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Molecular characterization of vembur sheep (*Ovis aries*) of south India based on microsatellites

S. Pramod, P. Kumarasamy*, A. Rosalyn Mary Chandra, P. Sridevi and P. S. Rahumathulla
Department of Animal Genetics and Breeding, Madras Veterinary College
Tamilnadu Veterinary and Animal Sciences University, Chennai – 600 007, India
pksamy2000@gmail.com*

Abstract: The Vembur sheep is a mutton breed, adapted to semi arid habitat and distributed in and around Thoothukudi, Virudhunagar and Tirunelveli districts of Tamilnadu, India. Microsatellite characterization yielded a total of 147 alleles in the sampled population for 25 marker loci under investigation, with a mean of 5.88 ± 0.29 alleles per locus. Number of alleles observed on each locus varied between two and nine. The effective number of alleles observed with a mean of 4.0950 ± 0.23 alleles per locus. The mean PIC value was 0.6905 ± 0.02. The frequency of majority of the loci returned highly significant chi-square values suggesting departure from HWE. The observed heterozygosity (Ho) values varied between 0.1333 and 1.0000, while the expected (He) values ranged from 0.4847 to 0.8537. The mean values were 0.5202 ± 0.04 and 0.7339 ± 0.02 respectively. F_{IS} estimates were negative for three loci indicating more heterozygous nature of the population. The mean value observed was 0.2954 ± 0.31.

Keywords: Vembur sheep, microsatellites, PIC, $F_{IS,}$ India.

Introduction

Sheep were probably first domesticated in the Fertile Crescent, ~8000 to 9000 years ago. Archaeological information revealed two independent areas of sheep domestication in Turkey- the upper Euphrates valley in eastern Turkey, and central Anatolia (Peters *et al*., 1999). It was suggested that, three species of wild sheep viz., the urial, *Ovis vignei*, the argali, *Ovis ammon*, and the Eurasian mouflon, *Ovis musinom/orientalis* were ancestors of domestic sheep. The Indian Council for Agricultural Research (ICAR) estimates the existence of 40 sheep breeds in India. The strategy employed to classify a large number of sheep and goat breeds in India was based on identifiable morphological characteristics that were distinct from other populations in the vicinity, particularly those with a local name (Acharya, 1982; Shrestha, 2005).

The Vembur sheep (Ganesakale & Rathnasabapathy, 1973; Acharya, 1982) is distributed among the three southern districts of Tamilnadu, namely Thoothukudi, Virudhunagar and Tirunelveli (Fig. 1). The population estimated in 1977 was 0.251m (Acharya, 1982). The present population of the breed assessed by SEVA, a non-governmental organization working for conservation of indigenous breeds was around 2,000. Cross breeding, change in land use pattern, availability of alternate employment and subsequent reduction in the number of herdsmen are all suggested as reasons for severe reduction in population. Genetic characterization of Vembur sheep was performed using 25 microsatellite markers recommended by Food and Agricultural Organization for diversity studies on ovines (FAO, 2004)

Material and methods

Blood samples were collected from 50 Vembur sheep (Fig. 2), unrelated by ancestry from the breeding tract and genomic DNA was isolated by phenol-chloroform method (Sambrook *et al*., 1989).

Amplification and genotyping

The stock DNA was diluted in sterile ultra filtered water so as to contain 20-50 ng of genomic (material) DNA per microlitre. The primers were diluted to 10 pM in ultra filtered water. The reaction mixture consisted of Master mix (Bangalore Genei) 10µl; genomic DNA 3 µl; 0.8 µl of each forward and reverse primer (5 pM) and 5.4 µl of ultra filtered water in a reaction volume of 20 µl. The loci BM8125, CSSM31, HUJ616, OarCP20, OarFCB128, OarFCB48, OarHH41, OarJMP8, RM4, TGLA137 and TGLA377 were amplified by PCR programme with respective annealing temperatures (Bishop *et al*., 1995) for 35 cycles. The loci BM1314, BM6506, BM6526, BM757, BM827, CSSM47, OarAE129, OarJMP29, OarCP34, OarFCB20, OarHH35, OarHH47, OarHH64 and OarVH72 were amplified with a 'touch down' programme suggested by FAO (2004), with an additional extension step of 72ºC for 45 S for 25 cycles at 48ºC. The PCR products were separated on a 6% denaturing polyacrylamide gel and silver-stained using a simplified method (Lujiang Qu *et al*., 2005). The silver-stained gels were analysed by Diversity Database software (Bio-Rad, USA) for scoring the alleles. The product sizes were determined with the help of 10 bp DNA ladder (Invitrogen, USA) as a standard marker. The genotypes were scored based on the presence of a single band (homozygotes) or double bands (heterozygotes) in the gel.

