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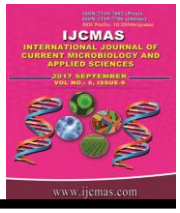

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Genetic Diversity Analysis of Kilakarsal Sheep by Microsatellite Markers

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A B S T R A C T

Kilakarsal sheep population variability and structure was investigated genetically utilizing FAO recommended 25 microsatellite markers. Estimates of genetic variability such as effective number of alleles and gene diversities revealed substantial genetic variation detected by microsatellite markers. A total of 241 alleles were detected and the actual number of observable alleles ranged from five (BM6526) to a maximum of 16 (MAF70). The mean number of alleles (allelic diversity) was 9.64. The effective number of alleles which is lower than the observed number of alleles, was between 2.279 (OarCP20) and 8.510 (MAF70) with a mean of 4.786 alleles. The average observed heterozygosity (H_o) and expected heterozygosity (H_e) were 0.690 and 0.782 respectively. However, the study evidenced a significant departure from Hardy-Weinberg equilibrium in 13 loci. Such lack seems to be caused by a rather high level of inbreeding (F_{IS}=0.097).

Introduction

Kilakarsal sheep, one among of the registered breeds is distributed in the southern districts of Tamil Nadu. They are medium sized with compact body conformation and reared for meat purpose. The distinct breed characters include dark tan coat dorsally and black colour ventrally in the belly and inner side of legs. Rams have well developed twisted horns while ewes are polled.

The present study is carried out to estimate the inbreeding level in the field animals thus how various forces of genetic change are modifying the foundation genetic structure of the population. This will provide genetic information to be used for conservation and improvement of this population.

Materials and Methods

Blood samples of fifty genetically unrelated sheep were collected from breeding tract of Kilakarsal sheep to make them representative of the population. Genomic DNA was isolated as per the method described by Miller *et al.*, (1988) with minor modifications. The isolated DNA samples were quantified by agarose gel electrophoresis and visualized by UV spectrophotometer. After checking the quality and quantity, DNA was diluted to a final concentration of 50ng/µl using Tris EDTA buffer and stored at -20°C.

A battery of 25 microsatellite markers was selected based on the guidelines of ISAG (International Society of Animal Genetics) and FAO's DADIS (Domestic Animal

Diversity Information System). Polymerase Chain Reaction (PCR) was carried out on about 50ng genomic DNA in a 15µl reaction volume using Mastercycler ep gradients (Eppendorf, Germany). The reaction mixture consisted of one µl template DNA, 0.5 µl of primers (forward and reverse), 7.5 µl of 2 X PCR mastermix and 5.5 µl of triple distilled water.

The PCR protocol used with initial denaturation of 94^{o}C for 5 min, 3 cycles of 94^{o}C for 45 seconds, annealing for 35 seconds (temperature ranged from 51^{o}C to 61.5^{o}C), extension of 35 seconds at 72^{o}C, 35 cycles, a final extension at 72^{o}C for 10 min. The genotypes were scored by the ABI Genetic Analyzer and GeneMapper^{Tm} version 4.0 (Applied Bio-systems, Germany). Of the 25 microsatellite loci, all loci amplified successfully and produced definite banding patterns.

The original microsatellite allelic data is available from the first author upon request. For 25 microsatellite loci, genetic variation of the breed and breed structure was elucidated using allele frequencies, observed and effective number of alleles, observed and expected heterozygosities and within breed heterozygosity deficit (estimated using Wrights fixation index). All the estimates were derived using POPGENE version 3.1 program (Yeh *et al.*, 1999). Allelic frequencies were utilized for the calculation of the Polymorphism Information Content (PIC) values (Botstein *et al.*, 1980).

Results and Discussion

The allelic diversity and genetic variation are presented in table 1. A total of 241 alleles were detected. The number of alleles observed across the microsatellite loci varied from five (BM6526) to 16 (MAF70). The observed number of alleles across the loci was more than the effective number of alleles (4.78 ± 1.72) as expected. Total number of alleles observed in this study was higher than the values reported for five other Tamil Nadu sheep, Nilagiri 125 (Girish *et al.*, 2007), Vembur 147 (Pramod *et al.*, 2009), Madras Red 98 (Selvam *et al.*, 2009), Coimbatore 143 (Kumarasamy *et al.*, 2009) and Tiruchy Black 195 alleles (Kavitha, 2010) as well as this is higher than 190 alleles for the same breed as reported by Radha *et al.*, (2011).

However, direct comparisons with the earlier characterized breeds cannot be justified, as the method of genotyping in the earlier studies was manual (by silver staining) and a slightly different set of markers was used.

