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Genetic Diversity in Katchaikatty Sheep based on Microsatellites Polymorphism

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ABSTRACT

Background: Sheep is one of the important species of livestock in India contributing to meat production. India has 44 distinct breeds of sheep, out of which 10 are found in Tamil Nadu. The present study describes the characterization of recently registered breed-Katchaikatty sheep, genetic variability and genetic structure of the population by microsatellite marker analysis.

Methods: The genomic DNA from 50 unrelated Katchaikatty sheep was amplified with 25 FAO recommended fluorescent tagged ovine-specific microsatellite markers and genotyped on an automated Genetic Analyzer. Microsatellite fragment sizing was performed by Gene Mapper software. In each locus, observed (n_o) and effective number of microsatellite alleles (n_e), allele frequencies, observed (H_o) and expected (H_e) heterozygosity, heterozygosity deficit estimate (F_{IS}) and Chi-square (X^2) test for Hardy-Weinberg equilibrium (HWE) were assessed by POPGENE software and polymorphism information content (PIC) was calculated. Mode-shift analyses were performed for testing the genetic bottleneck by bottleneck software.

Result: The study revealed a total of 144 alleles across all the loci. The number of alleles at each locus varied from one to 10 with a mean of 5.76 alleles. The PIC values in the microsatellite loci ranged from 0.34 (OarAE129) to 0.83 (OarFCB48) with an average of 0.59, which showed a high genetic polymorphism. Six out of 25 microsatellite loci studied were in Hardy-Weinberg equilibrium and 19 out of 25 loci showed positive F_{IS} values. The overall mean observed and expected heterozygosity were 0.50 and 0.65 respectively and the estimates elucidated a substantial genetic variability in Katchaikatty sheep population. Mode shift analysis revealed a normal L-shaped curve, describing that the Katchaikatty population had not experienced any genetic bottleneck and remained in mutation-drift equilibrium.

Key words: Genetic bottleneck, Katchaikatty sheep, Microsatellites, Polymorphism.

INTRODUCTION

Livestock production constitutes a very important component of the agricultural economy of developing countries, with multipurpose uses, such as skins, fibre and fertilizer. Small ruminants play an important role in Indian economy and provide livelihood to two-third of rural community, especially in areas where crop and dairy farming are not economical. Furthermore, livestock are closely linked to the social and cultural lives of several million resource-poor farmers for whom animal ownership ensures varying degrees of sustainable farming and economic stability. Sheep is one of the important species of livestock in India contributing to meat production at almost nine per cent. India has a rich diversity of sheep genetic resources, with about 74.26 million sheep (Report, 2019) and 44 distinct breeds distributed in the different agro-climatic regions of the country.

Tamil Nadu ranks fifth among the Indian states in sheep population (Report, 2019) and has a rich repository of sheep genetic resources. Earlier the state had been reported to have eight descript sheep breeds (Ganesakale and Rathnasabapathy, 1973; Acharya, 1982) and a few non-listed sheep breeds namely Ramnad type (Pattanam sheep), Katchaikatty and Chevadu. Now, Katchaikatty and Chevadu have been recognized and registered.

The Katchaikatty sheep are distributed in the villages of Vadipatti block of Madurai district in Southern Tamil Nadu and are reared by *Konar* and *Pallar* communities. However, a few Katchaikatty flocks are also found in Melaneelithanallur block of Tirunelveli district (Ravimurugan *et al*., 2012). Like most of the sheep breeds of this region, Katchaikatty sheep also belong to the meat type as their wool is extremely coarse and hairy. The animals are adapted to their breeding tract and the flocks are stationary with the average size of 43. Katchaikatty sheep is black in color, moderate in size with long tail. The head is medium in length and breadth, ears are very short and stumpy. Rams have twisted horns

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and approximately five per cent of the ewes have thin small horns while the rest are polled. The neck is well set to thorax, thick and broad in males and slender in females. The legs are medium-sized, straight and squarely set under the body. Animals have typical meaty conformation and the mean body weight reported was 34.42±1.97 kg in rams and 28.14±0.7 kg in ewes (Report, 2006; Ravimurugan *et al*., 2012). The population of Katchaikatty sheep reported was 1506 (Report, 2022). Ram fighting (baiting) is an important event in and around the villages of Madurai district for which these rams are exclusively maintained and for this cultural importance, this breed fetches more market price than other sheep breeds of Tamil Nadu.

