

Genetic variability and bottleneck analyses of Kanniadu goat breed based on microsatellite markers

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ABSTRACT

Genetic variation at 25 microsatellite loci was examined in Kanniadu goats of Tamil Nadu. The Kanniadu goat is a prolific meat breed thriving well in the tropical draught conditions. The observed number of alleles ranged from 5 (RM4) to 13 (RM088, OarE129) with an average value of 8.64 ± 0.48 . The effective number of alleles ranged from 1.45 (ILSTS34) to 7.89 (ILSTS033 and OMHC1) with the overall mean value of 4.22 ± 0.34 . The average observed and expected heterozygosity values were 0.53 ± 0.03 and 0.73 ± 0.02 , respectively. The polymorphic information content value ranged from 0.30 to 0.86. These high values of PIC indicated higher polymorphism in the breed. Within population inbreeding estimate ($F_{is} = 0.25$) showed moderate level of inbreeding, which warranted adoption of appropriate breeding strategies under field conditions. The high level of genetic variability, however, suggested the scope for further genetic improvement of Kanniadu goats.

Key words: Diversity, Goat, Kanniadu, Loci, Microsatellite markers

Kanniadu is a native breed of Tamil Nadu (mainly Ramanathapuram and Trinnelveli districts). The Kanniadu goat is a prolific meat breed thriving well in the tropical drought conditions. They are medium size animals, predominantly black with white or brown stripes on either



Fig. 1. A Kanniadu doe in the pasture.



Fig. 2. A Kanniadu sire in the pasture.

side of the face and white or brown under belly and inner sides of legs (Figs 1, 2). Ears are long and pendulous. Males have horns and udder is small. The special features of Kanniadu goats are early sexual maturity, high prolificacy (67% of multiple births) and low adult mortality. The genetic variability in these goats has not been studied and therefore, the present investigation was planned to study the genetic variation in this breed so as the corrective measures may be undertaken, if required.

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MATERIALS AND METHODS

Molecular techniques

Genomic DNA was isolated from blood samples of 48 unrelated animals of the breed as per Sambrook *et al.* (1989). A battery of 25 microsatellite markers as used by Dixit *et al.* (2008) was selected to generate allelic data.

Statistical analysis

For 25 microsatellites loci analyzed, observed and expected heterozygosity estimates, observed and effective numbers of alleles were calculated by using POPGENE software (Yeh *et al.* 1999). F-statistics were determined after Weir and Cockerham (1984) as used in F-Stat software (Gaudet 1995).

Finally the bottleneck hypothesis was investigated using BOTTLENECK 1.2.01 (Cornuet and Luikart 1996). The BOTTLENECK tests for the departure from mutation drift equilibrium based on heterozygosity (not heterozygote), excess or deficiency. This does not require information on historical population sizes or level of genetic variations. It requires only measurement of allele's frequency from 5–20 polymorphic loci in a sample of approximately 20–30 individuals. The bottleneck compares heterozygosity

expected (H_E) at Hardy-Weinberg equilibrium to the heterozygosity expected (H_{eq}) at mutation drift equilibrium in same sample, that has the same size and the same number of alleles. All the 3 models of mutation were used to calculate H_{eq} : the strict one step-wise mutation (SSM) model, the infinite allele (IAM) model and 2-phase (TPM) model.

RESULTS AND DISCUSSION

The various measures of genetic variation in Kanniadu breed of goat are presented in Tables 1, 2. A total of 216 alleles were observed for 25 loci. The observed number of alleles ranged from 5 (RM4) to 13 (RM088, OarE129) with an average value of 8.64 ± 0.48 . The effective number of alleles ranged from 1.45 (ILSTS34) to 7.89 (ILSTS033 and OMHC1) with the overall mean value of 4.22 ± 0.34 . All the loci had lower values for effective number of alleles in comparison to observed number of alleles. The allele size ranged from 100bp (ILSTS082) to 208bp (OMHC1). These values are higher than those obtained by Ganai and Yadav (2001) for Sirohi, Jamunapari and Barbari. These values are also higher than the values obtained from the study of Sadeghi *et al.* (2009). Our values are comparable with the values obtained for Swiss goat breeds (Saitbekova *et al.* 1999). The

