

MOLECULAR GENETIC CHARACTERIZATION OF PUNGANUR CATTLE

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ABSTRACT

Eleven dinucleotide microsatellite markers were used for characterization of Punganur cattle by multiplexing. The mean genomic DNA in blood was 4.338 mg/ml and the mean optical absorbance ratio (OD 260 nm/280 nm) was 1.803. All the 11 markers studied were polymorphic with the number of alleles ranging from 4 to 9 and the mean number of alleles per locus was 6.0. The mean observed and expected heterozygosities were 0.684 and 0.666, respectively, while the mean PIC was 0.628. The marker TGLA 122 was found to be most polymorphic with a PIC estimate of 0.809, while the marker ETH 225 was the least polymorphic with a PIC value of 0.308. The overall mean coefficient of inbreeding was -0.001 and the Punganur cattle population studied was found to be under Hardy-Weinberg equilibrium.

Punganur is a dual-purpose zebu cattle breed native of Andhra Pradesh and is one of the dwarf breeds of cattle in India. Although few studies were made on the biochemical and cytogenetic markers of this breed, the levels of polymorphism detected were not sufficient and they have limitations in identifying breed specificity. Microsatellites are short sequence repeats of 2 to 4 nucleotides, distributed randomly throughout the genome. They are highly polymorphic and can be typed easily using Polymerase Chain Reaction (PCR) and they are markers of choice for estimating the genetic variation (MacHugh *et al.* 1994). The present study was undertaken with an objective to characterize Punganur breed of cattle using the microsatellite markers for undertaking the breed conservation.

MATERIALS AND METHODS

Blood samples (3 ml) from a total of 23 Punganur cattle (12 male and 11 female) maintained at the Livestock Research Station, Palamaner, Andhra Pradesh and from the farmers' herds in its breeding tract were collected from the external jugular vein. The genomic DNA was isolated and the PCR amplification was done with 11 dinucleotide microsatellite primers supplied along with Stock marks cattle paternity PCR typing kit. The locus wise chromosome location, primer sequence, chromosome location and the dye used for labeling the markers in multiplexing panel are given in Table 1. The genomic DNA samples were amplified in a reaction volume of 7 µL containing 1.5 µL of PCR buffer, 2.0 µL of DNTP mixture, 0.25 µL of *Ampli*

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taq DNA polymerase, 2.75 μ L of primer mixture, 0.5 μ L of deionized distilled water and 1 μ L of template DNA (10 ng/ μ L). A thermal cycling protocol of 95° C for 15 min for initial denaturation, followed by 31 cycles of 94° C for 45 seconds for denaturation, 61° C for 45 seconds for primer annealing and 72° C for 60 seconds for primer extension, ending with an extension phase of 72° C for 60 min was used. The denaturing Poly Acrylamide Gel Electrophoresis (PAGE) was performed in ABI Prism 377 Automated DNA sequencer. For each locus, the allele frequency, mean number of alleles per locus and percentage of polymorphic loci were computed. The heterozygosity and Polymorphism Information Content (PIC) were estimated as per Nei (1973) and Nei (1978), respectively. The coefficient of inbreeding (F_{IS}) was calculated by using FSTAT programme as per Goudet (1995). For each locus, the deviation of expected genotype frequency from the observed genotype frequency was tested by Chi-square test.

RESULTS AND DISCUSSION

The overall mean optical absorbance ratio (260 nm/280 nm) of the genomic DNA samples was 1.803, which was similar to the ratio recommended for the pure DNA preparations by Sambrook and Russell (2001). The mean quantity of DNA obtained was 4.338 μ g/ml of blood, which was higher than the mean of 3.333 μ g/ml reported by Muralidhar (2003) in Ongole and Deoni cattle. The agarose gel electrophoresis revealed the intact single band for each DNA sample, which indicated that there was no shearing of genomic DNA during isolation from blood samples (De *et al.* 2000).

