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Microsatellite analysis of Kangayam cattle (*Bos indicus*) of Tamilnadu

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Abstract: Assessment of genetic variability in Kangayam breed of cattle in Tamilnadu, South India was carried out using 25 bovine microsatellite markers. The mean number of alleles was 4.04 ± 0.09 with a range of 2 to 6 and the allele size ranged from 94 to 300 bp. The frequency distribution of alleles in the breed was from 0.0104 to 0.9167. The estimated heterozygosity was 0.6183 ± 0.01 and the PIC was 0.5628 ± 0.03. The overall mean within-population inbreeding estimate (F_{IS}) value (-0.084) suggested excess of heterozygotes in the population. In addition, higher PIC value indicated the scope for maintaining variation in the population and strategies to take meaningful conservation.

Keywords: Cattle, Kangayam, Microsatellites, PIC

Introduction

The Kangayam breed of cattle of Tamilnadu is best known for its superior draught qualities, adaptation to poor nutrition and longevity (Kandasamy, 2001). Bullocks are primarily used for transport of agricultural produce, besides being used for various agricultural operations. As per the estimate of 1996, the size of Kangayam population in the breeding tract was 0.479 million. However, replacement of Kangayam cattle in few areas of the breeding tract with exotic crosses is evident. Though the population size is more, the future of Kangayam breed is secured only if meaningful conservation strategies are followed to ensure genetic variability. The variability at DNA level would provide valuable information on genetic structure of the breed. The genetic variability in different zebu cattle breeds of India like Red Kandhari and Deoni (Sodhi *et al*., 2005), Hallikar (Naveen Kumar *et al*., 2006) and Umblachery (Karthickeyan *et al*., 2007) had already been elucidated. Therefore, the present study was undertaken using the microsatellites, which are powerful genetic markers for biodiversity evaluation, to characterize the Kangayam breed.

Materials and methods

Kangayam breed

The Kangayam cattle is distributed in Erode, Dindigul, Karur, Coimbatore, Salem and Namakkal districts of Tamilnadu state in South India. The adult animals are medium in size with grey-colored body. The bulls have grey body color with dark grey to black markings on the head, neck, hump, shoulders and quarters. Horns are longer, curving outwards and backwards, then inwards and almost complete a circle or ellipse at the point where they approach the tips. A pair of bullocks has the capacity to haul a total load of 3787 ± 51.4 kg of sugarcane load over a distance of 10 to 20 km without taking rest (Kandasamy, 2001).

The blood samples (48 numbers) collected at random from these animals in several areas of the main breeding tract were subjected to microsatellite analysis during 2005-06. Genomic DNA was isolated using a routine high salt method (Miller *et al*., 1988) and the quantity and quality of the DNA were analyzed by spectrophotometric measurements.

PCR amplification and microsatellite analysis

A total of 25 microsatellite markers (Table 1) were utilized as per the suggestions of FAO in the Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans for global management of cattle genetic resources using reference microsatellites (FAO, 2004). These markers were amplified in the target DNA samples using thermal cycler (MJ Peltier). PCR reaction mixture (20μl) containing 50-100ng of template DNA; 1.5mM $MgCl_2$; 5 picomoles each of forward and reverse primers; 0.75units of Taq DNA polymerase (Invitrogen, USA) and 100mM dNTPs was prepared. Amplification was carried out with initial denaturation at 94°C for 5 minutes; followed by 30 cycles of denaturation (94°C for 45 seconds), annealing (51°C to 58°C for 45 seconds for various primers) and extension (72°C for 45 seconds).

The PCR products were electrophoresed in 6 % denaturing polyacrylamide gel at a voltage of 1200 to 1400 for a period of 2 to 3 hours, depending upon the size of PCR products. Single-stranded 10 bp DNA ladder (Invitrogen, USA) was loaded (0.25μg) in one of the wells as a molecular weight marker. The genotyping was done after subjecting the gel to silver-staining procedure (Cominicini *et al*., 1995).

Sizing of fragments was done using Diversity Database (BioRad) software and by manual verification. Allele frequencies were estimated by direct counting. The observed heterozygosity was calculated as the actual percentage of heterozygotes occurring in the sample population. The effective number of alleles, 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 allele number, size and frequency, polymorphism information content and expected heterozygosity for different microsatellite loci are presented in Table 1.