Molecular characterisation plays a major role in uncovering the history, estimating the level of diversity, distinctiveness and genetic structure of farm animal breeds as well as populations. It can serve as an aid in the genetic management of small populations for avoiding excessive inbreeding. The development of tools for the analyses of deoxyribonucleic acid (DNA) that had taken place in the last few decades increased enormously the capacity to characterise variation within and between breeds or populations. The restricted traditional characterisation by means of phenotypic attributes can now be complemented by an increasingly available number of molecular markers and the development of sophisticated statistical techniques for the analyses. The characterizations by molecular markers potentially indicate the genetic variability both within and between populations and also contribute for establishing conservation priorities. Molecular genetic markers are the inherited allelic variations at a locus that can be used to understand the genetic events. The molecular markers used for such objectives are generally microsatellites (simple tandem repeats, STR), amplified fragment length polymorphism (AFLP), variable number of tandem repeats (VNTR), random amplified polymorphic DNA (RAPD), single strand conformation polymorphisms (SNP) and restriction fragment length polymorphisms (RFLP). Amongst the different molecular markers, microsatellites, having very short sequence motifs (1-6 base pair in length), are the markers of choice as they are highly polymorphic, densely distributed in the genome, co-dominant and are inherited in Mendelian fashion as well as ease of genotyping, which make them valuable for molecular characterization (Queller *et al*., 1993; Fan *et al*., 2008). By characterization using specific microsatellite markers, the degree and pattern of genetic differences within and between populations or breeds in each livestock species could be determined, which would help in the genetic improvement and conservation programmes (Forbes *et al*., 1995; Nahas *et al*., 2008; Bozzi *et al*., 2009). Considering the population size, adaptation and the need for utility in the area in which it is distributed, this study describes the molecular characterization of Katchaikatty sheep, genetic variability and genetic structure of the population by microsatellite marker analysis.

MATERIALS AND METHODS

Blood samples (10 ml) from 50 unrelated rams and ewes of Katchaikatty sheep across the breeding tract were collected from the jugular vein of animal using vacutainers containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant. The genomic DNA was isolated from the blood samples by Phenol-Chloroform method (Sambrook *et al*., 1989) and the DNA samples were diluted to reach a final concentration of 25-50 ng/µl. Then the genomic DNA was amplified by polymerase chain reaction (PCR) with 25 fluorescently tagged ovine-specific microsatellite markers (Table 1), which adhere to the guidelines of the International Society for Animal Genetics (ISAG), Food and Agriculture Organisation (FAO, 2004; FAO, 2011). Only 5' end of forward primers of each primer was labeled with FAM, HEX, ROX and TAMRA fluorescent compounds to enable multiplexing and analysis on automated sequencer. The PCR amplifications were carried out in 15 µl reactions containing 3 µl of template DNA (20-50 ng/µl), 1.5 µl of PCR assay buffer (10X), 1.2 µl of $MgCl_2$ (2 mM), 1 µl of dNTPs (each at 200 µM), 0.5 µl of (10 picomoles) each of forward and reverse primers and 0.2 µl of Taq DNA Polymerase in Eppendorf thermocycler. The PCR reaction cycle was accomplished by initial denaturation at 95°C for 5 min, then denaturation at 95°C for 45 sec, primer annealing temperature (54-62°C) for 45 sec and an extension for 45 sec at 72°C, repeating the cycle for 30-40 times (varies according to the locus) and a final extension for 10 minutes at 72°C. Genotyping was carried out on an automated ABI PISM 3730XL Genetic Analyzer (Applied Biosystems, USA). Microsatellite fragment sizing was performed by the GeneMapper™ software version 4.0 which provided the size of the allele, genotype (based on peak height) and observed number of alleles (n_o). In each locus, microsatellite allele frequencies, effective number of alleles (n_e), observed (H_o) and expected (H_e) heterozygosity and heterozygosity deficit estimate (F_{IS}) and Chi-square (X^2) test for Hardy-Weinberg equilibrium (HWE) were assessed using the POPGENE software version 1.31 (Yeh *et al*., 1999). The polymorphism information content (PIC) was calculated based on the frequencies in which the alleles occurred at each locus (Nei, 1978). Mode-shift analyses were performed by using the bottleneck software version 1.2.02 (Cornuet and Luikart, 1996). Three mode shift tests *viz*. Sign-rank test, Standardised differences test and Wilcoxon test were utilised in each of the three models of mutation, Infinite Allele Model (IAM), Two Phase Model (TPM) and Stepwise Mutation Model (SMM) with the null hypothesis of mutation-drift equilibrium.

