3 to 9 and 102 to 294 bp in Amritmahal and Hallikar breeds. In another study (Karthickeyan *et al*., 2006), the Krishna Valley breed of south India revealed 3 to 7 number and 94 to 300 bp size of alleles in these loci, which corroborate with the findings of the present study. In general, the number and sizes of microsatellite alleles observed fall within the range mentioned in the Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans, published by FAO.

These alleles occurred at a minimum frequency of 0.0111 (140 bp allele in ETH225) and a maximum frequency of 0.9375 (194 bp allele in ETH152 locus). Similar ranges in the frequency of alleles were observed in the other Indian breeds of cattle viz. Amritmahal, Hallikar and Krishna Valley

Table 1: Microsatellite allele frequency, Polymorphism Information Content (PIC) and heterozygosity in Umblachery breed of cattle

			Range				
Locus	n_a	n_e	Allele size (bp)	Allele frequency	HWE (Chi-square value)	PIC	He
ILSTS005	4	3.39	182-194	0.1125-0.4000	4.286	0.6517	0.7047
ILSTS006	4	2.30	290-300	0.1111-0.6222	5.676	0.5258	0.5647
ILSTS011	3	2.96	254-268	0.2826-0.3804	20.234**	0.5877	0.6619
ILSTS030	3	1.81	152-156	0.2778-0.6889	1.669	0.3727	0.4472
ILSTS033	4	3.23	138-150	0.1026-0.4359	20.299**	0.6368	0.6900
ILSTS034	5	3.55	156-166	0.1154-0.4487	19.863*	0.6820	0.7186
ILSTS054	4	3.98	132-148	0.2237-0.2763	12.090	0.7016	0.7486
INRA005	6	4.89	134-150	0.0682-0.2727	37.841**	0.7652	0.7955
INRA032	5	3.66	166-188	0.0349-0.3721	61.894**	0.6789	0.7266
INRA035	5	3.21	102-120	0.0208-0.4583	10.439	0.6402	0.6884
INRA063	2	1.20	180-186	0.0938-0.9062	1.125	0.1556	0.1699
ETH003	4	2.44	102-116	0.0119-0.5119	21.798**	0.5094	0.5901
ETH010	4	2.81	210-220	0.0921-0.4605	27.313**	0.5796	0.6447
ETH152	2	1.13	194-200	0.0625-0.9375	0.176	0.1103	0.1172
ETH225	6	3.34	138-160	0.0111-0.4556	53.923**	0.6572	0.7002
HEL001	4	1.86	100-110	0.0319-0.7021	46.809**	0.4152	0.4611
HEL005	4	2.06	150-166	0.0625-0.6667	38.737**	0.4763	0.5141
HEL009	5	3.69	148-164	0.0795-0.4205	62.381**	0.6903	0.7293
BM1818	3	2.97	262-278	0.2949-0.3718	10.997*	0.5895	0.6637
BM2113	4	3.34	136-148	0.0745-0.3830	17.411*	0.6434	0.7005
MM8	3	2.59	138-146	0.1458-0.4375	7.166*	0.5317	0.6137
HAUT024	4	2.63	124-136	0.0595-0.4643	36.482**	0.5452	0.6196
HAUT027	3	2.94	156-168	0.2639-0.3750	15.608**	0.5849	0.6593
CSRM060	5	3.34	94-112	0.0909-0.4773	20.354*	0.6646	0.7007
CSSM066	4	3.54	180-186	0.1452-0.3548	10.512	0.6658	0.7175
Overall mean/range	4.00±0.11	2.91±0.09	94-300	0.0111-0.9375		0.5625±0.03	0.6139±0.02

n_a-Observed No. of alleles; n_e-Effective No. of alleles; HWE-Hardy-Weinberg Equilibrium; He-Expected Heterozygosity; *-Significant; **-Highly significant

(Prabhu, 2004; Kumar *et al*., 2006; Karthickeyan *et al*., 2006). However, the higher frequency of allele (in ETH 152) observed in the present study as well as in the other Indian breeds of cattle indicates that the preponderance of this particular allele (194 bp) in the Indian zebu cattle population.