The allelic diversity is a reasonable parameter of genetic variation which revealed that Kilakarsal harboured a good amount (9.64 ± 2.82) of genetic variation as this breed possessed more than four alleles (Li *et al.*, 2002). The allelic diversity in this study were observed to be higher than 7.6 reported for the same breed (Radha *et al.*, loc.cit) probably because of genotyping accomplishment under this study by automated DNA sequencer rather than by silver staining technique and also different set of markers used.

The average expected genetic diversity within the population ranged from 0.5613 (OarCP20) to 0.8825 (MAF70). The average genetic variation (H_o=0.762 ± 0.088) in Kilakarsal sheep was higher than that reported for the genetic variation in Nellore and Pattanam (H_o= 0.658 and 0.666) by Ramachandran (2012). In assessing diversity estimates from different studies, it should be mentioned that the values are not directly comparable as different microsatellite sets were used by different workers. These values have only suggestive indication of diversity in the populations. Expected heterozygosity is considered to be a better estimator of the genetic variability in a population.

Table.1 Number of alleles, observed and expected heterozygosities, polymorphism information content, Shannon's information index, F_{IS}, Hardy-Weinberg equilibrium (HWE) and Nei's genetic distance values for Kilakarsal sheep

Microsatellite Marker	n_a	n_e	H_o	H_e	PIC	I	F_{IS}	HWE		D
								χ^2	d.f	
BM757	12	5.7143	0.7000	0.8462	0.797	2.0399	0.1515	53.08^{NS}	66	0.8250
BM827	6	3.1375	0.6000	0.6987	0.6293	1.3393	0.1195	10.10^{NS}	15	0.6813
BM1329	9	5.4422	0.9500	0.8372	0.7911	1.8518	-0.1639	49.60^{NS}	36	0.8163
BM6506	11	5.1613	1.0000	0.8269	0.7696	1.9124	-0.2403	55.05^{NS}	5	0.8063
BM6526	5	3.1250	0.5500	0.6974	0.6223	1.2760	0.1912	9.53^{NS}	10	0.6800
BM8125	8	3.9604	0.4500	0.7667	0.7118	1.6375	0.3980	73.82**	28	0.7475
CSSM31	13	5.4795	0.9500	0.8385	0.6969	2.0575	-0.1621	115.11**	78	0.8175
ILSTO33	6	3.2000	0.6000	0.7051	0.6449	1.3964	0.1273	19.07^{NS}	15	0.6875
MAF65	9	2.9851	0.6000	0.6821	0.6445	1.5598	0.0977	80.23**	36	0.6650
MAF70	16	8.5106	0.8000	0.9051	0.5119	2.4353	0.0935	108.70^{NS}	120	0.8825
MAF209	8	3.0303	0.6500	0.6872	0.6387	1.4882	0.0299	43.76*	28	0.6700
MCM527	9	4.3478	0.5000	0.7897	0.7442	1.7546	0.3506	67.54**	36	0.7700
OarCP20	6	2.2792	0.7000	0.5756	0.4892	1.0524	-0.2472	42.52**	15	0.5613
OarFCB20	11	4.3243	0.5500	0.7885	0.7467	1.8614	0.2846	101.92**	55	0.7688
OarCP34	11	6.6116	0.9500	0.8705	0.8314	2.1337	-0.1193	116.24**	55	0.8488
OarFCB48	12	8.0808	0.9500	0.8987	0.8267	2.2563	-0.0842	55.79^{NS}	66	0.8225
OarFCB128	7	3.8095	0.4500	0.7514	0.7002	1.5675	0.3898	35.45*	36	0.7375
OarFCB304	6	2.3324	0.6500	0,5859	0.5209	1.1371	-0.0379	3.17^{NS}	21	0.5713
OarAE129	12	4.5455	0.3500	0.8000	0.7312	1.9734	0.5513	140.39**	66	0.7800
OarJMP29	14	7.0796	0.7500	0.8805	0.6741	2.2871	0.1266	80.10^{NS}	91	0.8588
OarHH35	8	4.8780	0.4500	0.8154	0.7652	1.7364	0.4340	71.07**	28	0.7950
OarHH47	10	4.3478	0.7000	0.7897	0.7504	1.8563	0.0909	61.21^{NS}	45	0.7700
OarVH72	9	5.5556	0.8000	0.8410	0.7979	1.8964	0.0244	60.95**	36	0.8200
SRCRSP5	11	4.3716	0.7500	0.7910	0.6176	1.8910	0.0276	121.75**	55	0.7712
SRCRSP9	12	7.3394	0.8500	0.8859	0.7888	2.1792	0.0159	66.45^{NS}	66	0.8638
Mean	**9.64**	**4.7860**	**0.6900**	**0.7824**	**0.6977**	**1.7831**	**0.0979**	-	-	**0.7628**
S D	**2.8267**	**1.7253**	**0.1843**	**0.0903**	**0.09**	**0.3640**	-	-	-	**0.0880**

