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MICROSATELLITE ANALYSIS OF ONGOLE CATTLE (BOS INDICUS) OF A.P.

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Abstract: Eighteen dinucleotide markers were used to assess the genetic diversity in Ongole cattle, a dual purpose breed maintained at Livestock research station, cattle project, Lam farm, Guntur, A.P. The observed number of alleles at each locus ranged from three to twelve with a total of 136 no. of alleles with a mean number of 7.5 across all the loci. The mean effective number of alleles was found to be 4.44. The size and frequencies of alleles ranged from 82 to 308 bp and from 0.026 to 0.765 respectively. The Polymorphism Information Content (PIC) values varied from 0.36 to 0.86 with a mean value of 0.68. The mean F_{IS} value was 0.506. Genetic bottleneck hypotheses revealed that the population is non-bottlenecked.
Keywords: Genetic variation, Ongole cattle, Microsatellites, Bottleneck analysis.

Introduction

Ongole is the dual purpose breed of cattle belongs to short horned group of zebu with majestic-looking, huge in size, extremely docile, faster growth rate and suitable for harsh tropical conditions. Mechanization and indiscriminate breeding among native stocks lead to the dilution of Ongole cattle germplasm. DNA-based molecular markers have high level of polymorphism and successfully used to evaluate genetic variation of populations [Almeida *et al*., 2000]. Hence the present study was carried out using microsatellite markers to understand the genetic constitution of Ongole cattle population which helps to formulate breeding strategy. Usually in a population at mutation-drift equilibrium, there is approximately an equal probability that a locus shows heterozygosity excess or deficit. Populations which have experienced a recent reduction of their effective population size exhibit a correlative reduction of the allele numbers and heterozygosities at polymorphic loci [Piry *et al*. 1999]. Hence, bottleneck analysis was conducted to find out whether the Ongole cattle population is at mutation-drift equilibrium or not.

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Materials and methods

Blood samples were collected from 20 Ongole cattle and Genomic DNA was isolated by standard Phenol-Chloroform method [Sambrook and Russell, 2001]. The purity and concentration of DNA samples were estimated by UV spectrophotometer. The mean yield of DNA was 2.96µg/ml. The quality of DNA samples were also checked by agarose gel (1%) electrophoresis. A total of 18 microsatellite markers chosen randomly from the list recommended by FAO were used in the present study.

These microsatellites were amplified using a thermal cycler with a PCR reaction mixture (12.5µ1) containing 25 mM $Mgcl_2$, 10mM dNTPs, 10x buffer, 60pM of each primer, 50ng of template DNA and 1 unit of Taq DNA polymerase. The annealing temperature of each primer was standardized by running gradient PCR. The PCR products were checked on 2% agarose gel electrophorosis, genotyped using 6% denaturing polyacrylamaide gel and then visualized after silver staining. The allele sizes were determined with the help of 50 bp DNA ladder as a standard marker. Individual samples were scored by using quantity one and genotyper 2.3 software verified manually either as homo or heterozygote for each loci. Microsatellite allele frequencies, effective number of alleles, expected heterozygosity and within population heterozygosity deficiency were calculated using the POPGENE 1.31 software version [Yeh *et al.* 1999]. Polymorphism information content was calculated using PIC calculator. BOTTLENECK version 1.2.02 was used to know the presence of bottleneck effect in the investigated Ongole cattle population [Piry *et al.* 1999].

Results and discussion

The allele number, size, frequency, polymorphism information content, Observed and expected heterozygosity, within population inbreeding estimates for different microsatellite loci are presented in Table1. A total of 136 alleles were observed with number of alleles ranged from 3 to 12 with a mean allelic number of 7.5 per microsatellite locus which was higher compared to the values reported in literature by Muralidhar, 2004 (3.5). However Nei, 1987 stated that the mean number of alleles observed for different population is a reasonable indicator of genetic variation and mutation–drift equilibrium. The effective number of alleles at various loci ranged from 1.642 to 7.62 with a mean effective number of 4.44 alleles per locus. The size and frequency of alleles ranged from 82 to 308 bp and 0.026 to 0.765 which was on par with the findings of Karthikeyan *et al.* 2009 in Kangayam breed.

The observed heterozygosity values ranged from zero to one with a mean of 0.36 which was in accordance with the statement given by Takezaki and Nei (1996) that the average

heterozygosity must be between 0.30 to 0.80 in a breed to be a useful marker for measuring genetic variation. The overall observed heterozygosity was lesser than the expected heterozygosity which might be due to the fact that samples collected from the unrelated animals which were maintained as closed populations and also be due to the presence of more homozygous individuals in the samples analyzed.