RESULTS AND DISCUSSION

Many studies conducted in recent years on the genetic variability and diversity of the native sheep breeds utilize the microsatellites endorsed in 2011 for use in the genetic studies of sheep diversity analysis and suggested by the

Table 1: Microsatellite markers [primer sequence (5′ to 3′), type of repeat, labeling dye and allele size] studied in Katchaikatty sheep.

Locus	Primer sequence	Type of repeat	Fluorescent dye	Allele size (bp)
BM757	P1: TGGAAACAATGTAAACCTGGG	$(GT)_n$	FAM	177-197
	P2: TTGAGCCACCAAGGAACC			
BM8125	P1: CTCTATCTGTGGAAAAGGTGGG	$(CA)_n$	FAM	108-120
	P2: GGGGGTTAGACTTCAACATACG			
BM1314	P1: TTCCTCCTCTTCTCTCCAAAC	$(AC)_n$	HEX	141-159
	P2: ATCTCAAACGCCAGTGTGG			
BM6526	P1: CATGCCAAACAATATCCAGC	$(CA)_n$	TET-CE	153-169
	P2: TGAAGGTAGAGAGCAAGCAGC			
OarFC1B128	P1: CAGCTGAGCAACTAAGACATACATGCG	$(GT)_n$	TAMRA	109-119
	P2: ATTAAAGCATCTTCTCTTTATTTCCTCGC			
BM827	P1: GGGCTGGTCGTATGCTGAG	$(CA)_n$	HEX	216-224
	P2: GTTGGACTTGCTGAAGTGACC			
OarCP34	P1: GCTGAAGAATGTGATATGTTCAGG	$(GT)_n$	FAM	113-121
	P2: GGGACAATACTGTCTTAGATGCTGC			
CSSM47	P1: TCTCTGTCTCTATCACTATATGGC	$(GT)_n$	TET-CE	128-132
	P2: CTGGGCACCTGAAACTATCATCAT			
OarFCB48	P1: GAGTTAGTACAAGGATGACAAGAGGCAC	$(GT)_n$	TAMRA	142-162
	P2: GACTCTAGAGGATCGCAAAGAACCAG			
OarHH41	P1: TCCACAGGCTTAAATCTATATAGCAACC	$(CA)_n$	HEX	121-135
	P2: CCAGCTAAAGATAAAAGATGATGTGGGAG			
OarVH72	P1: CTCTAGAGGATCTGGAATGCAAAGCTC	$(GT)_n$	FAM	125-131
	P2: GGCCTCTCAAGGGGCAAGAGCAGG			
BM6506	P1: GCACGTGGTAAAGAGATGGC	$(CA)_n$	FAM	195-209
	P2: AGCAACTTGAGCATGGCAC			
OarHH47	P1: TTTATTGACAAACTCTCTTCCTAACTCCACC	$(CA)_n$	TET-CE	128-142
	P2: GTAGTTATTTAAAAAAATATCATACCTCTTAAGG			
OarHH64	P1: CGTTCCCTCACTATGGAAAGTTATATATGC	$(GT)_n$	TAMRA	133
	P2: CACTCTATTGTAAGAATTTGAATGAGAGC			
OarAF129	P1: AATCCAGTGTGTGAAAGACTAATCCAG	$(CA)_n$	HEX	147-163
	P2: GTAGATCAAGATATAGAATATTTTTCAACACC			
OarJMP8	P1: GGGATGATCTTCTGTCCAAATATGC	$(GT)_n$	TET-CE	120-130
	P2: ATTTGCTTTGGCTTCAGAACCAGAG			
OarCP20	P1: GATCCCCTGGAGGAGGAAACGG	$(GT)_n$	TAMRA	71-83
	P2: GGCATTTCATGGCTTTAGCAGG			
OarJMP29	P1: GTATACACGTGGACACCGCTTTGTAC	$(CA)_n$	FAM	123-137
	P2: GAAGTGGCAAGATTCAGAGGGGAAG			
INRA63	P1: GACCACAAAGGGATTTGCACAAGC	$(AC)_n$	FAM	170-192