n_a – observed number of alleles, **n_e**- Effective number of alleles, **H_o**- Observed heterozygosity, **H_e**- Expected heterozygosity, **PIC**- Polymorphism Information Content **I**- Shannon's Information Index**,** ***F_{IS}*** **-** Within population inbreeding estimate, **χ2-** Chi- square value, **d.f-** Degrees of freedom and **D-** Nei's genetic distance.
NS-Not Significant *- Significant **- Highly significant

The high mean (both observed and expected) values estimated in the present study showed that Kilakarsal sheep possess a substantial level of genetic diversity that might be used in planning breeding strategies.

The within breed inbreeding estimate (F_{IS}) reported in most of the Tamil Nadu sheep breeds indicated various levels of inbreeding as 0.004 in Mecheri sheep (Prema *et al.*, 2008), 0.295 in Vembur breed (Pramod *et al.*, loc.cit) and 0.147 in Kilakarsal sheep (Radha *et al.*, loc.cit). Heterozygote deficiency analysis revealed significant deviation from Hardy-Weinberg equilibrium ($P<0.01$) at several loci. The exact basis of this departure is difficult to explain, however, the presence of low frequency of null alleles segregating at the loci. There are other more potent forces than null alleles that cause heterozygote deficiency e.g. within population inbreeding, mainly due to use of limited number of sires. The Shannon information index (1.783 ± 0.364) and polymorphic information content (0.697 ± 0.090) showed that most of the loci were highly informative indicating polymorphism across the loci, thus suggesting the suitability of these markers for genetic diversity studies in other sheep breeds of India.

Kilakarsal sheep had substantial genetic variation as indicated by high allele and genetic diversity. The breed lost its genetic identity due to excessive gene flow resulted from the invading effect by rams of Chevaadu breed of sheep. The study, however, also suggests that such an uncontrolled mating is avoided for maintaining pure germplasm of Kilakarsal, which possessed unique alleles.

References

Barker, J.S.F., 1994. In 'A global protocol for determining genetic distance among domestic livestock breeds' Proceedings of the world congress held at University of Guelph, pp. 501.

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. *Am. J. Hum. Genet*, 32: 314-331.

Girish, H., Sivaselvam, S.N., Karthickeyan, S.M.K. and Saravanan, R. 2007. Molecular characterisation of Nilagiri sheep (*Ovis aries*) of south India based on microsatellites. *Asian Aust. J. Anim*., 20: 633-637.

Kavitha, S.T., 2010. Molecular characterization of Tiruchy Black Sheep. M.V.Sc. Thesis. Tamil Nadu Veterinary and Animal Sciences University, Chennai.

Kumarasamy, P., Prema, S., Ganapathi, P., Karthickeyan, S.M.K. and Kanakaraj, P. (2009). Molecular characterization of Coimbatore breed of sheep (*Ovis aries*) in south India. *Icfai. Univ. J. Genet. Evol*, 2: 55-56.

Li, M.H., Jhao, S.H.E., Bian, C., Wang, H.S., Wei, H., Liu, B., Yu, M., Fan, B., Chen, C.L., Zhu, M.J., Li, S.J., Xiong, T.A and Li, K. 2002. Genetic relationship among twelve Chineese indigenous goat populations based on microsatellite analysis. *Genet. Selection Evol*, 34: 729-744.

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. Res*., 16: 1215.

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 J. Sci. Technol*., 2: 55-58.

Prema, S., Sivaselvam, S.N. and Karthickeyan, S.M.K. 2008. Evaluation

of genetic diversity in Mecheri sheep (Ovis aries) of Tamil Nadu using microsatellite markers. *Indian J. Biotechnol*., 7: 401-403.

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

Ramachandran, A., 2012. M.V.Sc. Thesis. Tamil Nadu Veterinary and Animal Sciences University, India.

Selvam, R., Rahumathulla, P. S., Sivaselvam, S. N., Karthickeyan, S. M. K. and Rajendran, R. 2009. Molecular genetic characterization of Madras Red sheep in Tamil Nadu, India using microsatellite markers. *Livest. Res. Rural Dev., 21: 50.*

Yeh, F.C., Boyle, T., Rongcai, Y., Ye, Z. and Xian, J.M. 1999. Popgene, Version 1.31. A Microsoft Windows Based Freeware for Population Genetic Analysis. University of Alberta, Edmonton.

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