The locus wise number of alleles, allele size and frequency, heterozygosity, Polymorphism Information Content (PIC), along with the Chi-square value for testing the difference between the observed and expected genotype frequencies are given in Table 2. The genescan image showing

polymorphism of microsatellite markers is given in Fig 1. All the eleven-microsatellite markers used in the present study were found to be polymorphic with the number of alleles ranging from 4 (ETH 10 and ETH 225) to 9 (TGLA 122), which revealed the existence of considerable genetic variability among the animals sampled for exploitation by selection. A total of 66 alleles were produced by eleven markers with a mean of 6.0 alleles per locus, which was in agreement with a mean 6.5 alleles per locus reported by Beja-Pereira *et al.* (2003) in Iberian and French cattle breeds, while it was lower than the mean of 8.4 alleles per locus obtained by MacHugh *et al.* (1997) in Taurine and zebu cattle populations. The higher percentage of polymorphic loci observed in the present study might be due to the dinucleotide repeats.

The overall mean observed and expected heterozygosities were 0.684 and 0.666, respectively and ranged from 0.304 to 1.000 and 0.334 to 0.829, respectively, indicating higher polymorphism of the microsatellite loci in the population. The mean heterozygosities observed in the present study were in agreement with those reported by Heyen *et al.* (1997).

The overall mean estimate of PIC obtained in the present study was 0.628 and it ranged from 0.308 for ETH225 to 0.809 for TGLA122, which were within the range of the estimates reported by Heyen *et al.* (1997). The locus ETH 225 had the lowest number of alleles and least heterozygosity and hence, it was least informative as revealed by its lowest PIC estimate. In contrast, the locus TGLA122, which amplified the maximum number of alleles had the highest heterozygosity and PIC values, was observed to be the most informative among all the 11 loci studied. The overall mean coefficient of inbreeding in the present study was -0.001 and it ranged from -0.225 to 0.512. A total of seven loci recorded negative inbreeding coefficients indicating absence of inbreeding with respect to these loci, which could be due to the fact that the Punganur cattle utilized in the present study were procured from different locations in its breeding tract.

Table 1
Locus-wise chromosome location, primer sequence and dye used for the microsatellite markers used in the study

Locus	Chromosome location	Primer sequence (5' → 3')	Dye used	Reference
TGLA227	18	CGA ATT CCA AAT CTG TTA ATT TGC T ACA GAC AGA AAC TCA ATG AAA GCA	Fam (blue)	Georges and Massey (1992)
BM2113	2	GCT GCC TTC TAC CAA ATA CCC CTT CCT GAG AGA AGC AAC ACC	Fam (blue)	Bishop <i>et al.</i> (1994)
TGLA53	16	GCT TTC AGA AAT AGT TTG CAT TCA ATC TTC ACA TGA TAT TAC AGC AGA	Fam (blue)	Georges and Massey (1992)
ETH10	5	GTT CAG GAC TGG CCC TGC TAA CA CCT CCA GCC CAC TTT CTC TTC TC	Fam (blue)	Toldo <i>et al.</i> (1993)
SPS115	15	AAA GTG ACA CAA CAG CTT CTC CAG AAC GAG TGT CCT AGT TTG GCT GTG	Fam (blue)	Moore and Byrne (1993)
TGLA126	20	CTA ATT TAG AAT GAG AGA GGC TTC T TTG GTC TCT ATT CTC TGA ATA TTC C	Joe (green)	Georges and Massey (1992)
TGLA122	21	CCC TCC TCC AGG TAA ATC AGC AAT CAC ATG GCA AAT AAG TAC ATA C	Joe (green)	Georges and Massey (1992)
INRA23	3	GAG TAG AGC TAC AAG ATA AAC TTC TAA CTA CAG GGT GTT AGA TGA ACT C	Joe (green)	Vaiman <i>et al.</i> (1994)
ETH3	19	GAA CCT GCC TCT CCT GCA TTG G ACT CTG CCT GTG GCC AAG TAG G	Ned (yellow)	Toldo <i>et al.</i> (1993)
ETH225	9	GAT CAC CTT GCC ACT ATT TCC T ACA TGA CAG CCA GCT GCT ACT	Ned (yellow)	Steffen <i>et al.</i> (1993)
BM1824	1	GAG CAA GGT GTT TTT CCA ATC CAT TCT CCA ACT GCT TCC TTG	Ned (yellow)	Bishop <i>et al.</i> (1994)

Table 2.
Locus wise number of alleles, allele size, allele frequency, heterozygosity, Polymorphism Information Content (PIC) and Chi-square values of microsatellite loci

