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GENETIC DIVERSITY AND BOTTLENECK ANALYSIS OF SHEEP BASED ON MICROSATELLITE MARKERS

R. Selvam* and P. Kathiravan[1]

Department of Animal Genetics and Breeding

Veterinary College and Research Institute, Tirunelveli- 627 358, Tamil Nadu

[1]Animal Production and Health Laboratory

Joint FAO/IAEA Division, International Atomic Energy Agency (IAEA) Laboratories

A-2444 Seibersdorf, Austria

**E-mail address: selvam.r@tanuvas.ac.in*

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ABSTRACT

The genetic diversity of Madras Red sheep at Post Graduate Research Institute in Animal Sciences, Kattupakkam (Tamil Nadu) and Mecheri sheep at Mecheri Sheep Research Station, Pottaneri (Tamil Nadu) was analysed using FAO recommended microsatellite markers. Genetic variation at 10 microsatellite loci, population structure and genetic bottleneck analysis were done to provide genetic information. A total of 98 and 86 alleles were scored for Madras Red and Mecheri sheep, respectively. The polymorphism information content ranged from 0.609 to 0.902 for all the microsatellite loci. The overall observed and estimated heterozygosity for all loci combined were 0.972 and 0.785 for Madras Red and 0.982 and 0.774 for Mecheri sheep, respectively. The average and total heterozygosity of subpopulation was 0.779 and 0.821, respectively. The coefficient of gene differentiation was 0.049 between the breeds which indicates that the genetic diversity is low between the Madras Red and Mecheri breeds of sheep with respect to microsatellite loci studied. The study revealed no genetic bottleneck in recent past and this was confirmed by mode shift test which gave L-shaped distribution for proportion of alleles, indicating that the population is stable with respect to population size. The panel of microsatellites used was highly informative for molecular characterization and could be used for exploitation of genetic diversity of the related breeds for conservation.

Key words: Bottleneck analysis, Genetic diversity, Microsatellite, Polymorphism information content

The sheep breeds of Tamil Nadu (India) are well-known for their heat tolerance, mutton production and adaptability to the local agro-ecological conditions (Ganesakale and Rathnasabapathy, 1973). Earlier survey in the native tract of these breeds revealed that the population of indigenous breeds of sheep has declined considerably due to indiscriminate breeding with non-descript and exotic breeds (Acharya, 1999). At present, there is genetic dilution of these breeds and a few of them are threatened with extinction. Genetic characterisation of native breeds is the first step to safeguard the valuable germplasm and it requires basic knowledge of genetic variation that can be effectively measured within and between populations (Tantia and Vij, 2000). Among the various molecular markers, microsatellites are the markers of choice for characterising breeds of domestic animals as they are abundantly distributed throughout the mammalian genome and are easy to genotype. They have a large number of alleles, a high level of heterozygosity and are inherited in a Mendelian fashion. These characteristics make them valuable for genome mapping, linkage analysis and phylogenetic studies (Koreth et al., 1996).

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Effective number of breedable population is very important for maintaining genetic variability within a population. Bottleneck occurs when populations undergo severe reductions in effective population size (Spencer et al., 2000). Drastic decline of a population can affect within breed genetic variability especially allelic diversity, as reduction in effective population size and the consequent genetic drift could lead to loss of many rare alleles (Bradshaw et al., 2007). Hence, the occurrence of bottleneck needs to be monitored genetically. Such a genetic monitoring of population bottleneck in domestic livestock has become more feasible with the use of microsatellites markers (Arora and Bhatia 2009; Kathiravan et al., 2009). Successful demonstrations of genetic basis of demographic bottleneck events using microsatellite markers have been reported in various species like cattle (Kathiravan et al., 2009; Ganapathy et al., 2012; Thiagarajan, 2012), sheep (Girish et al., 2007; Radha et al., 2011) and goat (Thiruvenkadan et al., 2014). The present study was undertaken to characterise Madras Red and Mecheri sheep breeds of Tamil Nadu based on microsatellite polymorphism, to find out the genetic difference between these sheep breeds and to evaluate mutation drift equilibrium by microsatellite genotyping and consequently the occurrence of genetic bottleneck. This study will be of immense use in identifying genetic uniqueness of the breed, tracing the evolutionary origin and formulating effective conservation strategies for genetic diversity within breeds.

