Indian Journal of Animal Sciences **84** (9): 1023–1026, September 2014/Short communication

Microsatellite based genetic characterization of Kanni Adu, Kodi Adu and Salem Black goats of Tamil Nadu

M JEYAKUMAR[1], A K THIRUVENKADAN[2], R SARAVANAN[3], P KATHIRAVAN[4], S PANNEERSELVAM[5] and S MALMARUGAN[6]

Veterinary College and Research Institute, Namakkal, Tamil Nadu 637002 India

Received: 29 December 2013; Accepted: 9 May 2014

Key words: Goat breeds, Genetic characterization, Microsatellite loci

Goat is a multi-functional animal and plays a significant role in the economy and nutrition of landless, small and marginal farmers in India. India has 23 recognized goat breeds adapted to different agro-climatic conditions. These different goat breeds represent important genetic resources because of their special economic and ecological characteristics. Tamil Nadu is situated in southern part of India and bestowed with Kanni Adu, Kodi Adu and Salem Black goat breeds (Acharya 1982, Thiruvenkadan *et al*. 2000, Thiruvenkadan and Karunanithi 2006, Thiruvenkadan 2012). These breeds are well adapted to their environment and are important economic resource. Genetic variation within and between breeds is warranted to differentiate them on a genetic basis. The resulting genetic information may assist in choosing the best conservation and improvement options for these genetic resources. Advances in biotechnology offer possibilities of improving, utilizing and conserving present domestic animal diversity. Molecular markers have been shown to be an efficient tool in quantification of genetic diversity of various populations. Microsatellite markers are best suited to characterize the genetic variability within and between populations because of their high variability, distribution throughout the genome, co-dominant inheritance and neutrality to selection (Boyce *et al*. 1996). For goats, microsatellites were used for parentage testing, estimation of genetic diversity, differentiation, and relationship between and within populations (Luikart *et al*. 1999, Saitbekova *et al*. 1999, Barker *et al*. 2001, Ganai and Yadav 2001, Dixit *et al*. 2009, Serrano *et al*. 2009). An assessment of genetic variability in domestic goats is a first step towards conservation of genetic resources for maintaining breeding options. The goat breeds of Tamil Nadu are mainly defined by their geographical position, morphological characteristics and production performance (Thiruvenkadan *et al*. 2000, Thiruvenkadan and Karunanithi 2006, and Thiruvenkadan 2012) however, no systematic study has been made on genetic characterization using microsatellite markers. Hence, an analysis of genetic structure and relationships of 3 goat populations of Southern India based on microsatellite loci is discussed here.

Blood samples were collected from unrelated healthy animals of Kodi Adu (n=50); Kanni Adu (50) and Salem Black (50) goats at random from their respective home tract. The herdsmen were interviewed in detail to minimize the sampling of closely related animals. Blood samples were collected in vacutainers containing EDTA as anticoagulant, and DNA was extracted from blood samples using a modified high salt method (Miller *et al.* 1988). A set of 25 goat-specific heterologous microsatellite markers (OARFCB20, SRCRSP5, ILSTS029, OARJMP29, CSRD247, MAF70, ILSTS087, MCM527, OARAE129, ETH225, OARFCB304, ILSTS005, MAF65, ILST008, ETH10, BM1329, SRCRSP23, SPS113, MAF209, BM6444, OARFCB48, ILSTS011, SRCRSP8, SRCRSP9 and ILSTS033) earlier identified for evaluating goat biodiversity (Barker *et al*. 2001, Dixit *et al*. 2010) were utilized to estimate various genetic diversity parameters. Only forward primers of each pair were labelled with 1 of the 2 fluorophore i.e. 6-carboxyflouroscien (FAM) dye phosphoramidites and hexachloro-6-carboxy flouroscient (HEX) dye phosphoramidites. Polymerase chain reaction (PCR) amplification of microsatellite loci was carried out in a 15 µl reaction volume containing 1.5 mm $MgCl_2$, 200 mm dNTPs, 50 ng of forward/reverse primer, approximately 100 ng of genomic DNA and 0.5 U of Taq DNA polymerase. PCR was carried out in a thermal cycler using cycling conditions as: 5 min at 94°C, followed by 30 cycles of 45 sec at 94°C, 35 sec at annealing temperature (depending upon locus), 35 sec at 72°C and final extension at 72°C for 10 min. The genotyping of microsatellite markers was made