Molecular genetic analysis

Allele frequencies, effective number of alleles, test of Hardy-Weinberg equilibrium (HWE), observed and expected heterozygosity and F-statistics were calculated using the Popgene version 1.31 (Yeh *et al*.,1999). The polymorphism information content (PIC) for each marker was determined separately for the groups of animals using the following equation:

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - 2\left[\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} p_i^2 p_j^2\right]$$

where p_i is the frequency of the i^{th} allele, and n is the number of alleles (Botstein *et al*., 1980).

Results and discussion

Number of observed alleles

A total of 147 alleles were delineated from the sampled population with a mean of 5.88 ± 0.29 alleles per locus. Number of alleles observed on each locus varied between two (OarHH47) and nine (HUJ616). Among

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other Tamilnadu breeds, 125 alleles were reported for Nilagiri (Girish *et al*., 2007) in 25 loci with a mean of 5 per locus and 143 alleles with a mean of 6.88 ± 0.85in 27 loci for Coimbatore sheep (Kumarasamy *et al.,* 2009) which were lower than the findings of the present study. Allele numbers in Madras Red and Mecheri (Prema *et al.,* 2008; Selvam *et al.,* 2009), were 189 and 195 respectively which were higher than that for Vembur sheep.

In Jalauni sheep (Arora *et al*., 2008) 148 alleles were observed over 25 loci, with a mean of 5.92 which is comparable to Vembur sheep. In Garole sheep, the observed number of alleles reported was 6.2 (Sodhi *et al*., 2003), which is higher than that of the present study. In Nali and Chokla sheep breeds (carpet wool breeds of Northwestern India), a total of 138 and 133 alleles respectively were reported. The number of observed allele ranged from 3 to 10 in Nali and 2 to 8 in Chokla with the respective means of 5.52 and 5.32 alleles (Sodhi *et al*., 2005), which is similar to the observation of the present study.

In Magra breed of sheep, another carpet wool breed of Northwestern India, the number of observed alleles ranged from 3 to 10 with a mean of 5.7 (Arora & Bhatia, 2006). In exotic sheep breeds, a wide range of number of observed alleles were reported (2 to 4, Gutierrez-Espelata *et al*., 2000 and 7 to 28, Pariset *et al*., 2003).

Effective number of alleles

The effective number of alleles in Vembur sheep over 25 loci ranged from 1.9406 (OarHH47) to 6.8368 (HUJ616) with a mean of 4.095 ± 0.23 alleles per locus (Table 1). The effective number of alleles at each locus provides information on predominant alleles. Among other Indian breeds, values lower than that of Vembur sheep were observed by many authors, 3.7 in Jalauni sheep (Arora *et al*., 2008); 2.18 in Nilagiri sheep (Girish *et al*., 2007); 3.34 in Nali sheep (Sodhi *et al*., 2005); 3.27 in Chokla sheep (Sodhi *et al*., 2005); 3.64 in Muzzafarnagri breed (Arora & Bhatia, 2004). Madras Red and Mecheri breeds (Selvam *et al.,* 2009; Prema *et al.,* 2008) and Coimbatore sheep (4.93, Kumarasamy, *et al.,* 2009) had higher mean values than that of Vembur sheep.

Polymorphism information content

PIC values for Vembur sheep (Table 1) ranged from 0.3712 (OarHH47) to 0.8360 (HUJ616) with a mean of 0.6905 ± 0.02. 24 out 25 loci (96 per cent) had PIC values greater than 0.5 which prove their utility for genetic diversity studies. OarHH47 was found to be reasonably informative (0.50>PIC>0.25). Among the Indian sheep breeds, the PIC values ranged from 0.533 to 0.808 in Muzzafarnagri sheep (Arora & Bhatia, 2004) and 0.210 to 0.831 and 0.346 to 0.768 in Nali and Chokla sheep breeds respectively (Sodhi *et al*., 2005), 0.347 to 0.849 in Magra sheep (Arora & Bhatia, 2006), 0.4587 to 0.8277 in Nilagiri sheep, and 0.24 to 0.82 in Jalauni sheep (Arora *et al*., 2008) using ovine-specific microsatellite markers.

