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GENETIC DIVERSITY AND BOTTLENECK STUDIES IN PUNGANUR CATTLE THROUGH MICROSATELLITE MARKERS

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Abstract: In the present study a total of seventeen microsatellite loci were used for the assessment of genetic diversity and bottleneck analysis in Punganur cattle maintained at LRS, Palamaner, Sri Venkateswara Veterinary University, Tirupati. A total of 115 alleles with a mean of 6.76 were observed. The mean effective number of alleles was 3.83 with size and frequencies ranged from 76 to 216 bp and 0.024 to 0.905 respectively. The mean Polymorphism Information Content value was 0.62 with mean F_{IS} value of 0.51. No mode shift was detected in the frequency distribution of alleles and a normal L-distribution was obtained indicating that the population is non-bottlenecked.
Keywords: Punganur cattle, Microsatellite markers, Genetic diversity, Bottleneck analysis.

Introduction

Punganur is a one of the dwarf and dual purpose cattle breed of A.P. Characterization at phenotypic and molecular level is a preliminary step in conservation of the animal genetic resources. Since the population size is declining in number, efforts have been in progress now for its conservation. The analysis of genetic diversity by using microsatellite markers provides information about the presence of bottleneck in the population.

Materials and Methods

Blood samples were collected from 21 Punganur cattle and Genomic DNA was isolated as per procedure of Sambrook and Russell, 2001. A total of 17 microsatellite markers chosen randomly from the list recommended by FAO 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 PCR products were genotyped using 6% denaturing polyacrylamaide gel and then visualized after silver staining. The allele sizes were determined with the help of 50 bp DNA marker. Samples were scored by using quantity one and genotyper 2.3 software, verified manually

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either as homo or heterozygote for each loci. Allelic profile was 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 reduction of size in the investigated Punganur population [Piry *et al.* 1999].

Results and discussion

The allele number, size and frequency, PIC, Observed and expected heterozygosity, within population inbreeding estimates for 17 number of microsatellite loci are presented in Table 1. The size and frequency of alleles ranged from 76 to 216 bp and 0.024 to 0.90 and were on par with values reported by Karthikeyan *et al.* (2009). The estimate of heterozygosity indicates the diversity within the breed. The overall observed heterozygosity is lesser than the expected heterozygosity, which might be due to the presence of more homozygous individuals in the samples analyzed. 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 long term selection for primary traits of importance. The PIC values observed in most of the loci are more or less similar with the results reported in Kangayam cattle (0.1505 to 0.7453; Karthikeyan *et al*., 2009). The inbreeding coefficient measures the reduction of heterozygosity within the population. F_{IS} values more than zero reveal closer relationship between the individuals of a population and there is an excess of homozygotes (0.51) which is due to assortative mating, linkage with loci under selection, small sample size or the presence of null alleles (Muralidhar *et al*. 2004).

Bottleneck Analysis:

When a population experiences reduction of its effective population size, the reduction in the allele number is at a faster rate than reduction in heterozygosity, *i.e*. when the locus is at mutation-drift equilibrium, the observed heterozygosity is larger than the expected heterozygosity from the observed number of alleles. Three tests (sign test, standard difference test and wilcoxon test) under three mutation models namely infinite allele model (IAM), two phase model (TPM), step-wise mutation model (SMM) used to find the heterozygosity excess using the bottleneck programme and results were shown in Table 2. Results revealed that under sign rank test, observed heterozygosity excess (H_{oe}) was significantly less than the expected heterozygosity excess (H_{ee}) under IAM ($P \leq 0.05$), TPM and SMM ($P \leq 0.01$) models of microsatellite evolution and hence population is not in mutation drift equilibrium. It was

found that T_2 values were significantly negative in all 3 models, indicating magnitude of heterozygote deficiency, which means absence of genetic bottleneck.

Non significant P-value under Wilcoxon sign rank test revealed the heterozygosity excess under IAM, TPM and SMM models, agreed the acceptance of mutation-drift equilibrium (Deepika *et al*., 2012).

In addition qualitative graphical method (Mode-shift indicator test) of Luikart *et al.* (1998) was also used to visualize the allele frequency spectra. The microsatellite alleles were classified into 10 frequency classes and found normal L-shaped distribution where alleles with low frequencies (0.01-0.1) are the most abundant (fig 1.) which reflects that the population has not undergone any recent reduction in size.

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

Locus	Observed alleles (n_a)	Effective number of alleles (n_e)	Allele size	Allele frequency	PIC	Heterozygosity		F_{IS}
						H_o	H_e	
ETH010	12	6.416	176-216	0.028-0.306	0.83	0.44	0.84	0.47
TGLA122	6	4.333	136-182	0.077-0.308	0.73	0.00	0.77	1.00
INRA035	2	1.208	110-112	0.095-**0.905**	0.16	0.00	0.17	1.00
INRA063	3	1.851	170-184	0.053-0.684	0.39	0.00	0.46	1.00
HEL001	4	1.495	118-128	0.048-0.810	0.31	0.00	0.33	1.00
TGLA126	7	6	118-130	0.071-0.238	0.81	0.90	0.83	-0.09
INRA005	4	1.664	138-146	0.048-0.762	0.38	0.00	0.40	1.00
INRA032	5	2.609	197-204	0.024-0.548	0.56	0.24	0.62	0.61
ETH225	6	3.472	134-158	0.024-0.429	0.67	0.95	0.71	-0.34
CSRM60	12	6.945	76-114	0.024-0.286	0.84	0.81	0.86	0.05
BM1824	7	6.438	179-197	0.071-0.190	0.82	0.05	0.84	0.94
TGLA053	6	3.841	146-180	0.038-0.385	0.70	0.85	0.74	-0.14
INRA037	9	5.517	112-146	0.025-0.300	0.80	0.40	0.82	0.51
ETH003	13	4.455	102-130	0.024-0.429	0.76	0.52	0.78	0.32
TGLA227	4	1.740	88-100	0.026-0.737	0.39	0.16	0.43	0.63
MM12	8	4.198	102-142	0.026-0.421	0.74	0.63	0.76	0.17
HAUT024	7	2.985	142-156	0.025-0.525	0.63	0.30	0.67	0.55
Mean	**6.76**	**3.83**			**0.62**	**0.36**	**0.65**	**0.51**

Table 2. Mutation–drift equilibrium, heterozygosity excess/deficiency under three mutation models in Punganur cattle population

Model		Infinite alleles model (IAM)	Two-phase model (TPM)	Step wise mutation model (SMM)
Sign test (No. of loci with heterozygosity excess)	Expected	10.09	10.14	10.08
	Observed	6	3	2
	Probability	0.038*	0.000**	0.000**
Standardized differences test	T_2 values	-3.705	-5.524	-8.659
	Probability	0.000**	0.000**	0.000**
Wilcoxon sign rank test	Probability of heterozygosity excess	0.960	0.997	0.999

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