Present address: [1]Veterinary Assistant Surgeon (drjaikumar007@gmail.com), Department of Animal Huasbandry, [2]Professor and Head (drthirusiva@gmail.com), Department of Animal Husbandry Statitics and Computer Applications, [3]Assistant Professor (mrsagb@gmail.com), [5]Professor and Head (panneer57@rediffmail.com), Department of Animal Genetics and Breeding. [4]Livestock Geneticist/Breeder (kathirvet@yahoo.co.in), Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria. [6]Associate Professor (micromals@rediffmail.com), Department of Veterinary Microbiology,Veterinary College and Research Institute, Tirunelveli.

Table 1. Diversity estimates at each microsatellite locus in goat breeds of Tamil Nadu

Locus	Allele size range	Number of observed alleles	Heterozygosity			F_{IS}	PIC
			Observed	Expected	Nei		
Kodi Adu	95 to 254	11.52±0.95	0.660±0.045	0.846±0.018	0.843±0.018	0.229±0.046	0.817±0.023
Kanni Adu	97 to 254	11.24±0.87	0.677±0.041	0.857±0.016	0.853±0.016	0.215±0.040	0.829±0.020
Salem Black	95 to 254	10.14±0.83	0.631±0.041	0.820±0.024	0.816±0.024	0.233±0.044	0.786±0.023

by capillary electrophoresis using automatic genetic analyzer. All the 25 microsatellite loci amplified successfully in 3 goat breeds. Test for linkage disequilibrium between different combinations of loci under study showed significant deviations in four loci alone and hence they were removed and the remaining 21 loci were retained for analysis. The data were captured using ABI 3130 Data collection Software Version 4.0. Analysis of molecular variance (AMOVA), F_{st}, pair-wise difference and the exact tests for deviation from Hardy–Weinberg equilibrium were computed using Arlequin version 3.1 (Excoffier *et al*. 2005). POPGENE (Yeh *et al*. 1999) was used to calculate allele frequencies, observed number of alleles (No), effective number of alleles (Ne), observed heterozygosity (Ho) and expected heterozygosity (He). Polymorphism information content was estimated using GenAlEx program (Peakall and Smouse 2006). As admixture in the breeding tract of goats is very common and some populations are known to admixtures, authors used a multivariate procedure viz. principal component analysis (PCA) to represent population relationships. The PCA is done using the allelic frequencies as variables using R software with the ade4 package (Ihaka *et al*. 1996, and Chessel *et al*. 2004).

The microsatellite markers used in this study were polymorphic in all the 3 breeds. Kodi Adu and Kanni Adu had the highest mean number of alleles of 11.52 and 11.24 respectively. The mean observed number of alleles noticed in the present study is comparable with the values reported for Kutchi, Sirohi and Mehsana goat breed of India (Dixit *et al*. 2009). The overall mean polymorphism information content (PIC) values observed in Kodi Adu, Kanni Adu and Salem Black goats were 0.817±0.023, 0.829±0.020 and 0.786±0.023 respectively. The PIC values observed in 3 breeds are comparable with those obtained in other Indian breeds (Ramamoorthi *et al*. 2009, Dixit *et al*. 2010, Vijh *et al*. 2010). In all the 3 goat populations, the PIC values were above 0.5 at all the studied loci indicating their high information content and goodness for genetic diversity study (Botstein *et al*. 1980). A more appropriate measure of genetic variation within a population is gene diversity i.e. average expected heterozygosity. This study revealed that the genetic variability within breeds is relatively high as evidenced by the high mean expected heterozygosity of 0.846, 0.857 and 0.820 for Kodi Adu, Kanni Adu and Salem Black goats. Our diversity estimates are within the range found in Kutchi, Mehsana and Sirohi goats of India (Dixit *et al*. 2009) however, lower than the present values were also reported for the Barbari, Jamunapari, Black Bengal,