Allele size and frequency

The number of alleles was ranging from 2 to 6 with a mean of 4.04 ± 0.09 per microsatellite locus. A total number of 101 alleles were observed in these polymorphic loci in the breed. The lowest number of two alleles was observed in the locus INRA063 and highest value of 6 alleles in locus CSRM060. In contrast, the mean effective number of alleles was estimated to be 2.90 ± 0.08, ranging from 1.19 (ETH152) to 4.56 (ILSTS054). The number of alleles observed in the

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present study is in accordance with Umblachery cattle, the other draught breed of cattle in Tamilnadu (karthickeyan *et al*., 2007). Further, the allele numbers are more or less similar to other draught breeds of South India such as Amritmahal (2 to 8; Prabhu, 2004), Hallikar (3 to 9; Naveen Kumar *et al*., 2006) and Krishna Valley (Karthickeyan *et al*., 2006). However, the mean number of alleles noticed in Kangayam is lesser than the number reported in Sahiwal (5.2) and Deoni (5.9) breeds of cattle found in Northern parts of India (Mukesh *et al*., 2004).

Table 1 Allele frequency, polymorphism information content (PIC) and heterozygosity of microsatellite loci in Kangayam breed of cattle

Sl. No.	Locus	n_a	n_e	Range		PIC	He	F_{is}
				Allele size (bp)	Allele frequency			
1.	*ILSTS005*	4	2.99	182 - 194	0.1562 - 0.4167	0.6014	0.6615	-0.2842
2.	ILSTS006	4	3.04	286 - 300	0.0208 - 0.3750	0.6022	0.6710	0.0996
3.	ILSTS011	4	2.89	254 - 274	0.0104 - 0.3333*	0.5824	0.6539	-0.3701
4.	ILSTS030	3	2.03	152 - 156	0.0435 - 0.6087	0.4151	0.5066	-0.2015
5.	ILSTS033	5	3.63	138 - 158	0.0244 - 0.3659	0.6756	0.7246	-0.2791
6.	ILSTS034	5	3.84	156 - 166	0.0319 - 0.5311**	0.6947	0.7397	0.0508
7.	ILSTS054	5	4.56	132 - 148	0.1250 - 0.2625	0.7453	0.7809	0.0396
8.	INRA005	4	3.28	134 - 150	0.0833 - 0.3750**	0.6374	0.6951	0.3406
9.	INRA032	4	3.37	166 - 188	0.0889 - 0.3778	0.6474	0.7032	0.0204
10.	INRA035	4	3.31	102 - 120	0.0761 - 0.3152	0.6404	0.6980	0.0968
11.	INRA063	2	1.44	180 - 186	0.1875 - 0.8125	0.2583	0.3047	0.1795
12.	ETH003	4	3.55	102 - 116	0.1829 - 0.4024**	0.6700	0.7183	0.3549
13.	ETH010	4	2.92	210 - 220	0.0729 - 0.4792**	0.6011	0.6582	-0.3294
14.	ETH152	3	1.19	194 - 204	0.0417 - 0.9167	0.1505	0.1562	-0.0667
15.	ETH225	5	3.14	138 - 160	0.0233 - 0.4186**	0.6231	0.6814	-0.3992
16.	HEL001	4	1.75	100 - 110	0.0349 - 0.7326*	0.3904	0.4283	0.2399
17.	HEL005	3	2.46	150 - 158	0.1744 - 0.5465**	0.5236	0.5930	0.4510
18.	HEL009	5	2.90	148 - 164	0.0217 - 0.4783**	0.5975	0.6552	-0.4266
19.	BM1818	3	2.94	262 - 278	0.2841 - 0.3977*	0.5860	0.6599	-0.3088
20.	BM2113	4	2.47	136 - 148	0.0543 - 0.5652	0.5383	0.5952	-0.1322
21.	MM8	3	2.70	138 - 146	0.2262 - 0.4881**	0.5573	0.6290	-0.5899
22.	HAUT024	5	4.05	124 - 136	0.0114 - 0.2614**	0.7080	0.7531	-0.0864
23.	HAUT027	3	2.27	156 - 168	0.1596 - 0.5957	0.4962	0.5598	-0.1403
24.	CSRM060	6	3.80	94 - 114	0.0435 - 0.4348**	0.7045	0.7365	-0.1806
25.	CSSM066	5	1.96	180 - 186	0.0111 - 0.6556	0.4222	0.4909	-0.1771
Overall mean / Range		4.04 ± 0.09	2.90 ± 0.08	94 - 300	0.0104 - 0.9167	0.5628 ± 0.03	0.6183 ± 0.01	-0.0840 ± 0.05

n_a = Observed number of alleles; n_e = Effective number of alleles; H_e = Expected Heterozygosity; * $p < 0.05$; ** $p < 0.01$

The size of microsatellite alleles in Kangayam cattle ranged from 94 (CSRM060) to 300 (ILSTS006) bp. The allele sizes observed are in accordance with the sizes of some highly informative markers (ILSTS005, ILSTS006, ILSTS011 and ILSTS033) described for *Bos taurus* cattle (Kemp *et al*., 1995). These microsatellite alleles were present in the population of Kangayam breed at a minimum frequency of 0.0104 (264 bp allele in ILSTS011) to a maximum of 0.9167 (194 bp allele in ETH152 locus). The allele with 194 bp in the ETH152 locus was found to be the predominant allele in the population.

