Genetic diversity analysis of Katchaikatty Black – An endangered sheep breed from Tamil Nadu

REENA ARORA[1✉], ANAND JAIN[1], RAJA K N[1], MANDEEP KAUR[1], ASHISH KUMAR[1],
SONIKA AHLAWAT[1] and REKHA SHARMA[1]

ICAR-National Bureau of Animal Genetic Resources, Karnal, Haryana 132 001 India

ABSTRACT

The study presents the genetic diversity of Katchaikatty Black, an endangered and culturally significant sheep breed of Tamil Nadu. A panel of 25 microsatellite markers recommended for Indian sheep was used for genotyping. Considerable genetic variation in terms of allele diversity and heterozygosity was observed within the investigated breed. A total of 174 distinct alleles were detected across the analyzed microsatellite loci with an average of 6.96 alleles per locus. The average observed and expected heterozygosity values were 0.600 and 0.706, respectively. The positive heterozygote deficiency (F_{IS}) value of 0.171, estimated for this breed may be due to the possibility of Wahlund effect resulting from sampling from different breeding flocks, i.e. different villages and flocks in the same area. A normal L-shaped curve suggested absence of genetic bottleneck in this breed. The information generated will be useful in guiding conservation and management programmes for Katchaikatty Black sheep.

Keywords: Genetic diversity, Indian sheep, Microsatellite markers, Tamil Nadu

Sheep have been associated with wealth and prosperity since their domestication in the pre-agricultural era. These animals are involved in the social and cultural stratum of human life and have served as supporting pillars of the agricultural sector. India is endowed with several sheep breeds with potential to meet the demands for food and livelihood in future. At present there are 44 registered sheep breeds in the country and Tamil Nadu is the proud owner of 10 breeds which include Coimbatore, Mecheri, Kilakarsal, Madras Red, Ramnad White, Tiruchi Black, Vembur, Nilagiri, Chevaadu and Katchaikatty Black. Among these well-known mutton type sheep of Tamil Nadu, Katchaikatty sheep has carved its own place as a mutton breed of cultural significance. This breed derives its name from the Katchaikatty village in Vadipatti Taluka of Madurai district, from where it originated. The Pallar and Konar communities in Madurai district of Tamil Nadu have been rearing the Katchaikatty Black sheep for mutton since decades. The animals are medium in size with a black coat and the rams are especially maintained for fighting events (Ravimurugan *et al.* 2012). Despite its socio-economic relevance, Katchaikatty Black sheep has been listed as an endangered breed in the National Breed Watch list (Pundir *et al.* 2022). The dwindling numbers of this population warrant an immediate action for its conservation. Genetic characterization is an essential step towards conservation

Present address: [1]ICAR-National Bureau of Animal Genetic Resources, Karnal, Haryana. ✉Corresponding author email: rejagati@gmail.com; reena.arora@icar.gov.in

and evaluation of breed potential, which in turn leads to the sustainable improvement. Microsatellite markers have widely been used for genetic diversity analysis of sheep globally (Arora *et al.* 2011a, 2011b, Ben Sassi-Zaidy *et al.* 2022, Dayo *et al.* 2022). These markers are valuable tools used for inferring within and between breed genetic diversity. Keeping in view the endangered status, this study was undertaken with the aim to estimate the level of genetic diversity in Katchaikatty Black sheep, so that immediate steps may be taken for its restoration and conservation.

MATERIALS AND METHODS

Samples: Blood samples of 34 unrelated animals of Katchaikatty Black sheep were collected from Madurai district of Tamil Nadu. Blood sampling was coordinated with owners and veterinary officers. Care was taken to avoid the possibility of mixing of blood with other populations or breeds by taking samples from distinct flocks exhibiting specific characteristics. All ethical norms were followed during collection of blood samples.

Genotyping with microsatellite markers: Genomic DNA was isolated from blood samples using the standard phenol chloroform extraction method (Sambrook *et al.* 1989). Twenty five microsatellite markers recommended for Indian sheep were selected for the analysis of genetic diversity. The forward primer for each marker was fluorescently labelled with either FAM, NED, VIC or PET dye. Details of microsatellite genotyping are given in Arora *et al.* (2011a). The genotyping was carried out on ABI 3100 automated DNA sequencer using LIZ 500 as internal size

standard. Allele sizing was done using GENEMAPPER software (Applied Bio system, USA).