Table 2. F-statistics estimates at each microsatellite locus across three goat breeds of Tamil Nadu

Locus	F_{IT}	F_{IS}	F_{ST}	P-values
BM1329	0.123	0.112	0.012	0.011
BM6444	0.113	0.078	0.038	0.000
CSRD247	0.452	0.421	0.054	0.000
ETH10	0.343	0.321	0.033	0.004
ETH225	0.174	0.159	0.017	0.005
ILSTS005	0.234	0.222	0.016	0.016
ILSTS008	0.409	0.389	0.032	0.015
ILSTS029	0.222	0.195	0.033	0.001
ILSTS033	0.428	0.375	0.085	0.000
ILSTS087	0.116	0.085	0.034	0.000
MAF209	0.575	0.533	0.088	0.000
MAF70	0.084	0.069	0.016	0.001
MCM527	0.555	0.520	0.072	0.000
OARAE129	0.249	0.228	0.027	0.000
OARFCB20	0.279	0.258	0.028	0.000
OARFCB304	0.212	0.199	0.016	0.001
OARFCB48	0.189	0.192	–0.004	0.755
SPS113	0.122	0.114	0.008	0.039
SRCRSP5	0.200	0.187	0.016	0.002
SRCRSP8	0.106	0.103	0.003	0.159
SRCRSP9	0.085	0.068	0.019	0.001
Overall	0.245±0.033	0.222±0.031	0.030±0.005	0.000

Marwari and Sirohi goats of India (Rout *et al*. 2008). The high genetic diversity observed in the present populations could be explained by overlapping generations, mixing of populations from different geographical locations, natural selection favouring heterozygosity or subdivision accompanied by genetic drift. The effect of these factors is more pronounced when the effective population is very large. Takezaki and Nei (1996) determined that for markers to be useful for measuring genetic variation, they should have an average heterozygosity ranging from 0.3 to 0.8 in the population. This again confirmed that the markers used in this study were appropriate for measuring genetic variation.

The mean observed heterozygosity was lower than the expected in all the studied breeds and deviation from HWE was significant in most of the loci studied (18 loci in Kodi Adu and Kanni Adu goats and 17 loci in Salem Black goats). It is in accordance with the previous studies in other goat breeds of India (Kumar *et al*. 2005, Verma *et al*. 2007, Dixit *et al*. 2011). In general, the heterozygosity deficit within a population, as measured by Wright's F_{IS}, was positive in all the loci studied. The heterozygote deficiency observed at these loci might be due to genetic drift, null alleles,

selection against heterozygotes or inbreeding. However, distinguishing among them is difficult.

The overall means for global F-statistics pooled over 3 breeds obtained from jack-kniffing over loci were significantly different from zero (Table 2). The significant positive value of F_{is} ranged from 0.068 (SRCRSP9) to 0.533 (MAF209) with an overall mean of 0.222±0.031. The F_{is} value observed was comparable to those previously reported in Marwari (0.264), Kutchi (0.230), Barbari (0.202) and Sangamneri goats (0.245) of India (Kumar *et al*. 2005, Dixit *et al*. 2007, Sharma *et al*. 2008, Verma *et al*. 2010). Genetic differentiation among sub-populations (F_{ST}) was low (3%). Similar F_{ST} values were found in Europe, Middle East and Spanish goat breeds (Serrano *et al*. 2009). The low level of genetic differentiation (F_{ST}) among the three breeds under study implied that 97% of the total genetic variation corresponds to differences among individuals within breed and 3% of the total genetic variation corresponds to the breed.