Table 1. Measures of genetic variation in Kanniadu breed of goat heterozygosity

Locus	Sample size (diploid)	Observed number of alleles	Effective number of alleles	Observed	Expected	Nei's
ILST008	88	10	2.48	0.50	0.60	0.59
ILSTS059	96	9	3.27	0.52	0.70	0.69
ETH225	90	7	2.67	0.15	0.63	0.62
ILST044	92	12	5.60	0.69	0.83	0.82
ILSTS002	90	8	5.99	0.51	0.84	0.83
OarFCB304	26	10	5.36	0.46	0.84	0.81
OarFCB48	22	7	3.72	0.18	0.76	0.73
OarHH64	52	7	5.24	0.65	0.82	0.80
OarJMP29	82	9	3.44	0.26	0.71	0.71
ILSTS005	88	6	2.71	0.50	0.63	0.63
ILSTS019	96	7	3.94	0.72	0.75	0.74
OMHC1	30	12	7.89	0.40	0.90	0.87
ILSTS087	84	7	4.69	0.57	0.79	0.78
ILSTS30	96	7	4.40	0.72	0.78	0.77
ILSTS34	96	6	1.45	0.35	0.31	0.31
ILSTS033	30	11	7.89	0.26	0.90	0.87
ILSTS049	96	8	2.82	0.47	0.65	0.64
ILSTS065	94	7	4.28	0.61	0.77	0.76
ILSTS058	96	6	2.68	0.95	0.63	0.62
ILSTS029	96	10	2.70	0.66	0.63	0.62
RM088	86	13	5.16	0.67	0.81	0.80
ILSTS022	90	7	2.81	0.53	0.65	0.64
OarE129	92	13	6.85	0.78	0.86	0.85
ILSTS082	82	12	5.14	0.58	0.81	0.80
RM4	78	5	2.26	0.46	0.56	0.55
Mean	79	8.64	4.22	0.53	0.73	0.71
S.E.		0.48	0.34	0.03	0.02	0.02

Table 2. F-stat. analysis for microsatellite loci in Kanniadu goats

Locus	Shannon Information index	PIC	Gene diversity	Allelic richness	F _{is}	P-value
ILST008	1.41	0.57	0.60	3.01	0.17	0.02
ILSTS059	1.59	0.67	0.70	3.39	0.26	0.00
ETH225	1.24	0.56	0.63	2.79	0.75	0.00
ILST044	2.01	0.80	0.83	4.11	0.16	0.01
ILSTS002	1.88	0.81	0.84	4.10	0.39	0.00
OarFCB304	1.96	0.79	0.86	4.28	0.46	0.00
OarFCB48	1.60	0.70	0.79	3.72	0.77	0.00
OarHH64	1.77	0.78	0.82	3.97	0.21	0.02
OarJMP29	1.55	0.66	0.72	3.30	0.63	0.00
ILSTS005	1.23	0.58	0.64	2.90	0.21	0.01
ILSTS019	1.50	0.70	0.75	3.39	0.03	0.38
OMHC1	2.24	0.86	0.92	4.74	0.56	0.00
ILSTS087	1.68	0.75	0.79	3.75	0.28	0.00
ILSTS30	1.60	0.73	0.78	3.61	0.06	0.22
ILSTS34	0.71	0.30*	0.31	1.96	-0.11*	1.0
ILSTS033	2.21	0.86	0.92	4.74	0.71	0.00
ILSTS049	1.30	0.58	0.65	2.90	0.26	0.00
ILSTS065	1.65	0.73	0.77	3.63	0.20	0.00
ILSTS058	1.13	0.55	0.63	2.68	-0.51*	1.00
ILSTS029	1.47	0.60	0.63	3.14	-0.04*	0.80
RM048	1.95	0.78	0.81	3.97	0.17	0.01
ILSTS022	1.26	0.58	0.65	2.86	0.18	0.04
OARE129	2.20	0.83	0.86	4.39	0.09	0.07
ILSTS082	1.92	0.78	0.81	3.97	0.28	0.00
RM4	1.08	0.51	0.56	2.62	0.18	0.06
mean	1.60	0.68	0.73	3.51	0.25	

* F_{is}, significant heterozygosity

high allelic diversity observed is probably due to no selection pressure and choice of samples based on random sampling.

The polymorphic information index (PIC) values ranged from 0.30 (ILSTS34) to 0.86 (OMHC 1, ILSTS 033) (Table 2). Except locus ILSTS34, all the loci had PIC values were greater than 0.50. These high values of PIC indicated higher polymorphism and greater heterogeneity in the breed. PIC estimated in the present study are comparable with those values obtained in Chinese goat breeds, which ranged from 0.746 to 0.800 (Yang *et al.* 1999) using ovine microsatellite markers and with Tali goats having average PIC value 0.704 (Sadeghi *et al.* 2009). In contrast, lower PIC values were obtained for Korean (0.350), Chinese (0.620) and Saanen (0.570) goats (Kim *et al.* 2002). Based on the PIC values, the microsatellite markers can be well utilized for molecular characterization of goat breeds.