	P2: AAACCACAGAAATGCTTGGAAG			
CSRD247	P1: GGACTTGCCAGAACTCTGCAAT	$(AC)_n$	HEX	212-232
	P2: CACTGTGGTTTGTATTAGTCAGG			
CSSM31	P1: CCAAGTTTAGTTACTTGTAAGTAGA	$(AC)_n$	FAM	129-167
	P2: GACTCTCTAGCACTTTATCTGTGT			
HSC	P1: CTGCCAATGCAGAGACACAAGA	$(GT)_n$	FAM	267-285
	P2: GTCTGTCTCCTGTCTTGTCATC			
MAF214	Pl: AATGCAGGAGATCTGAGGCAGGGACG	$(GT)_n$	TAMRA	192-228
	P2: GGGTGATCTTAGGGAGGTTTTGGAGG			
OarCP49	P1: CAGACACGGCTTAGCAACTAAACG	$(AC)_n$	HEX	82-102
	P2: GTGGGGATGAATATTCCTTCATAAGG			
OarHH35	P1: AATTGCATTCAGTATCTTTAACATCTGGC	$(CA)_n$	HEX	117-135
	P2: ATGAAAATATAAAGAGAATGAACCACACGG			

ISAG, FAO. The allele size observed and type of repeat for the recommended microsatellite markers studied in Katchaikatty sheep are presented in Table 1.

In Katchaikatty sheep, a total of 144 microsatellite alleles were observed in the present study. The number of alleles (n_o) at each locus varied up to 10 (CSSM31) with a mean of 5.76 alleles across all loci while one locus (OarHH64) was found to be monomorphic. The effective number of alleles (n_e) ranged from one (OarHH64) to 6.43 (OarFCB48) with a mean of 3.40 across all loci, which showed high genetic polymorphism (Table 2). Less number of microsatellite alleles were reported in earlier studies as 125 in Nilagiri (Girish *et al*., 2007), 126 in Muzzafarnagari (Arora and Bhatia, 2004) and 131 in Kheri (Bhatia and Arora, 2008) while more number of alleles were also recorded as 148 in Jalauni (Arora *et al*., 2008), 165 in Patanwadi, 160 in Dumba, 181 in Marwari (Jyotsna *et al*., 2010) and 196 in Kilakarsal (Radha *et al*., 2011). A comparable number of alleles was reported as 143 in Coimbatore (Kumarasamy *et al*., 2009) and 147 in Vembur (Pramod *et al*., 2009). The number of alleles reported in exotic sheep was considerably low as five to 20 in Swiss sheep breeds (Saitbekova *et al*., 2001), seven to 22 in Turkish sheep breeds (Guiterrez-Gil *et al*., 2006), 10 to 23 in European sheep breeds (Handley *et al*., 2007), 11 to 33 in Alpine sheep breeds (Dalvit *et al*., 2008), seven to 25 in Greek sheep breeds (Ligda *et al*., 2009) and eight to 21 in Italian sheep breeds (Bozzi *et al*., 2009).