MATERIALS AND METHODS

The study was carried out in Madras Red sheep maintained at Post Graduate Research Institute in Animal Sciences, Kattupakkam (Tamil Nadu) and Mecheri sheep maintained at Mecheri Sheep Research Station, Pottaneri (Tamil Nadu). Madras Red sheep is a medium-sized, hairy and meat type breed mainly distributed in northern districts of Tamil Nadu. Mecheri sheep is medium-sized with a compact body and covered with short hairs and distributed in north-western zone of Tamil Nadu (Karunanithi et al., 2005). Blood samples were randomly collected from 50 animals in each breed belonging to both the sexes and unrelated by ancestry. Genomic DNA was isolated by a rapid non-enzymatic method (Lahiri and Nurnberger, 1991). The purity and concentration of DNA samples were estimated by spectrophotometer. The concentrations of DNA samples were also checked by agarose gel electrophoresis. A total of 10 microsatellite primer sets specific for sheep were used as recommended by Food and Agriculture Organisation. The amplified polymderase chain reaction (PCR) products were checked on 1.0% agarose gel by UV illumination (Bio-Rad, USA). The samples showing amplification were used for polyacrylamide gel electrophoresis. The gel was stained by silver staining as described by Comincini et al. (1995) with a few modifications. The silver-stained gels were analysed by Diversity Database software (Bio-Rad, USA) for sizing of various allelic products in both the breeds. The genotyping of animals was done based on the size of PCR products resolved through polyacrylamide gels and their sizes were assessed on the basis of 10 bp molecular DNA marker (Invitrogen, USA). The genotypes of animals were scored based on the presence of a single band (homozygotes) or double bands (heterozygotes) in the gel.

The occurrence of single or double bands was estimated by direct counting and allele frequencies were calculated. The polymorphism information content (PIC) was calculated using the individual frequencies in which the allele occurred at each loci (Nei, 1973). The observed heterozygosity was calculated as the actual percentage of heterozygotes occurring in the sample population. The expected heterozygosity of each microsatellite locus was measured as per Nei (1973). The chi-square test was carried out from observed and the expected numbers to check whether the population was in Hardy-Weinberg equilibrium (HWE) or not. Genetic diversity within breeds was measured as the estimate of heterozygosity while diversity among breeds was measured as coefficient of gene differentiation. Three different tests, viz., Sign rank, Standardized differences and Wilcoxon tests using 3 models of microsatellite evolution (infinite allele model- IAM, two phase model- TPM and stepwise mutation model- SMM)) were utilised to investigate whether the Madras Red and Mecheri sheep populations have undergone recent bottleneck or were in mutation drift equilibrium (Piry et al., 1999).

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RESULTS AND DISCUSSION

The numbers and sizes of the alleles for the 10 microsatellite loci in Madras Red and Mecheri sheep are presented in Table 1. The mean number of alleles reflected high level of genetic variability in the investigated populations. All the markers exhibit more alleles which indicate that the microsatellite loci screened in this study is appropriate for analysing the genetic variation in the population. At Oar JMP 8 locus, the total number of alleles in both the breeds was 11, while Madras Red and Mecheri had 8 and 9 alleles respectively. The allele sizes for total population ranged from 119 to 139 bp, while it was 125 to 139 bp for Madras Red and 119 to 139 bp for Mecheri. The number of alleles identified at Oar AE 129, Oar CP 34, Oar FCB 128 and Oar JMP 29 loci by Saitbekova et al. (2001) were 7, 8, 8 and 20 in Swiss breeds of sheep, respectively. Using the same set of microsatellite primers, the number of alleles identified in the present study was found to be almost similar in Madras Red sheep. In Mecheri, the number at Oar CP 34 locus was more (13). Similar reports were also made by Diez-Tascon et al. (2000) for Oar CP 34 and Oar FCB 128 and Arranz et al. (2001) for Oar CP 34.