The pair-wise Nei's genetic distance, Cavalli-Sforza and Edwards chord distance, pair-wise F_{ST} among 3 goat breeds of Tamil Nadu are presented in Table 3. The genetic data

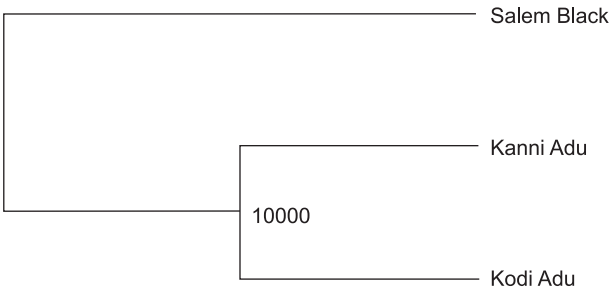

Fig. 1. Dendrogram based on pair-wise Cavalli-Sforza and Edwards chord distance following UPGMA algorithm with 10000 bootstrap re-sampling.

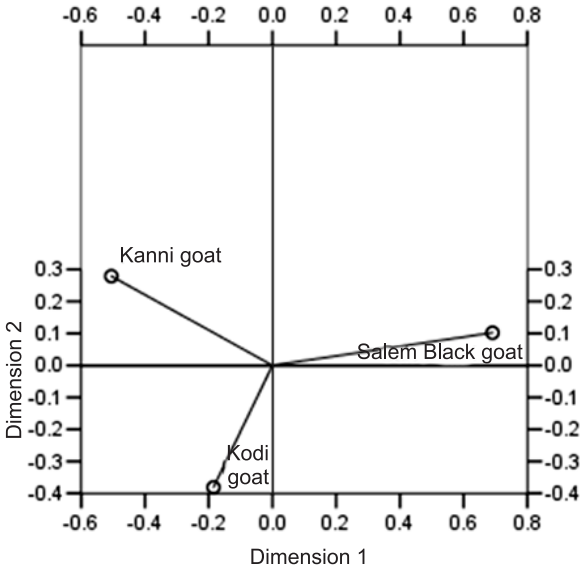

Fig. 2. Multi-dimensional scaling display of pair-wise FST among 3 goat breeds of Tamil Nadu (S-stress=0.000).

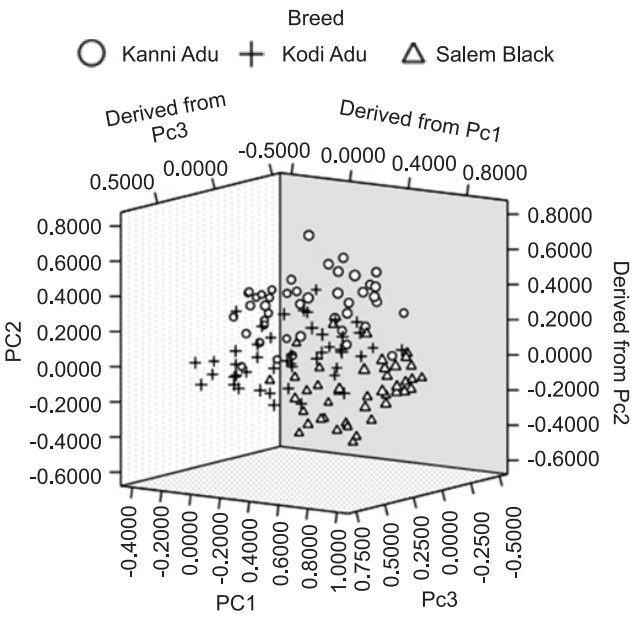

Fig. 3. Three dimensional scatterplot of first 3 principal components derived from inter-individual Cavalli-Sforza and Edwards chord distance of 3 goat breeds.