Genetic diversity can be measured as the amount of actual or potential heterozygosity (H_o). Expected heterozygosity (H_e) is considered to be a better estimator of the genetic variability in a population. The observed heterozygosity ranged from 0.03 (CSSM47) to 0.91 (OarVH72) with a mean value of 0.50 (excluding the monomorphic locus), which could be due the selective outbreeding practice in Katchaikatty flock. The expected heterozygosity ranged from 0.40 (OarAE129) to 0.85 (OarFCB48) with a mean value of 0.65 (Table 2). Mean observed and expected heterozygosity observed in the Katchaikatty were comparable respectively with the values reported in Muzzafarnagari, 0.65 and 0.69 (Arora and Bhatia, 2004); Nilagiri sheep, 0.76 and 0.72 (Girish *et al*., 2007); Jalauni sheep, 0.58 and 0.69 (Arora *et al*., 2008); Coimbatore sheep, 0.74 and 0.81 (Kumarasamy *et al*., 2009); in Vembur

Table 2: Observed (n_o) and effective number (n_e) of alleles, observed (H_o) and expected (H_e) heterozygosity, within population inbreeding estimate (F_{IS}), test for hardy-weinberg equilibrium (HWE) and polymorphism information content (PIC) at microsatellite loci in Katchaikatty sheep.

Locus	Chromosome location	n_o	n_e	H_O	H_e	F_{IS}	HWE (X^2 value)	PIC
BM757	9	5	3.13	0.64	0.69	0.06	12.7953^{NS}	0.63
BM8125	17	7	3.31	0.44	0.71	0.36	47.4465**	0.65
BM1314	22	9	6.21	0.22	0.85	0.74	251.9468**	0.82
BM6526	26	7	4.59	0.71	0.79	0.09	34.8512*	0.75
OarFCB128	2	3	2.00	0.34	0.51	0.31	4.6442^{NS}	0.39
BM827	3	3	1.94	0.26	0.40	0.46	11.8663**	0.38
OarCP34	5	4	3.10	0.49	0.68	0.27	17.2062**	0.62
CSSM47	2	3	2.36	0.03	0.59	0.94	54.7664**	0.51
OarFCB48	17	9	6.43	0.66	0.85	0.22	59.3866**	0.83
OarHH41	10	5	1.98	0.19	0.49	0.60	124.6482**	0.44
OarVH72	25	4	2.92	0.91	0.67	-0.38	39.5422**	0.59
BM6506	1	4	2.86	0.78	0.66	-0.20	26.8284**	0.58
OarHH47	18	7	3.44	0.45	0.72	0.37	45.5275**	0.66
OarHH64	4	1	1.00	0.00	0.00	-	-	-
OarAE129	5	3	1.65	0.28	0.40	0.28	17.8099*	0.34
OarJMP8	6	6	3.87	0.52	0.75	0.29	121.5568**	0.69
OarCP20	21	5	1.69	0.33	0.41	0.18	35.3920**	0.38
OarJMP29	24	6	3.25	0.56	0.69	0.18	22.1101^{NS}	0.64
INRA63	14	6	3.40	0.61	0.71	0.13	29.9880*	0.67
CSRD247	14	7	4.23	0.77	0.77	-0.01	12.8177^{NS}	0.73
CSSM31	23	10	5.28	0.88	0.82	-0.93	38.7895^{NS}	0.78
HSC	20	8	4.93	0.75	0.80	0.06	29.8391^{NS}	0.77
MAF214	16	6	2.05	0.61	0.52	-0.20	99.5770**	0.47
OarCP49	17	8	6.19	0.75	0.85	0.11	46.8693*	0.82
OarHH35	4	8	3.26	0.36	0.70	0.47	101.7246**	0.67
Mean		5.76	3.40	0.50	0.65	0.21		0.59