In general, PIC values are suggestive of polymorphic nature of the microsatellite loci analysed that reveals its usefulness in diversity studies of a breed. PIC depends upon the number of alleles and their relative population frequencies. In the present study, all the 10 microsatellite loci showed relatively higher PIC values indicating the rich polymorphic nature of these loci in both the breeds (Table 1). Based on these PIC values, the microsatellite markers can be well utilized for molecular characterization of sheep breeds. It was observed that the populations of both the breeds were not in Hardy-Weinberg equilibrium proportions (P<0.01) for all the microsatellite loci except three (BM 8125, Oar JMP 8 and TGLA 377). At BM 8125 and TGLA 377 loci, the Mecheri sheep population alone was found to be in equilibrium. At Oar JMP 8 locus, both Madras Red and Mecheri populations were in equilibrium. A population is said to be within HWE only when it is able to maintain its relative allele frequencies. The disequilibrium proportions observed in most of the loci in this study might be due to both the systematic and dispersive processes operating in the population. This deviation from HWE may also be attributable to non-amplifying alleles that may be present in the samples analysed. Similar PIC values and departure from HWE was also reported in Nilagiri (Girish et al., 2007), Coimbatore (Kumarasamy et al., 2009), Vembur (Pramod et al., 2009) and Kilakarsal (Radha et al., 2011) sheep breeds of Tamil Nadu.

Table 1. Number of alleles,allele size and polymorphism information content (PIC) and Hardy-Weinberg equilibrium (HWE) in Madras Red and Mecheri sheep

Locus	Breed	Alleles		PIC	HWE	
		Number	Size (bp)		Chi-square value	d.f.
BM 8125	Madras Red	7	96 - 122	0.789	109.524**	21
	Mecheri	6	114 - 124	0.705	19.054^{NS}	15
CSSM 31	Madras Red	19	122 - 164	0.902	373.165**	171
	Mecheri	15	122 - 164	0.867	249.269**	105
Oar AE 129	Madras Red	9	144 - 168	0.777	189.511**	36
	Mecheri	6	144 - 154	0.848	51.131**	15
Oar CP 34	Madras Red	8	115 - 125	0.807	63.360**	28
	Mecheri	13	111 - 147	0.871	251.343**	78
Oar FCB 128	Madras Red	9	106 – 122	0.737	116.791**	36
	Mecheri	6	108 - 122	0.762	95.570**	15
OAE HH 35	Madras Red	12	110 - 134	0.871	315.432**	66
	Mecheri	9	114 - 134	0.810	151.838**	36
Oar JMP 29	Madras Red	11	124 - 148	0.856	122.916**	55
	Mecheri	12	128 - 148	0.878	225.649**	66
Oar JMP 8	Madras Red	8	125 - 139	0.835	31.556^{NS}	28
	Mecheri	9	119 - 137	0.846	35.011^{NS}	36
RM 4	Madras Red	8	146 - 156	0.777	101.484**	28
	Mecheri	6	134 - 144	0.782	565.876**	15
TGLA 377	Madras Red	7	92 - 105	0.739	83.026**	21
	Mecheri	4	96 - 102	0.609	15.831^{NS}	6