Table 3. Pair-wise Nei's genetic distance (upper triangle), Cavalli-Sforza and Edwards chord distance (within parenthesis), pair-wise F_{ST} (lower triangle) among three goat breeds of Tamil Nadu

	Kanni Adu	Kodi Adu	Salem Black
Kanni Adu	0	0.166 (0.346)	0.216 (0.395)
Kodi Adu	0.024	0	0.173 (0.354)
Salem Black	0.039	0.029	0

revealed that the smallest difference is between Kodi Adu and Kanni Adu; and the Salem Black goat was distinctly different from the Kodi Adu and Kanni Adu goats. The phylogenetic analysis indicated (Figs 1, 2) that breeds were grouped according to their geographical locations. The highest geographical distance between Salem Black and Kodi Adu as well as Kanni Adu goats corresponds to the highest genetic distance. A similar observation of population clustering according to their geographic origin was reported in earlier studies (Fatima *et al*. 2008, Rout *et al*. 2008). This showed that geographically adjacent populations are genetically related. The closer genetic relationship between Kodi Adu and Kanni Adu goats perhaps due to founder effect and interbreeding near bordering areas; which increases the possibility of gene flow between these breeds. The principal component analysis supported the grouping of animals and the genetic distance between breeds was significant (Fig. 3). PCA revealed separation of breeds more clearly between populations of different geographical locations. Although, the breeding tracts of goats are overlapping and no strict breeding policy is adopted to maintain standard breeds in the region, they still maintain genetic distances in their natural habitat, therefore it is necessary to combine genetic data with geographical

positioning and to assess the genetic relationship by geostatistical models in future studies.

SUMMARY

In the present study, assessment of genetic diversity of Kodi Adu, Kanni Adu and Salem Black goats of Tamil Nadu, India was made using 21 microsatellite markers. The mean number of alleles observed per locus in Kodi Adu, Kanni Adu and Salem Black goats were 11.52, 11.24 and 10.14 respectively. The mean expected heterozygosity was 0.846 in Kodi Adu, 0.857 in Kanni Adu and 0.820 in Salem Black goats. All the population of different breeds showed an overall significant heterozygote deficit (F_{IS}) and the F_{IS} values estimated in Kodi Adu, Kanni Adu and Salem Black goats were 0.229, 0.215 and 0.233 respectively. The measures of standard genetic distance between pairs of breeds indicated that the lowest genetic distance was between Kanni Adu and Kodi Adu (0.166) and the largest genetic distance was between Kanni Adu and Salem Black (0.216) goats. Both a phylogenetic tree and principal component analysis showed the distribution of breeds in two major clusters with respect to their geographic distribution. All measures of genetic variation revealed substantial genetic variation in each of the populations studied, thereby showing good scope for exploiting genetic variability of these breeds for further improvement of performance.

REFERENCES

Acharya R M. 1982. *Sheep and Goat Breeds of India*. FAO Animal Production and Health Paper 30, Food and Agriculture Organization of the United Nations, Rome. pp. viii + 190.

Barker J S F, Tan S G, Moore S S, Mukherjee T K, Matheson J L and Selvaraj O S. 2001. Genetic variation within and relationships among populations of Asian goats (*Capra hircus*). *Journal of Animal Breeding and Genetics* **118**: 213–33.

Botstein D, White R L, Skolnick M and Davis R W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics* **32**: 314–31.

Boyce W M, Hedrick P W, Muggli-Cockett N E, Kalinowski S, Penedo M C and Ramey R. R. 1996. Genetic variation of major histocompatibilty complex and microsatellite loci: a comparison in Bighorn sheep. *Genetics* **145**:421–33.

Chessel D, Dufour A B and Thioulouse J. 2004. The ade4 package. 1–one table methods. *The R newsletter* **1**: 5–10.

Dixit S P, Aggarwal R A K, Verma N K, Vyas M K, Rana J, Sharma A and Chander R. 2011. Genetic variability and bottleneck analyses of Kanniadu goat breed based on microsatellite markers. *Indian Journal of Animal Sciences* **81**: 40–43.

Dixit S P, Verma N K, Aggarwal R A K, Kumar S, Chander R, Vyas M K and Singh K P. 2009. Genetic structure and differentiation of three Indian goat breeds. *Asian-Australasian Journal of Animal Sciences* **22**: 1234 –40.