Statistical analysis: The GENALEX-6.5 (Peakall and Smouse 2012) software was used to estimate allele frequencies, observed and effective number of alleles, observed and expected heterozygosity, within breed heterozygote deficiency (F_{IS}) of the Katchaikatty Black sheep and genetic distances between breeds. The deviations from the Hardy-Weinberg equilibrium (HWE) were tested using GENEPOP-3.2a (Raymond and Rousset 1995). BOTTLENECK (version 1.2.02) software (Piry *et al.* 1999) was used in this study for detection of genetic bottleneck in the investigated sheep population. The Nei's (1978) genetic distances were used for construction of phylogenetic tree using POPULATIONS 1.2.28 software (Langella 2002). Bootstraps of 1000 replicates were used for testing the robustness of the tree topology.

RESULTS AND DISCUSSION

Genotypes for the 25 microsatellite loci were recorded for Katchaikatty Black sheep. One hundred and seventy four alleles were detected across all the 25 markers used. The informativeness of the microsatellite markers was assessed by their Polymorphism Information Content (PIC) value (Botstein *et al.* 1980). It was observed that 88% of the markers used were highly informative with PIC value ≥ 0.5 while remaining 12% were moderately informative (0.25 ≥ PIC ≤ 0.5). The observed allele number ranged from 3 (BM6506) to 11 (OarFCB48) while the effective number of alleles varied from 1.424 (CSSM47) to 7.728 (OarCP49). The average allele diversity (Na) and effective allele number (Ne) were 6.96 and 4.052, respectively (Table 1).

Seven loci (BM1314, BM6506, CSSM47, OarCP49, OarAE129, OarFCB48, OarJMP8) showed significant deviations from HWE (p<0.001). Two of these loci (BM1314 and CSSM47) showed a considerable deficiency of heterozygotes and were therefore eliminated from further analyses. The deviation from HWE and high heterozygote deficiency exhibited by BM1314 and CSSM47 markers may be due to the presence of non-amplifying or null alleles. Null alleles may lead to a false observation of excess homozygotes. It was not possible to estimate the extent of null alleles in the present study, as no pedigree records of animals were available as random sampling was done from the distribution area.

The allele diversity estimates observed in Katchaikatty Black sheep were higher than those described for Coimbatore (Hepsibha *et al.* 2014) but lower than that of Kilakarsal, Ramnad White and Chevaadu breeds (Radha *et al.* 2011, Raja *et al.* 2012, Ravimurugan and Panneerselvam 2014). However, comparison with Vembur (Pramod *et al.* 2009), Mecheri (Prema *et al.* 2008), Madras Red (Selvam *et al.* 2009) and Nilgiri (Girish *et al.* 2007) sheep breeds was not feasible as the analysis was done by manual methods. The results indicated adequate genetic variability is present in Katchaikatty Black sheep breed.

Table 1. Summary of genetic diversity measures for Katchaikatty Black sheep

Locus	Na	Ne	Ho	He	F_{IS}
BM757	5	3.401	0.750	0.706	-0.062
BM827	5	4.141	0.810	0.759	-0.067
BM1314	8	2.375	0.133	0.579	0.770
BM6506	3	2.101	0.370	0.524	0.293
BM6526	8	4.408	0.722	0.773	0.066
BM8125	8	4.639	0.536	0.784	0.317
CSRD247	9	6.178	0.667	0.838	0.205
CSSM31	10	5.341	0.630	0.813	0.225
CSSM47	5	1.424	0.133	0.298	0.552
HSC	7	5.011	0.714	0.800	0.108
INRA63	6	2.945	0.778	0.660	-0.178
MAF214	5	1.816	0.563	0.449	-0.252
OarAE129	3	2.323	0.000	0.569	1.000
OarCP49	10	7.728	1.000	0.871	-0.149
OarCP20	6	2.903	0.600	0.656	0.085
OarCP34	8	3.947	0.600	0.747	0.196
OarFCB48	11	5.262	0.786	0.810	0.030
OarFCB128	8	5.400	0.889	0.815	-0.091
OarHH35	8	3.675	0.714	0.728	0.019
OarHH41	6	2.946	0.542	0.661	0.180
OarHH47	8	4.899	0.654	0.796	0.178
OarHH64	5	4.050	0.591	0.753	0.215
OarJMP8	6	4.401	0.600	0.773	0.224
OarJMP29	10	7.200	0.792	0.861	0.081
OarVH72	6	2.776	0.423	0.640	0.339
Mean	6.96	4.052	0.600	0.706	0.171

Na, observed alleles; Ne, effective number of alleles; He, observed heterozygosity; He, expected heterozygosity; F_{IS}, Heterozygote deficiency.