*** - Significant (P<0.01); NS – Non- significant (P>0.05)*

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Genetic diversity can be measured as the amount of actual or potential heterozygosity and the diversity within the breed is measured as the estimate of heterozygosity. For all loci combined, an average heterozygosity of 0.779 was estimated. As presented in Table 2, the total heterozygosity obtained for these loci were varied from 0.642 (TGLA 377) to 0.876 (Oar JMP 29 and RM 4). The average total heterozygosity of 0.821 was obtained in this study when all these loci were combined. The high heterozygosity values obtained in this study were in accordance with those reported in French Mutton Merino (Diez-Tascon et al., 2000), Swiss sheep breeds (Saitbekova et al., 2001), Garole (Sodhi et al., 2003), Nilagiri (Girish et al., 2007) and Muzzafarnagri (Arora and Bhatia, 2009). The estimated heterozygosity of sub-population values indicates the population dynamics for a particular microsatellite locus. The high mean heterozygosity values estimated in the present study could be attributed to low level of inbreeding, low selection pressure and large number of alleles. The genetic markers having a higher number of alleles per locus and a higher degree of heterozygosity are more useful for population and individual typing. The genetic diversity between breeds is estimated as the coefficient of gene differentiation from the heterozygosity values of sub-populations and total population. The overall genetic diversity value across all loci was estimated to be 0.049 (Table 2). This suggests that the genetic diversity is low between the Madras Red and Mecheri breeds with respect to microsatellite loci studied. It is discernible that these two breeds are genetically more related to each other because of the common evolutionary origin which is reflected from their geographic distributions.

Table 2. Observed (O) and estimated (E) heterozygosity and coefficient of gene differentiation in Madras Red and Mecheri sheep

Locus	Madras Red		Mecheri		Av. heterozygosity of sub-population (Hs)	Total heterozygosity (HT)	Population coefficient of gene differentiation
	O	E	O	E			
BM 8125	1	0.771	1	0.638	0.701	0.720	0.026
CSSM 31	0.978	0.883	1	0.855	0.872	0.874	0.002
Oar AE 129	0.952	0.735	0.977	0.826	0.781	0.840	0.070
Oar CP 34	1	0.805	0.939	0.867	0.833	0.859	0.030
Oar FCB 128	1	0.688	1	0.731	0.705	0.816	0.136
Oar HH 35	0.927	0.86	1	0.786	0.821	0.875	0.062
Oar JMP 29	1	0.856	0.978	0.878	0.867	0.876	0.010
Oar JMP 8	0.976	0.813	0.927	0.826	0.819	0.834	0.018
RM 4	0.923	0.75	1	0.754	0.752	0.876	0.122
TGLA 377	0.966	0.693	1	0.583	0.636	0.642	0.009
All loci	0.972	0.785	0.982	0.774	0.779	0.821	0.049

The results of bottlenecks analysis (Tables 3 and 4) revealed no genetic bottlenecks in these two sheep breeds populations in the recent past and this was confirmed by mode shift test which gave L-shaped distribution for proportion of alleles (Fig. 1). It is clear that the population has not undergone any reduction in the effective population size and remained at mutation drift equilibrium. The stepwise mutation model revealed absence of significant heterozygotes excess in Madras Red and Mecheri populations. Similarly the sheep populations of Kilakarsal (Radha et al., 2011), Vembur (Pramod et al., 2009) and Salem black goat (Thiruvenkadan et al., 2014) of Tamil Nadu were also reported to be non-bottlenecked.

Table 3. Estimated observed (H_e) and expected (H_{eq}) gene diversities in Madras Red and Mecheri sheep under different models of microsatellite evolution