Dixit S P, Verma N K, Aggarwal R A K, Patel A K, Kumar D, Sharma R and Ahalwat S P S. 2007. Phenotypic and genotypic characterization of Marwari breed of goat in hot-arid region of India. *Indian Journal of Animal Sciences* **77**: 395–99.

Dixit S P, Verma N K, Aggarwal R A K, Vyas M K, Rana J, Sharma A, Tyagi P, Arya P and Ulmek B R. 2010. Genetic diversity and relationship among southern Indian goat breeds based on microsatellite markers. *Small Ruminant Research* **91**: 153–59.

Excoffier L, Laval G and Schneider S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47–50.

Fatima S, Bhong C D, Rank D N and Joshi C G. 2008. Genetic variability and bottleneck studies in Zalawadi, Gohilwadi and Surti goat breeds of Gujarat (India) using microsatellites. *Small Ruminant Research* **77**: 58–64.

Ganai N A and Yadav B R. 2001. Genetic variation within and among three Indian breeds of goat using heterologous microsatellite markers. *Animal Biotechnology* **12**: 121–36.

Ihaka R and Gentleman R. 1996. R: a language for data analysis and graphics. *Journal of Computer Graphics Statistics* **5**: 299–314.

Kumar D, Dixit S P, Sharma R, Pandey A K, Sirohi G, Patel A K, Aggarwal R A K, Verma N K, Gour D S and Ahlawat S P S. 2005. Population structure, genetic variation and management of Marwari goats. *Small Ruminant Research* **59**: 41–48.

Luikart G, Biju-Duval M P, Ertugrul O, Zagdsuren Y, Maudet C and Taberlet P. 1999. Power of 22 microsatellite markers in fluorescent multiplexes for parentage testing in goats (*Capra hircus*). *Animal Genetics* **30**: 431–38.

Miller S A, Dykes D D and Polesky H F. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* **16**:1215.

Peakall R and Smouse P E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**: 288–95.

Rout P, Joshi M, Mandal A, Laloe D, Singh L and Thangaraj K. 2008. Microsatellite-based phylogeny of Indian domestic goats. *BMC Genetics* **9**: 11.

Saitbekova N, Gaillard C, Obexer-Ruff G and Dolf G. 1999. Genetic diversity in wiss goat breeds based on microsatellite analysis. *Animal Genetics* **30**: 36–41.

Serrano M, Calvo J H, Martinez M, Carcavilla A M, Cuevas J, Gonzalez C, Jurado J J and Tejada P D. 2009. Microsatellite based genetic diversity and population structure of the endangered Spanish Guadarrama goat breed. *BMC Genetics* **10**:61.

Takezaki N and Nei M. 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics* **144**: 389–99.

Thiruvenkadan A K. 2012 *Kodi Adu goat*- a monograph. International Foundation for Science, Sweden.

Thiruvenkadan A K and Karunanithi K. 2006. Characterization of Salem Black goats in their home tract. *Animal Genetic Resources Information* **38**: 67–75.

Thiruvenkadan A K. Panneerselvam S and Kandasamy N. 2000. Distribution, characteristics and production performance of Kanni Adu goats of Tamil Nadu. *Indian Journal of Animal Sciences* **70**: 723–27.

Verma N K, Dixit S P, Aggarwal R A K, Dangi P S and Joshi B K. 2010. Phenotypic and genotypic characterization of Sangamneri goat breed. *Indian Journal of Animal Sciences* **80**: 1109–14.

Verma N K, Dixit S P, Kumar D, Aggarwal R A K and Ahlawat S P S. 2007. Physical characteristics, performance status and genetic variation in Jakhrana breed of goat in its native tract. *Indian Journal of Animal Sciences* **77**: 390–94.

Yeh F C, Yang R, Boyle C, Timothy B J, Ye Z H and Mao J X. 1999. POPGENE version 1.32, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre. University of Alberta, Canada. http://www.ualberta.ca/fyeh/.