Heterozygosity is another measure for assessing genetic diversity within a population. The observed and expected heterozygosity values were high for all loci except CSSM47 and BM1314. The observed heterozygosity varied from 0.133 to 1.0 with an average of 0.6. The expected heterozygosity ranged from 0.298 to 0.871. The average observed (0.6) and expected (0.706) heterozygosity values of Katchaikatty Black sheep were relatively similar to those of other domestic sheep breeds investigated earlier (Yadav *et al.* 2011, Arora *et al.* 2010b, 2016; Yadav and Arora 2014, Yadav *et al.* 2017). No significant differences were observed between mean observed and expected heterozygosity values when tested using ANOVA (P>0.05), which suggested random mating in Katchaikatty Black. The high value of gene diversity indicated that the population had retained the presence of several alleles although at low frequencies. This further substantiated the presence of sufficient amount of genetic variability in this sheep breed that might be used in planning breeding strategies.

The Katchaikatty Black sheep breed exhibited a positive heterozygote deficit value (F_{IS}=0.171). Significant heterozygotes deficiencies have also been reported in several earlier investigated Indian sheep breeds

(Radha *et al.* 2011, Arora *et al.* 2011c). The heterozygote deficiency could be due to inbreeding or Wahlund effect (population substructure). Inbreeding is an unlikely explanation because of the presence of high gene diversity or heterozygosity in Katchaikatty Black sheep breed. The possibility of Wahlund effect or sub-structuring due to pooling of samples (within population) from different breeding flocks (e.g. different villages in the same area) appears to be more likely.

The decline in the number of Katchaikatty Black sheep may have impacted the number of breeding individuals. Since the effective population size directly affects the genetic diversity of a population (Luikart *et al.* 1998), it is imperative to assess whether the dwindling population has also undergone loss in genetic variation. The genetic bottleneck analysis was therefore performed to estimate whether genetic diversity was lost due to population decline. A mode shift test, under the assumption of two-phase model was carried out on the genotype data. The resulting L-shaped curve suggested no deficiency of rare alleles or absence of a recent decrease in the effective population size in Katchaikatty Black population (Fig. 1). Similar observations were reported for Indian sheep with declining populations (Arora *et al.* 2011a).
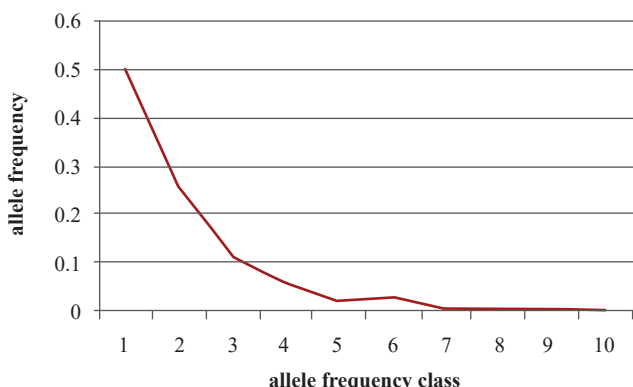

Fig. 1. Analysis of genetic bottleneck in Katchaikatty Black sheep showing normal L-shaped curve.

The genetic distances between Katchaikatty Black sheep and other breeds of the Southern Peninsula were estimated to determine their genetic relationship. Nei's genetic distances (Nei 1978) between Deccani, Madgyal, Tiruchy Black, Nellore, Chevaadu and Katchaikatty Black breeds were used to construct the phylogenetic tree using Unweighted Pair Group method with Arithmetic mean algorithm (UPGMA). The tree topology suggested a closer relationship of Katchaikatty Black sheep with Tiruchy Black and Chevaadu breeds (Fig. 2). Deccani and Madgyal breeds of Maharashtra formed a separate cluster, while Nellore from Andhra Pradesh was most distinct among the investigated breeds. The present results support the view that Katchaikatty may be the progenitor of Chevaadu sheep (Ravimurugan 2017).