Hardy-Weinberg Proportion

The Chi-square (χ^2) test for Hardy-Weinberg equilibrium revealed that the Umblachery population is not in equilibrium with respect to 17 out of 25 loci screened. The disequilibrium exhibited in most of loci revealed that there might be unobserved null alleles (those which could not be amplified) in those loci which have not been, hitherto, identified by other means. The deviation of 68% of the loci from equilibrium would be attributed to high mutation rates and size homoplasty, the inherent qualities of the microsatellites. Further, the disequilibrium could have resulted from the sampling from a range of distinct locations, within the breeding tract of the Umblachery, as suggested by Dorji *et al*. (2003).

Informativeness of Microsatellite Markers

The Polymorphism Information Content (PIC) value is the statistical assessment of informativeness of a marker. This value was ranging from 0.1103 (ETH152) to 0.7652 (INRA005) with a mean PIC of 0.5625±0.03. Except a few loci, all other loci showed high PIC values of more than 0.5 indicating more polymorphic information content in the breed. The polymorphism at any locus is created by increasing dinucleotide repeats and mutation. The higher number of alleles found in the INRA005 locus has exhibited high polymorphic information content and the lower number of alleles (2) in ETH152 with a higher frequency of 194 bp allele resulting in lower PIC value. In Ongole cattle,

the range of PIC between 0.15 and 0.79 was reported by Metta *et al*. (2004) using 10 different microsatellite markers which is in close agreement with present study. Almost similar range of PIC (0.13 to 0.80) was observed in the same study for Deoni cattle of India. While Kumar *et al*. (2006) observed slightly higher PIC range of 0.2322 to 0.8654 in Hallikar cattle using 19 microsatellite markers. Karthickeyan *et al*. (2006) reported PIC range of 0.2583 (ILSTS030) to 0.7975 (INRA035) with a mean of 0.6209±0.03 in Krishna Valley cattle for the same set of markers. In general, the population has got high polymorphism information content of 56% which indicates that these markers are highly informative for genetic assessment of Umblachery cattle.

With-in Population Genetic Variability

The heterozygosity is an appropriate measure of genetic variability within a population. In the present study, overall means for observed and expected heterozygosities were 0.6581±0.03 and 0.6139±0.02, respectively with the ranges of 0.9773 (HEL009) to 1.000 (ETH225) and 0.1172 (ETH152) to 0.7955 (INRA005). Majority of the loci had relatively higher expected heterozygosity, reflecting the existence of variation in the breed. The mean expected heterozygosity value is comparable to that of Sahiwal (0.61) and Hariana (0.66) and lower than that of Deoni (0.70), the other Indian cattle breeds studied by Mukesh *et al*. (2004). Whereas low average heterozygosity (0.46±0.1) was observed in Ongole cattle (Metta *et al*., 2004). In Krishna Valley cattle (Karthickeyan *et al*., 2006), the heterozygosity value of 0.6569±0.03 with higher range from 0.3047 (ILSTS030) to 0.8220 (INRA035) was observed. The high heterozygosity values observed in the present study indicate more number of polymorphic loci in Umblachery cattle. This implies the higher amount of genetic variability that can be still exploited in the population.

Within-Breed Genetic Diversity

The within-breed diversity was estimated using the F_{IS} (within population inbreeding estimate; Wright's Fixation Index) values as a measure of heterozygote deficiency. The negative F_{IS} value of -0.0487 over all loci reveals that the Umblachery breed is having a wide genetic variability with excess of heterozygotes and outbred in nature even though some of the loci exhibited the positive values. On the contrary, Metta (2004) reported high F_{IS} value (0.36) which resulted from small sample size (n = 17) in Ongole breed. In spite of the general belief that Umblachery breed has originated from Kangayam breed of cattle, the variability is maintained to a greater extent and their selection for short stature should have been done from a very wide ancestral population.

CONCLUSIONS

From the large number of polymorphic loci in Umblachery breed and the overall mean polymorphism information content of 56%, these markers are highly informative and can be utilized for characterization of domestic animal biodiversity. It also opens up the scope for exploiting the genetic variability in the population for conservation. Comparative analysis with other Indian draught cattle breeds will determine the genetic distance and evolutionary relationship of this breed with other zebu cattle of India.