Hardy-Weinberg equilibrium

Out of 25 loci screened, Kangayam population was in equilibrium proportion in 12 microsatellite loci as revealed from the Chi-square (χ^2) test for Hardy-Weinberg equilibrium. The disequilibrium exhibited in 13 loci revealed that there might be unobserved null alleles (those which could not be amplified) which have not been, hitherto, identified by other means. The deviation from equilibrium would have resulted from the use of frozen semen (from bulls kept at other farm) in the native breeding tract, as envisaged by Selvi *et al*. (2004) in Mafriwal cattle of Malaysia, besides sampling from a range of distinct locations within same broad geographical area (breeding tract) (Dorji *et al*., 2003).

Polymorphism information content

The statistical assessment of informativeness of a marker, referred to as polymorphism information content (PIC), ranged from 0.1505 (ETH152) to 0.7453 (ILSTS054) with a mean PIC of 0.5628 ± 0.03. This value is in close agreement with the range of PIC between 0.11 and 0.77 in Umblachery as reported by Karthickeyan *et al.* (2007) for the same set of markers. In another study, similar PIC values of 0.15 to 0.79 in Ongole and 0.13 to 0.80 in Deoni cattle were reported by Metta *et al*. (2004) who used 10 different sets of microsatellite markers. More or less similar ranges of PIC were observed in Hallikar (0.2322 to 0.8654; Naveen Kumar *et al*., 2006) and Krishna Valley (0.2583 to 0.7975; Karthickeyan *et al*., 2006) breeds of cattle in south India. In general, the population has got high polymorphism information content of 56 per cent which indicates that these markers are highly informative for characterization of Kangayam cattle. The number of alleles and their relative frequencies in the respective locus reflect the informativeness of the markers. The higher number of

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alleles found in CSRM060, ILSTS033, ILSTS034, ILSTS054, ETH003, HEL009 and HAUT024 loci have led to high polymorphic information content and the lower number of alleles in ETH152 and INRA063 with respective higher frequencies of 194 bp and 186 bp alleles resulting in lower PIC values. The mean PIC value further reflected the heterogeneity in the Kangayam population as they have wider geographical distribution.

Heterozygosity

The overall mean observed and expected heterozygosities were 0.6742 ± 0.02 and 0.6183 ± 0.01 and ranged from 0.1667 (ETH152) to 1.0000 (MM8) and 0.1562 (ETH152) to 0.7809 (ILSTS054), respectively. Though few loci exhibited lower heterozygosity values, majority of them had relatively higher expected heterozygosity, reflecting the existence of within breed genetic diversity. The mean expected heterozygosity value is comparable to 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 Krishna Valley (Karthickeyan *et al*., 2006), the other Indian cattle breeds. Whereas low average heterozygosity (0.46 ± 0.1) was observed in Ongole cattle (Metta *et al*., 2004). The high heterozygosity values observed indicates more number of polymorphic loci in the breed. Further, there exists gene flow among the herds of Kangayam within the wide breeding tract. This implies higher genetic diversity within the breed that can be still exploited in the population of Kangayam cattle.

Within-breed diversity

The inbreeding estimates were calculated using the F_{IS} values (Wright's Fixation Index) which revealed that the Kangayam breed is having a wide genetic variability and is outbred in nature as indicated by negative F_{IS} values in most of the loci (60 per cent; 15 loci). Some of the loci exhibited positive values which ranged from 0.02 to 0.45. However, the overall mean F_{IS} value observed across all the loci in the present study was -0.0840 indicating an excess of heterozygotes in the population as found in Umblachery (-0.0487) cattle (Karthickeyan *et al.,* 2007). On the contrary, Metta (2004) reported high F_{IS} value (0.36) which resulted from small sample size (n=17) in Ongole breed of cattle.

Conclusions

The study revealed large number of polymorphic microsatellite loci, high within breed genetic diversity besides the valuable information about the genetic structure of the Kangayam breed. It also opens up the scope for exploiting the genetic variability in the population for bovine conservation. Detailed studies on comparative analysis with other Indian draught and dual purpose cattle breeds will not only determine the genetic distance; but will also be useful to establish evolutionary relationship of this breed with other zebu cattle of India.

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