The results suggested that even though the Katchaikatty Black population is shrinking in numbers, a concurrent decline in the effective population size or genetic diversity
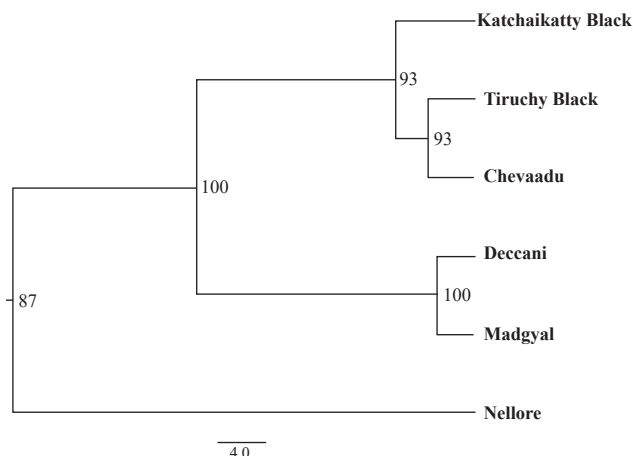

Fig. 2. Genetic relationship of Katchaikatty Black sheep with six sheep breeds of Southern Peninsular region. The UPGMA tree is derived from Nei's (1978) genetic distances. The numbers on the nodes indicate the percentage occurrence in 1000 bootstrap replicates.

is not evident. High positive value of F_{IS}, however, points to population sub-structuring. Genetic management strategies like segregating fighting and breeding rams will help in retaining the genetic diversity within the population. Establishment of farms for *in situ* conservation of this endangered breed is highly recommended. The information generated in this study may be utilized for planning future breeding and conservation strategies for Katchaikatty Black sheep breed.

REFERENCES

Arora R, Bhatia S and Mishra B P. 2010. Genetic structure analysis of Deccani sheep-A major breed from Maharashtra. *Indian Veterinary Journal* **87**(11): 1109–11.

Arora R, Bhatia S and Mishra B P. 2011a. Genetic variation and relationship of six Indian sheep breeds adapted to the northwestern arid zone of Rajasthan. *Biochemical Genetics* **49**(7-8): 449–57.

Arora R, Bhatia S, Mishra B P and Joshi B K. 2011b. Population structure in Indian sheep ascertained using microsatellite information. *Animal Genetics* **42**(3): 242–50.

Arora R, Bhatia S, Yadav D K and Mishra B P. 2011c. Current genetic profile of sheep breeds/populations from Northwestern semi-arid zone of India. *Livestock Science* **135**: 193–98.

Arora R, Kulkarni V S, Jain A and Yadav D K. 2016. Yalaga sheep - A microsatellite based genetic profile. *Indian Journal of Animal Sciences* **86**(10): 1155–58.

Ben Sassi-Zaidy Y, Mohamed-Brahmi A, Nouairia G,

Charfi-Cheikhrouha F, Djemali M and Cassandro M. 2022. Genetic variability and population structure of the Tunisian Sicilo-Sarde dairy sheep breed inferred from microsatellites analysis. *Genes (Basel)* **13**(2): 304.

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

Dayo G K, Houaga I, Somda M B, Linguelegue A, Ira M, Konkobo M, Djassi B, Gomes J, Sangare M, Cassama B and Yapi-Gnaore C V. 2022. Morphological and microsatellite DNA diversity of Djallonké sheep in Guinea-Bissau. *BMC Genome Data* **23**(1): 3.

FAO. 2007. Global plan of action for animal genetic resources and the Interlaken declaration. *Food and Agriculture Organization* of the United States, Rome, Italy. (http://www.fao.org/docrep/010/a1404e/a1404e00.htm).

Girish H, Sivaselvam S N, Karthikeyan S M K and Saravanam R. 2007. Molecular characterization of Nilagiri Sheep (*Ovis aries*) of South India based on microsatellites. *Asian-Australasian Journal of Animal Science* **20**(5): 633–37.

Hepsibha P, Karthickeyan S M K and Guru V. 2014. Microsatellite marker based assessment of genetic structure of Coimbatore breed of sheep *Ovis aries* in Tamil Nadu. *Indian Journal of Biotechnology* **13**: 203–06.