Hardy-Weinberg equilibrium

The results of the χ^2 test of goodness of fit (Table 1) proved that majority of the loci under investigation (19 out of 25) returned highly significant chi square values suggesting departure from HWE. The loci OarHH47,

OarJMP29 and TGLA377 revealed statistically significant departure from HWE, while the loci BM1314, OarCP34 and OarFCB20 were observed to be in HWE. The observations from Vembur sheep in this regard were in agreement with the findings of Madras Red and Mecheri (Prema *et al.,* 2008; Selvam *et al.,* 2009) and Coimbatore breeds (Kumarasamy *et al.,* 2009). The departure from HWE in majority of the loci could be attributed to shrinkage in population size and selection

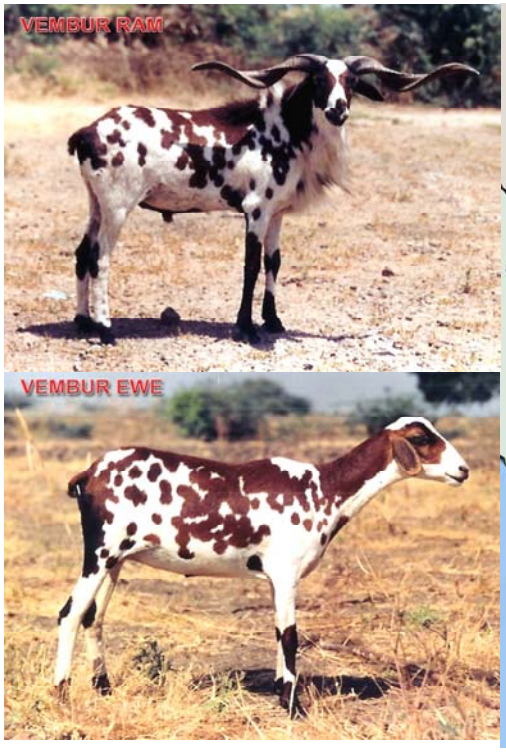

Fig. 2. Photographs of Vembur sheep

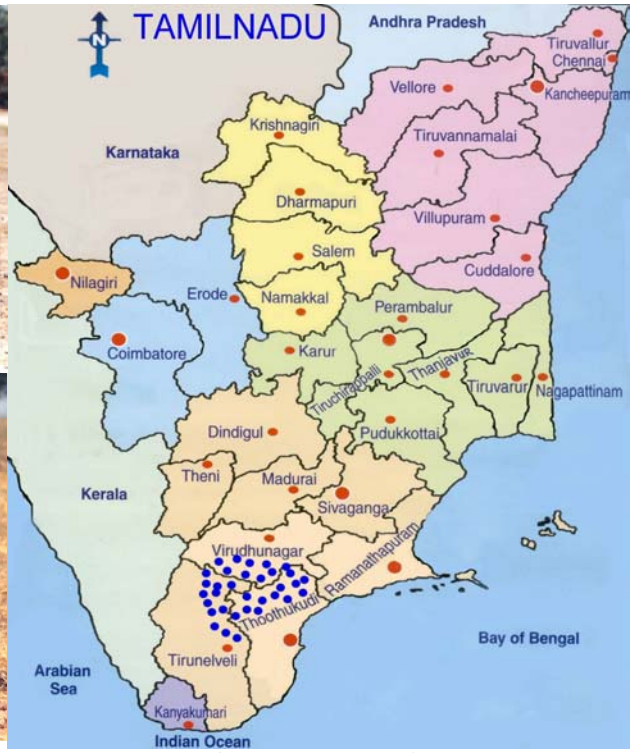

Fig. 1. Breeding tract of Vembur sheep

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Table 1. Number of observed and effective alleles, size of alleles, polymorphism information content, observed and expected heterozygosity at 25 microsatellite loci in Vembur sheep