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REFERENCES

Bostein, D., R.L. White, M. Skolnick and R.W. Davis, 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet., 32: 314-331.

Cominicini, S., P. Leone, I. Redaell, L. De Giuli and Y. Zhang, 1995. Characterization of bovine microsatellites by silver staining. J. Anim. Breed. Genet., 112: 415-440.

Dorji, T., O. Hanotte, M. Arbenz, J.E.O. Rage and W. Roder, 2003. Genetic diversity of indigenous cattle populations in Bhutan: Implications for conservation. Asian-Aust. J. Anim. Sci., 16: 946-951.

FAO, 2004. Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans for Global Management of Cattle Genetic Resources Using Reference Microsatellites Global Projects for the Maintenance of Domestic Animal Genetic Diversity (MoDAD). http://www.fao.org/dad-is/.

Karthickeyan, S.M.K., R. Saravanan and P. Thangaraju, 2006. Characterisation of Krishna Valley breed of cattle (*Bos indicus*) in south India using microsatellite markers. Livestock Research for Rural Development, 18, Article No. 151. (http://www.cipav.org.co/lrrd/lrrd18/11/kart18151.htm).

Kumar, S.N., M.R. Jayashankar, C.S. Nagaraja, M.G. Govindaiah, R. Saravanan and S.M.K. Karthickeyan, 2006. Molecular characterisation of Hallikar breed of cattle using microsatellite markers. Asian-Aust. J. Anim. Sci., 19: 622-626.

Kumarasamy, P., S.M.K. Karthickeyan, R. Rajendran, S.N. Sivaselvam, P. Thangaraju and A. Mahalinga Nainar, 2003. Karyological Studies on Umblachery Cattle. Abstract Presented in the National Seminar on Strategies for Genetic and Reproductive Improvement of Bovine, Held on 13-14 November, 2003 at Madras Veterinary College, Chennai, India, pp: 241-242.

Metta, M., S. Kanginakudru, N. Gudiseva and J. Nagaraju, 2004. Genetic characterization of Indian cattle breeds, Ongole and Deoni (*Bos indicus*) using microsatellite markers-a preliminary study. BMC Genetics, 5:16 (http://www.Biomedcentral.com/1471-2156/5/16).

Miller, S.A., D. Dykes and H.F. Polesky, 1988. A simple salting out procedure for extraction of DNA from human nucleated cells. Nucleic. Acids Res., 16: 1215.

Mukesh, M., M. Sodhi, S. Bhatia and B.P. Mishra, 2004. Genetic diversity of Indian native cattle breeds as analyzed with 20 microsatellite loci. J. Anim. Breed. Genet., 121: 416-424.

Prabhu, M.B.H., 2004. Phenotypic and molecular genetic characterisation of amritmahal cattle. M.Sc. Thesis, University of Agricultural Sciences, Bangalore, India, pp: 62-90.

Snedecor, G.W. and W.G. Cochran, 1989. Statistical Methods. 8th Edn., Iowa State University Press, Ames, Iowa, USA.

Sivaselvam, S.N., P. Kumarasamy, R. Rajendran, S.M.K. Karthickeyan, P. Thangaraju and A. Mahalinga Nainar, 2003. Heamoglobin, Transferrin and Albumin Polymorphisms in Umblachery Cattle (*Bos indicus*). Abstract Presented in the National Seminar on Strategies for Genetic and Reproductive Improvement of Bovine, Held on 13-14 November, 2003 at Madras Veterinary College, Chennai, India, pp: 244.

Thangaraju, P., A. Mahalinga Nainar and R. Rajendran, 2001. Umblachery-a Renowned Draught Cattle of Tamilnadu. Status Paper Presented at the Workshop on Indigenous Cattle and Their Role in the New Millennium, Organized by TANUVAS, IDA and NDRI on 24-25 March, 2001 at Erode, Tamilnadu, India, pp: TN-13-19.