The polymorphism information content values which provides the informativeness of a marker ranged from 0.36 to 0.86 with a mean value of 0.68 which confirmed that all the markers were found to be polymorphic in nature, The higher percentage of polymorphic loci might be due to the efficient breeding and management strategies followed in the breeding farm without any adverse effects of inbreeding, though the populations were under longterm selection for primary traits of importance. The loci possessing high PIC values (above 0.5) indicated that cattle specific microsatellite markers used were highly polymorphic and hence highly informative for genetic characterization of cattle breeds. The F_{IS} values ranged from -0.22 to 1.0 with a mean of 0.506 which revealed that there is a shortage of heterozygotes (50.6%), which is slightly higher than the results reported by Muralidhar *et al.* 2004 in Ongole cattle (0.36). The reason for deficiency of heterozygotes ($F_{IS}>0$) might be attributable to a number of causes like assortative mating, linkage with loci under selection, small sample size, Population heterozygosity or null alleles *etc*.

The results of bottleneck analysis using three tests viz., Sign rank test, Standardized 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 summarized in Table 2 which indicates the population is non-bottlenecked, *i.e.*, it has not undergone any recent reduction in the effective population size. The heterozygosity excess values obtained are non significant under all the three models indicating that the population is at mutation-drift equilibrium. SMM, which is the most suited model for microsatellite analysis, revealed absence of significant heterozygotes excess in Ongole cattle population and no mode-shift was detected in the frequency distribution of alleles and a normal L-shaped form was observed (Fig.1). similar findings were reported by Ganapathi *et al.* 2012 in Bargur cattle.

In conclusion, results of this study revealed that considerable genetic variability is existing in the Ongole cattle showing high number of alleles per microsatellite locus with high mean expected heterozygosity and absence of bottleneck in the population, which will help to formulate suitable breeding programme for the improvement of Ongole cattle.

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Table 1. Allele number, Size, Frequency, Polymorphism information content, Observed and expected heterozygosity, Within population inbreeding estimates

Locus	Observed alleles (n_a)	Effective number of alleles (n_e)	Allele size	Allele frequency	PIC	Heterozygosity		Within population inbreeding
						Observed	Expected	
ETH010	12	6.416	176-216	0.028-0.306	0.83	0.44	0.84	0.47
ILSTS005	5	1.642	180-192	0.029-0.765	0.36	0.18	0.39	0.55
TGLA122	8	2.700	138-182	0.028-0.583	0.61	0.22	0.63	0.65
INRA035	9	6.278	102-124	0.026-0.237	0.82	1.00	0.84	-0.19
INRA063	5	3.333	118-184	0.067-0.467	0.66	0.13	0.70	0.81
HEL001	12	6.416	116-154	0.028-0.306	0.83	0.44	0.84	0.47
TGLA126	6	4.102	116-144	0.026-0.342	0.72	0.32	0.76	0.58
INRA005	3	1.923	135-140	0.028-0.639	0.39	0.22	0.48	0.54
ILSTS006	4	2.348	300-308	0.111-0.611	0.53	0.00	0.57	1.00
ETH225	5	3.447	132-146	0.056-0.444	0.67	0.22	0.71	0.69
CSRM60	7	5.641	82-114	0.053-0.237	0.80	1.00	0.82	-0.22
BM1824	6	3.139	186-196	0.026-0.395	0.62	0.42	0.68	0.38
TGLA053	8	4.263	148-190	0.028-0.417	0.74	0.33	0.77	0.56
INRA037	12	6.416	116-154	0.028-0.306	0.83	0.44	0.84	0.47
ETH003	7	2.439	114-132	0.026-0.605	0.56	0.37	0.59	0.38
MM12	11	7.624	108-164	0.028-0.194	0.86	0.44	0.87	0.49
HAUT024	8	5.885	120-156	0.031-0.250	0.81	0.31	0.83	0.62
HAUT027	8	5.891	134-152	0.028-0.222	0.81	0.11	0.83	0.87
Mean	**7.5**	**4.44**			**0.68**	**0.36**	**0.72**	**0.506**
Range			82-308	0.026-0.765				

Table 2. Bottle neck analysis in three models of mutation

Model		IAM	TPM	SMM
Sign test (No. of loci with heterozygosity excess)	Expected	10.49	10.80	10.82
	Observed	9	8	4
Standardized differences test (T_2 values)		-3.580	-5.593	-8.910
Wilcoxon sign rank test (Probability of heterozygosity excess)		0.86774	0.96673	0.99968

Figure 1: L-shaped mode-shift graph showing lack of bottleneck in Ongole population.