Locus name	Number of alleles	Allele size (bp)	Allele frequency	Heterozygosity		PIC	Chi-square value
				Observed	Expected		
TGLA227	5	77	0.065	0.739	0.612	0.565	10.062
		79	0.565				
		81	0.217				
		83	0.130				
		89	0.022				
BM2113	7	129	0.043	0.739	0.718	0.677	11.324
		131	0.413				
		137	0.087				
		139	0.022				
		141	0.065				
		143	0.304				
		145	0.065				
TGLA53	7	159	0.545	0.304	0.630	0.586	91.480**
		161	0.045				
		163	0.023				
		164	0.068				
		167	0.250				
		177	0.023				
		179	0.045				
		210	0.348				
ETH10	4	210	0.348	0.739	0.688	0.624	4.296
		212	0.348				
		214	0.261				
		220	0.043				
SPS115	5	245	0.196	0.783	0.762	0.726	12.287
		247	0.326				
		249	0.065				
		253	0.065				
		255	0.283				
TGLA126	7	118	0.152	1.00	0.802	0.776	34.964
		120	0.087				
		122	0.239				
		124	0.304				
		126	0.043				
		128	0.087				
		130	0.087				
		137	0.283				
TGLA122	9	137	0.283	0.826	0.829	0.809	53.268
		143	0.043				
		145	0.065				
		147	0.043				
		149	0.217				
		152	0.065				
		153	0.065				
		157	0.065				
		161	0.152				
		197	0.065				

Table 2.
Locus wise number of alleles, allele size, allele frequency, heterozygosity, Polymorphism Information Content (PIC) and Chi-square values of microsatellite loci

Locus name	Number of alleles	Allele size (bp)	Allele frequency	Heterozygosity		PIC	Chi-square value
				Observed	Expected		
INRA23	6	197	0.196	0.609	0.577	0.536	6.758
		199	0.022				
		203	0.043				
		209	0.109				
		211	0.022				
		215	0.609				
ETH3	5	101	0.065	0.957	0.719	0.679	15.738
		111	0.152				
		113	0.435				
		115	0.217				
		117	0.130				
ETH225	4	146	0.043	0.304	0.334	0.308	28.052
		148	0.130				
		154	0.022				
		158	0.804				
BM1824	6	179	0.174	0.522	0.652	0.619	18.704
		181	0.109				
		183	0.543				
		189	0.043				
		195	0.065				
		197	0.065				
Overall				0.684	0.666	0.628	

**Significant at $P < 0.01$.

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The results of chi-square test indicated that there was no significant deviation of observed genotype frequencies from the expected genotype frequencies at all the loci, except at TGLA53 locus. This revealed that the population was under Hardy-Weinberg equilibrium. The significant deviation of genotype frequencies expressed by the TGLA53 locus could be caused by a point mutation as indicated by the variation in its allele size. MacHugh *et al.* (1994) also reported significant deviation of observed frequencies from the expected ones in 3 out of 12 loci studied in European cattle breeds.

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Fig.1

Genescan gel image showing polymorphisms of microsatellite markers of Punganur Cattle

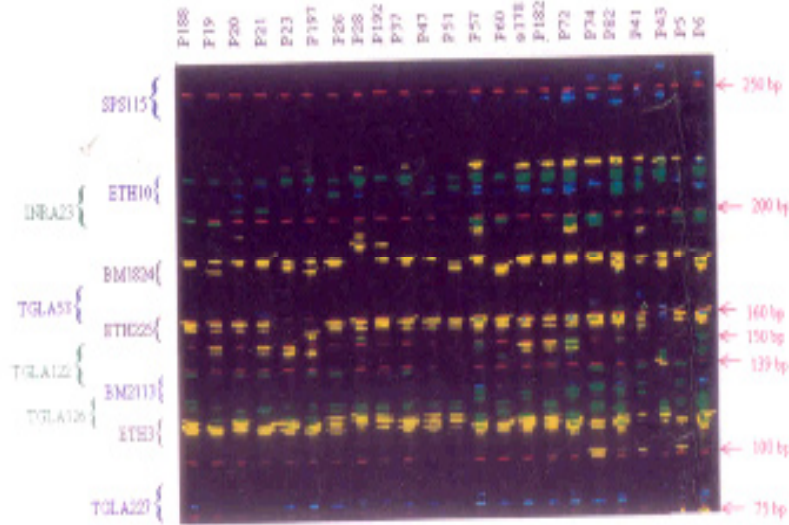


Fig. 1 Genescan gel image showing polymorphisms of microsatellite markers of Punganur cattle

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