Locus	Madras Red					Mecheri				
	n_o	H_e	H_{eq}			n_o	H_e	H_{eq}		
			IAM	TPM	SMM			IAM	TPM	SMM
BM8125	7	0.787	0.683	0.745	0.786	6	0.652	0.636	0.696	0.750
CSSM31	19	0.899	0.921	0.931	0.942	15	0.871	0.883	0.906	0.919
OarAE129	9	0.745	0.767	0.813	0.843	6	0.820	0.637	0.692	0.747
OarCP34	8	0.822	0.729	0.778	0.821	13	0.896	0.853	0.882	0.900
OarFCB128	9	0.702	0.761	0.812	0.841	6	0.747	0.631	0.701	0.751
OarHH35	12	0.886	0.838	0.870	0.891	9	0.802	0.766	0.813	0.843
OarJMP29	11	0.858	0.817	0.851	0.876	12	0.893	0.837	0.870	0.890
OarJMP8	8	0.830	0.729	0.781	0.820	9	0.844	0.767	0.809	0.842
RM4	8	0.763	0.728	0.782	0.818	6	0.770	0.638	0.696	0.747
TGLA377	7	0.707	0.686	0.742	0.787	4	0.596	0.486	0.550	0.609
Mean±S.E.	9.80 ±1.14	0.80 ±0.02	0.766 ±0.02	0.811± 0.02	0.843 ±0.02	8.60 ±1.16	0.789 ±0.03	0.713 ±0.04	0.762 ±0.04	0.80 ±0.03

n_o- Observed number of alleles; IAM- Infinite alleles model; TPM- Two phase model; SMM- Stepwise mutation model

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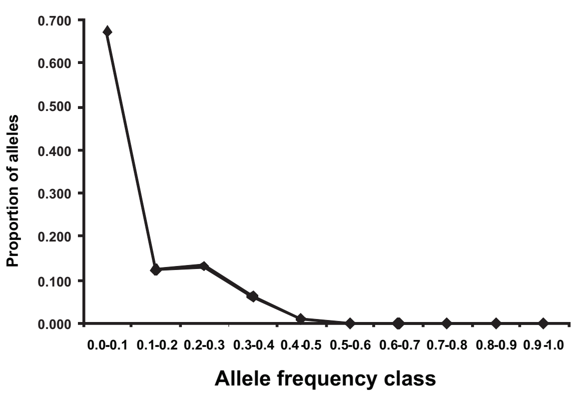
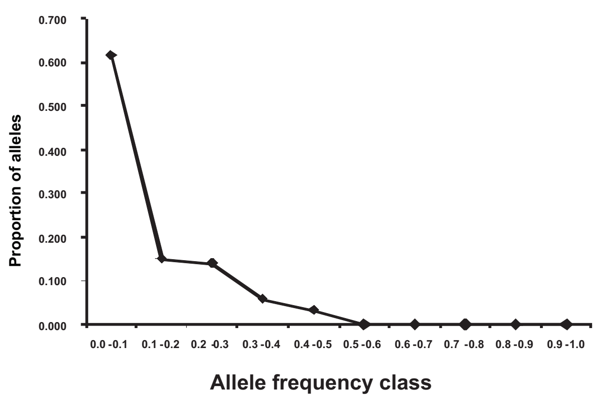
Table 4. Mutation drift equilibrium in Madras Red and Mecheri sheep

Test		Madras Red			Mecheri		
		IAM	TPM	SMM	IAM	TPM	SMM
Sign test	Expected no. of loci with H_e excess	5.99	5.99	5.88	6.07	6.11	5.92
	No. of loci with H_e excess	9	7	4	7	5	3
	No. of loci with H_e deficiency	1	3	6	3	5	7
	P value	0.046	0.381	0.186	0.399	0.340	0.061
Standardized differences test	T2 value	2.33	0.80	-1.51	1.00	-0.80	-4.36
	P value	0.010	0.213	0.065	0.160	0.212	0.000
Wilcoxon sign rank test	P value (one tail test for H_e excess)	0.001	0.116	0.839	0.138	0.688	0.993

H_e - Heterozygosity



Madras Red



Mecheri

Fig. 1. Qualitative tests for mode shift using allele frequency distribution indicate no evidence of genetic bottleneck in Madras Red and Mecheri sheep

Thus, low genetic difference between these two breeds suggested that Madras Red and Mecheri breeds are more closely related because of their probable common origin. No mode shift was detected in the frequency distribution of alleles and a normal L-shaped curve was observed, indicating that the population is non-bottlenecked and stable with respect to population size.

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