**Significant (P<0.01); *Significant (P<0.05); ^{NS} Not significant (P≥0.05).

sheep, 0.52 and 0.73 (Pramod *et al*., 2009); in Kilakarsal sheep, 0.60 and 0.72 (Radha *et al*., 2011).

The polymorphism information content (PIC) was described (Botstein *et al*., 1980) as a statical assessment of the informativeness of a marker. It depends upon the number of alleles and their relative population frequencies. The PIC values observed in the present study ranged from 0.34 (OarAE129) to 0.83 (OarFCB48) with a mean of 0.59 for all the 25 loci. Based on the PIC values, it was found that all markers except six (OarFCB128, BM827, OarHH41, OarAE129, OarCP20 and MAF214) used in the study showed values of more than 0.5 (Table 2), indicating that these microsatellite markers can effectively be used for molecular characterisation and genetic variability studies in sheep. Similar PIC values were found as 0.60 in Chokla, 0.60 in Nali (Mukesh *et al*., 2006) and 0.60 in Kheri sheep (Arora and Bhatia, 2008) while higher values were also observed in Jalauni, 0.64 (Arora *et al.,* 2008); Chottanagpuri, 0.63 (Bhatia *et al*., 2008) and Kilakarsal sheep, 0.83 (Radha *et al*., 2011). Majority of the loci under investigation (18 out of 25) showed significant departure from Hardy-Weinberg Equilibrium (HWE) while the microsatellite loci namely BM757, OarFCB128, OarJMP29, CSRD247, HSC and CSSM31 were observed to be in HWE. The deviation from HWE is due to the effect of systematic and dispersive forces on the genetic constitution. In previous studies on Nilagiri sheep, 17 of 25 loci were reported to be in HWE (Girish *et al*., 2007) whereas 19 out of 27 loci in Coimbatore sheep (Kumarasamy *et al*., 2009), 19 out of 25 loci in Vembur sheep (Pramod *et al*., 2009) and 17 out of 23 loci in Kilakarsal sheep (Radha *et al*., 2011) were reported to have significant deviation from HWE.

The within population heterozygote deficit estimate (F_{IS}) measures heterozygotes deficiency within population. The higher the values of F_{IS} indicates closer relationship between the individuals. The F_{IS} computed in the present study ranged from -0.38 (OarVH72) to 0.94 (CSSM47) with a mean of 0.21 across all the loci (Table 2). The positive F_{IS} values were observed at 19 loci and varied from 0.0619 (BM757) to 0.9421 (CSSM47). Five loci revealed negative F_{IS} values (F_{IS}<0) indicating the absence of heterozygote deficit in these loci (Table 2). The mean F_{IS} estimates reported as 0.12 in Jalauni sheep (Arora *et al*., 2008); 0.07 in Coimbatore sheep (Kumarasamy *et al*., 2009) and 0.16 in Kilakarsal sheep (Radha *et al*., 2011) were less than that observed in the present study, which might be due to the closed breeding in the flocks of Katchaikatty sheep.