The heterozygosity measurement (Table 1) clearly depicted the level of variability in the breed. The values of observed heterozygosity ranged from 0.15 (ETH225) to 0.95 (ILSTS058) with overall mean of 0.53±0.03. The expected heterozygosity varied from 0.31 (ILSTS34) to 0.89 (OMHC1 and ILSTS033) with an average value of 0.73±0.02. All the loci had greater expected heterozygosity than observed heterozygosity except 3 loci (ILSTS34, ILSTS058 and

ILSTS029). This indicated that 88% loci showed positive deviation from Hardy-Weinberg equilibrium (HWE). The higher values of expected heterozygosity were similar with the values of Chinese goat breeds (0.732) reported by Yang *et al.* (1999) and Li *et al.* (2008).

The Shannon Information index (Table 2) showed that all the loci were highly informative indicating the high polymorphism across the loci and index value ranged from 0.71 (ILSTS34) to 2.24 (OMHC1) with the average value of 1.60±0.38. The gene diversity analysis across all the studied loci varied from 0.31 (ILSTS34) to 0.92 (OMHC1, ILSTS033) with the average value of 0.73, which is comparable with Tali breed of goat (Sadeghi *et al.* 2009). The high variability was also reported for Mehsana, Marwari and Kutchi breeds of goat (Kumar *et al.* 2005, Aggarwal *et al.* 2007, Dixit *et al.* 2008). The allelic richness ranged from 1.96 (ILSTS34) to 4.74 (OMHC1, ILSTS033).

F-Stat analysis (Table 2) showed that 22 out of 25 loci had positive F_{is} values, which indicated significant heterozygote deficiency at these loci. However, one locus (ILSTS058) out of 3 loci, which had negative values for F_{is} indicated significant heterozygote excess. The deviation from HWE at these loci might be due to many causes such as existence of “null” alleles, high mutation rate and size

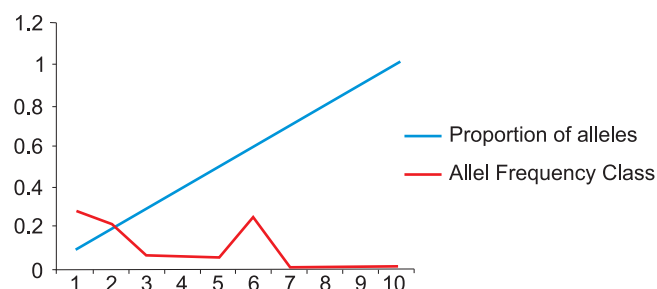


Fig. 3. Allele frequency distribution for all polymorphic studied microsatellite loci in Kanniadu breed of goat.

homoplasmy of microsatellite loci, besides the small size of studied population. Significant heterozygote deficiencies have also been reported in some studies on Indian goats (Kumar *et al.* 2005, Aggarwal *et al.* 2007, Verma *et al.* 2007, Dixit *et al.* 2008). The fairly high value of F_{is} (0.15) indicated that some of the loci in this breed were homozygous presumably resulting from the mating between relatives and consequent genetic drift.

The sign, standardized differences and Wilcoxon tests detected significant departure from mutation-drift-equilibrium in the population under all the mutation models except single step mutation (SMM) model (data not shown). The sign test detected significant heterozygosity excess at 21 and 20 loci under indefinite allele (IAM) and two phase (TPM) mutation models ($P < 0.001$), respectively but nonsignificant (17 loci with heterozygosity excess $P: 0.16$) under SMM. The standardized differences test also revealed significant departure from mutation-drift-equilibrium. The deviation was positive under IAM ($T_2 = 4.575$ $P < 0.001$), TPM ($T_2 = 2.954$ $P < 0.001$), and SMM ($T_2: 0.998$ $P = 0.159$). Microsatellites may tend to evolve under a model more similar to the SMM than IAM (Shriver *et al.* 1993, Valdes *et al.* 1993). Therefore, SMM should be used when analyzing microsatellite data to test for recent bottlenecks. All the tests under SMM revealed that there was mutation-drift equilibrium in the population. It may be concluded that the population has not undergone any recent genetic bottleneck. Furthermore, an analysis of allele frequency distribution also supported the mutation-drift equilibrium in Kanniadu goats (Fig. 3), as there was almost double L shaped curve which might have resulted due to shifting of the mode but not the mean.

In conclusion, study showed that all the microsatellite markers used were highly polymorphic and informative for molecular characterization of Kanniadu. There was substantial genetic variability across studied loci in Kanniadu goats and the population was not in Hardy Weinberg equilibrium but in mutation drift equilibrium. Therefore, appropriate breeding strategies should be designed under field conditions for its conservation and improvement.

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