Kavitha S T, Subramanian A, Sivaselvam S N, Thiagarajan R and Balasubramanian S. 2015. Genetic characterization of Tiruchy Black sheep of Tamil Nadu using microsatellite markers. *Indian Journal of Animal Research* **49** (3): 320–24.

Langella O. 2002. Populations 1.2.28. Available at: http:// www.cnrs-gif.fr/pge/bioinfo/populations/index.php#ancre_bibliographie.

Luikart G, Allendorf F W, Cornuet J M and Sherwin W B. 1998. Distortion of allele frequency distributions provides a test for recent population bottleneck. *Journal of Heredity* **89**: 238–47.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583–90.

Peakall R and Smouse P E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* **28**(19): 2537–9.

Piry S, Luikart G and Cornuet J M. 1999. BOTTLENECK a computer programme for detecting reductions in the effective size using allele frequencies. *Journal of Heredity* **90**: 502–03.

Pramod S, Kumarasamy P, Rosalyn Mary Chandra A, Sridevi P and Rahumathulla P S. 2009. Molecular characterization of Vembur sheep (*Ovis aries*) of South India based on microsatellites. *Indian Journal of Science and Technology* **2**: 55–58.

Prema S, Sivaselvam S N and Karthikeyan S M K. 2008. Evaluation of genetic diversity in Mecheri sheep (*Ovis aries*) of Tamil Nadu using microsatellite markers. *Indian Journal of Biotechnology* **7**: 401–03.

Pundir R K, Mishra A K, Narula H K, Yadav D K and Dige M S. 2022. National Breed watch list (2022) and way forward. *Proceedings of the National Symposium on Contemporary technology for Animal Genetic Resource (AnGR) Management*, pp.33-38. September 21-22. SOCDAB and ICAR-NBAGR, Karnal, Haryana.

Radha P, Sivaselvam S N, Kumarasamy P and Kumanan K. 2011. Genetic diversity and bottleneck analysis of Kilakarsal sheep by microsatellite markers. *Indian Journal of Biotechnology* **10**: 52–55.

Raja K N, Jain A, Singh G, Kumar L, Yadav H K and Arora R. 2012. Ramnad white sheep-phenotypic and genetic characterization. *Indian Journal of Animal Sciences* **82**(9): 1082–86.

Ravimurugan T, Thiruvenkadan A K, Sudhakar K and Panneerselvam S. 2012. Sheep genetic resources of Southern Tamil Nadu, India. *International Research Journal of Agricultural Science and Soil Science* **2**(3): 081–88.

Ravimurugan T. 2017. Chevaadu-A new registered sheep breed of Tamil Nadu, India. *International Journal of Current Microbiology and Applied Sciences* **6**(9): 1553–57.

Ravimurugan T and Panneerselvam S. 2014. Genetic diversity in Chevaadu, Kilakarsal and Madras Red sheep breeds of Tamil Nadu - Tested with microsatellite markers. *Indian Veterinary Journal* **91**(9): 14–17.

Raymond M and Rousset F. 1995. GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**: 249–49.

Sambrook J, Fritsch E F and Maniatis T. 1989. *Molecular Cloning: A Laboratory Manual*. 2nd ed. Cold Spring Harbor Laboratory Press. Cold Spring Harbour, New York.

Selvam R, Rahumathulla P S, Sivaselvam S N, Karthikeyan S M K and Rajendran R. 2009. Molecular genetic characterization of Madras red sheep in Tamil Nadu, India using microsatellite markers. *Livestock Research for Rural Development* **21**(4).

Yadav D K and Arora R. 2014. Genetic discrimination of Muzaffarnagri and Munjal sheep of northwestern semi-arid zone of India based on microsatellite markers and morphological traits. *Indian Journal of Animal Sciences* **84**(5): 527–32.

Yadav D K, Arora R and Jain A. 2017. Classification and conservation priority of five Deccani sheep ecotypes of Maharashtra, India. *PLoS ONE* **12**(9): e0184691.

Yadav D K, Arora R, Bhatia S and Singh G. 2011. Short tandem repeat based analysis of genetic variability in Munjal- the threatened sheep population of Northwestern India. *Indian Journal of Animal Sciences* **81**(2): 171–75.

42