Identifying populations that have experienced a severe reduction in size (*i.e*., bottleneck) is important because bottlenecks can increase the rate of inbreeding, loss of genetic variation, fixation of deleterious alleles and increase the probability of population extinction. It is especially important to identify recently bottlenecked population (within few dozen generations), because such populations may not have had time to adapt to the problems caused by the small population size and might have a high risk of extinction. Recently bottlenecked populations are likely to have lost rare alleles, but still contain substantial heterozygosity and genetic variation which are lost slowly (Luikart *et al*., 1998). It is often very difficult to identify recently bottlenecked populations because historical population sizes and level of genetic variation are seldom known. Allele frequency distribution (Mode shift indicator) discriminated the many bottlenecked populations from stable populations. In the present study, allele sizes obtained from Katchaikatty sheep was subjected to bottleneck analysis using the program, bottleneck applying three tests *viz*. Sign-rank test, Standardised differences test and Wilcoxon test in each of the three models of mutation, IAM, TPM and SMM. In a population at mutation shift equilibrium (*i.e*., the effective size of which has remained constant in the past), there is approximately an equal probability that a locus shows a heterozygote excess or deficit. The results are summraised in Table 3.

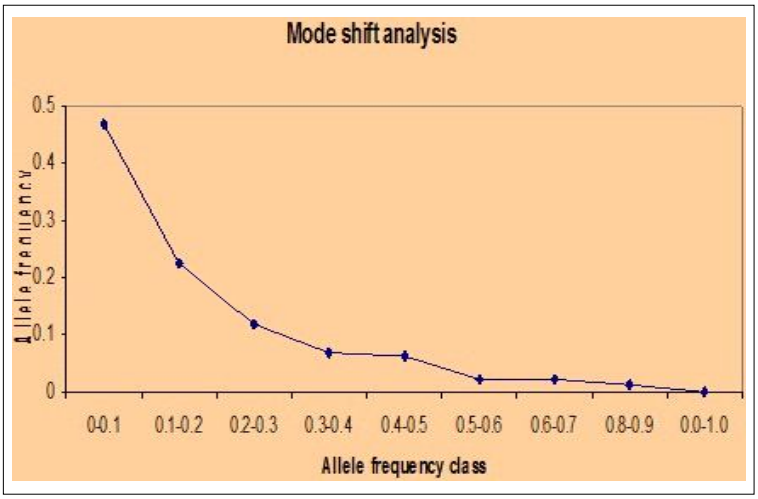

Fig 1: Mode shift analysis for genetic bottleneck in Katchaikatty sheep.

Table 3: Population bottleneck analysis of Katchaikatty sheep.

Model		IAM	TPM	SMM
Sign rank test	Expected	13.81	14.14	14.24
Number of loci with heterozygosity excess	Observed	21	19	13
Standardized differences test	T2 values	4.101	2.049	-1.684
Wilcoxon test	Probability of heterozygosity excess	0.00000	0.02113	0.60331

No mode-shift was detected in the frequency distribution of alleles and a normal L-shaped form was observed (Fig 1), which suggested that the Katchaikatty population had not experienced a genetic bottleneck, *i.e.*, it has not undergone any recent reduction in the effective population size and remained mutation-drift equilibrium. Similarly, the sheep populations of Muzzafarnagri (Arora and Bhatia, 2004), Bellary (Kumar *et al*., 2007), Jalauni (Arora *et al*., 2008), Vembur (Pramod *et al*., 2009), Coimbatore (Kumarasamy *et al*., 2010) and Kilakarsal (Radha *et al*., 2011) breeds were also not reported for bottleneck. Nilagiri sheep population was the only breed of Tamil Nadu which experienced the genetic bottleneck (Girish *et al*., 2009).

CONCLUSION

The molecular characterization estimates of the present study supported the usefulness of FAO-recommended ovine-specific microsatellite markers to assess the genetic variability of Katchaikatty sheep and to characterise the genetic structure of the population. The results also revealed that the Katchaikatty sheep population did not exhibit a decrease in genetic variation or effective population size. The information generated could further contribute to the designing of genetic management, in such that the genetic variation in the population should not be reduced and formulation of conservation programmes for the Katchikatty sheep.

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Conflict of interest

On behalf of all authors, I certify that there is no conflict of interest with respect to the manuscript of the research article submitted for publication.

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