locus	Obser-ved allele s	Effective Number of alleles	Allele size (bp)	PIC	Hardy-Weinberg equilibrium (χ^2 value)	Heterozygosity		Within population inbreeding estimate (F_{IS})
						Observed	Expected	
BM1314	8	6.0157	160-186	0.8129	21.85^{NS}	0.7500	0.8338	0.1005
BM6506	6	4.5453	180-200	0.7474	93.32**	0.3617	0.7800	0.5363
BM6526	6	4.3513	154-172	0.7382	44.86**	0.5625	0.7702	0.2697
BM757	7	5.0141	172-196	0.7717	205.20**	0.3750	0.8006	0.5316
BM8125	6	2.6982	110-124	0.5732	186.38**	0.1333	0.6294	0.7882
BM827	4	2.5538	208-230	0.5284	63.87**	0.8936	0.6084	-0.4687
CSSM31	5	4.1581	148-158	0.7202	45.96**	0.4000	0.7595	0.4733
CSSM47	6	2.6282	132-162	0.5480	36.97**	0.2766	0.6195	0.5535
HUJ616	9	6.8368	112-128	0.8360	80.89**	0.7609	0.8537	0.1088
OarAE129	8	4.3855	132-158	0.7325	76.63**	0.4565	0.7720	0.4086
OarCP20	5	3.4522	74-84	0.6662	46.48**	1.0000	0.7103	-0.4078
OarCP34	5	4.2237	112-128	0.7253	21.70^{NS}	0.6596	0.7632	0.1358
OarFCB128	5	4.2747	112-120	0.7266	83.04**	0.2609	0.7661	0.6595
OarFCB20	6	4.3229	92-116	0.7317	24.45^{NS}	0.5745	0.7687	0.2527
OarFCB48	5	4.1624	144-160	0.7196	47.21**	0.4667	0.7598	0.3858
OarHH35	6	4.4022	118-138	0.7394	142.33**	0.2000	0.7728	0.7142
OarHH41	8	4.8340	120-150	0.7628	269.10**	0.6364	0.7931	0.1977
OarHH47	2	1.9406	62-64	0.3712	7.95*	0.2750	0.4847	0.4326
OarHH64	5	3.5237	118-136	0.6780	35.28**	0.6087	0.7162	0.1501
OarJMP29	7	5.1601	124-148	0.7776	46.62*	0.8750	0.8062	-0.0853
OarJMP8	5	3.3508	110-134	0.6496	56.68**	0.3043	0.7016	0.5662
OarVH72	5	3.9293	124-134	0.6817	41.41**	0.6316	0.7455	0.1528
RM004	6	3.8535	138-148	0.7013	62.75**	0.4000	0.7405	0.4598
TGLA137	6	5.3928	138-156	0.7872	92.69**	0.6000	0.8146	0.2634
TGLA377	6	2.3655	76-100	0.5347	37.58*	0.5417	0.5773	0.2060
Mean	5.88	4.0950	62 - 230	0.6905	--	0.5202	0.7339	0.2954
	± 0.29	± 0.23		± 0.0212		± 0.227	± 0.087	± 0.309

process, which had been progressing in the population. Departure from HWE was also reported in Jalauni breed (Arora *et al*., 2008), Pag Island sheep breed (Ivankovic *et al*., 2005), Turkish sheep breeds (Soysal *et al*., 2005), West African Djallonke breed (Wafula *et al*., 2005), sheep breeds of Northern Spain (Alvarez *et al*., 2004), loci Baltic sheep breeds (Grigaliunaite *et al*., 2003), Sasi sheep (Rendo *et al*., 2003), and Uruguayan Corriedale (Tomasco *et al*., 2002).

Estimation of observed and expected heterozygosity

The observed heterozygosity (Ho) values varied between 0.1333 (BM1314) to 1.000 (OarCP20), while the expected values (He) ranged from 0.4847 (OarHH47) to 0.8537 (HUJ616). The mean values were 0.5202 ± 0.04 and 0.7339 ± 0.02 for Ho and He respectively (Table 1). When viewed in relation with figures for Tamilnadu breeds, the observed heterozygosity of 0.52 in Vembur sheep was lower than that for Nilagiri (Girish *et al*., 2007), Madras Red and Mecheri (Prema *et al.,* 2008; Selvam *et al.,* 2009). The expected heterozygosity value of 0.7339 for Vembur was comparable with that of Nilagiri but lower than both Madras Red and Mecheri. The deficiency of heterozygotes in the population may be due to selective breeding over the generations.

Within-population inbreeding estimate (F_{IS})

FIS values were negative for three loci (BM827, OarCP20 and OarJMP29) the presence of more heterozygotes at the loci. The remaining loci revealed FIS values ranging from 0.1005 in BM1314 to 0.7882 in BM8125, indicating presence of inbreeding of varying degrees. The mean F_{IS} value observed was 0.2954 ± 0.31 (Table 1) indicating the homozygous/inbreeding status of the population. F_{IS} estimates reported in most of the literature indicated various levels of inbreeding as 0.19 in Sarda sheep (Pariset *et al*., 2003), 0.066 in Spanish breeds (Alvarez et al., 2004), and 0.033 in Turkish sheep breeds (Soysal *et al*., 2005). Among the Indian sheep breeds, Arora and Bhatia (2004) reported a mean FIS value of 0.058 in Muzzafarnagri indicating a very low rate of inbreeding in that population. However, a high rate of inbreeding was reported in Nali and Chokla (0.397 and 0.299 respectively; Sodhi *et al*., 2005) and Magra (0.159; Arora & Bhatia, 2006) sheep breeds.

Conclusion

The genetic analysis of Vembur sheep returned several clues on the genetic status of its present population. The breed had allelic diversity, which indicates genetic variation. The mean PIC value of 0.69 justified the selection of markers for the study. The departure from HWE in majority of the loci can be attributed to shrinkage in population size and selection process which had been progressing in the population. *Ex*

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situ and *In situ* conservation of the breed are the best options suggested and the need of the hour for conservation of this breed.

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