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Genetic diversity in Kheri—A pastoralists developed Indian sheep using microsatellite markers

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The Kheri sheep were analyzed using 25 ovine microsatellite markers proposed by Food and Agriculture Organization, International Society for Animal Genetics (FAO-ISAG). All the used microsatellite markers amplified well and exhibited polymorphism. Wide range of variability depicted by number of observed alleles from 2 (BM6506, CSSM47 and OarCP20) to 10 (CSSM31 and OarJMP29), observed heterozygosity from 0.087 (OarCP20) to 1.000 (OarHH35), expected heterozygosity from 0.083 (OarCP20) to 0.828 (BM1314) and Polymorphism Information Content (PIC) from 0.079 (OarCP20) to 0.806 (BM1314) supported the utility of these microsatellite loci in measurement of genetic diversity indices in Indian sheep too. The mean number of observed and effective alleles was 5.3 and 3.3, respectively. The average observed heterozygosity values (0.582) compared to the average expected heterozygosity values (0.651) did not show significant differences in the selected population ($p>0.05$), which suggested random mating in Kheri. The allele diversity (average number of alleles per locus) and gene diversity (average expected heterozygosity) reflected high levels of genetic variability in Kheri sheep. Within population inbreeding estimate (Fis) was significant ($p<0.05$) and equal to 12.8%. The deficit of heterozygotes may be partly explained by Wahlhund effects at level of sampling (i.e. sampling at random from the whole population), although the main factor that appears to have provoked this lack of heterozygotes may be attributed to consanguinity that has resulted in accumulation of inbreeding in the breeding groups. This study contributes to the knowledge of genetic diversity of Kheri-the black brown faced meat, carpet wool sheep developed by migratory pastoralists in India.

Keywords: sheep, Indian indigenous sheep, Kheri, microsatellite, genetic diversity

Introduction

Kheri the black-brown faced meat, carpet wool sheep, distributed largely in Nagore, Jodhpur and Tonk districts of Rajasthan is considered to have originated from a crossbred base with unknown levels of inheritance of Marwari, Malpura and Jaisalmeri sheep of Rajasthan. Kheri sheep evolved in the farmers flock under the field conditions appears to be a need of migratory sheep breeders. These animals can sustain stress and on return of favourable condition regain faster growth resulting in better reproduction rate and growth of lambs as compared to prevalent breeds in the area. The Kheri sheep contributes substantially to the economy of the landless and marginal farmers in the arid zone where crop production is unpredictable due to scanty and erratic rainfall[1].

Awareness of the value of ovine genetic resources has stimulated the study of genetic diversity of native breeds. Among the different molecular markers, the microsatellites are considered as most suitable marker systems for the evaluation of paternity for tracking alleles through a population and biodiversity evaluation studies[2,3] owing to their abundance in the mammalian genome, high polymorphism and amenability for automation. The use of same set of microsatellites in ovines as per FAO's integrated global programme (1996)[4] to establish genetic relationships among the breeds would provide an excellent means for the joint analysis of future data with as much incorporation of the previous work as possible at national as well as international level.

In order to develop objective criteria for conservation and genetic improvement of various sheep breeds of north western arid and semi arid zones of India, we collected data for a panel of 25 ovine microsatellite polymorphisms in Kheri sheep under ongoing programme on 'Molecular genetic characterization of sheep,' at NBAGR, Karnal to have detailed knowledge of genetic variation within this sheep population exhibiting unique characteristics viz., hardiness, ability to walk long distances and adaptability to withstand drought and heat conditions.

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Material and Methods

Sampling

A total of 48 blood samples were randomly collected from genetically unrelated sheep across its breeding tract with the help of owners and veterinary officers.

Laboratory Techniques

Genomic DNA from blood samples was isolated and purified using the standard phenol chloroform extraction protocol. The 25 ovine microsatellite markers selected by the International Society of Animal Genetics (ISAG) under FAO's proposed list of 28 markers were genotyped and used for the analysis of genetic diversity in Kheri sheep. Microsatellites were amplified in a reaction mix containing 100 ng of genomic DNA, 200 μ*M* of each dNTP, 50 ng of each primer and 0.5 units of *Taq* DNA polymerase. PCR amplification was performed on a PTC-100 thermocycler (MJ Research Inc., USA). A common "Touchdown" PCR program suggested under FAO's MoDAD[4] project was used for amplification of all the studied markers. The cycling program involved 3 cycles of 45 sec at 95°C, 1 min at 60°C; 3 cycles of 45 sec at 95°C, 1 min at 57°C; 3 cycles of 45 sec at 95°C, 1 min at 54°C; 3 cycles of 45 sec at 95°C, 1 min at 51°C; 20 cycles of 45 sec at 92°C, 1 min at 48°C using PTC-200 M thermal Cycler (MJ Research, USA). The PCR products were separated on a 6% denaturing polyacrylamide gel and silver stained according to manufacturer's protocol (Promega, W1, USA). Genotype of each individual animal at 25 different loci was recorded by direct counting. Allelic size range was estimated using 10 bp sequencing ladder (Gibco BRL, Life Technologies, TM) as standard molecular weight marker.

Statistical Analysis

Allele frequencies, observed number of alleles, effective number of alleles, observed heterozygosity, expected heterozygosity and within breed heterozygosity deficit were estimated using POPGENE 1.31 and FSTAT 2.9.3.2 software packages, respectively[5,6]. Polymorphism information content (PIC) was calculated using the formula of Botstein *et al*[7]. Another software programme used in this study included BOTTLENECK[8] for existence of bottleneck effect in the investigated sheep population, if any.

Results and Discussion

The population statistics generated by the 25 microsatellites has been summarized in Table 1. Allele frequencies were available from the corresponding author on request. All loci were found to be polymorphic and a total of 131 alleles were detected across the 25 analyzed loci. Loci CSSM31 and OarJMP29 showed the highest number of alleles (na=10) and loci BM6506, CSSM47 and OarCP20 the lowest number of alleles (na=2) with an average of 5.3. The effective number of alleles (ne) was less than the observed (na) values ranging from 1.0 (OarCP20) to 5.7 (BM1314) with mean of 3.3. The number of alleles at different marker loci serves as a measure of genetic variability having direct impact on differentiation of breeds within a species. The level of variation depicted by number of alleles at each locus in the present study was similar to earlier reports in sheep and cattle breeds[9,10]. The comparison of ne with na at each locus provides information about the predominance of certain alleles in each breed.

The observed (Ho) and expected (He) heterozygosity values ranged from 0.087 (OarCP20) to 1.00 (OarHH35) and from 0.083 (OarCP20) to 0.828 (BM1314), respectively. The used microsatellites with wide range of heterozygosity reduced the risk of overestimating genetic variability, which might occur with microsatellites exhibiting only high heterozygosity. The high mean values of observed (0.582) and expected (0.651) heterozygosity were in accordance with mean observed heterozygosity counts reported in French mutton merino[11] (Ho=0.679); mean expected heterozygosity counts in six domestic Swiss sheep breeds[3] (He = 0.60 – 0.67); observed (Ho=0.603), expected (He=0.673) heterozygosity values in Garole–a highly prolific Indian microsheep[12] and Muzzafarnagri, the largest and heaviest mutton sheep breed from India[13] (Ho=0.603, He=0.673). Although varying across the loci, the mean values of observed heterozygosity were lower than the expected mean heterozygosity values. However, failure of significant differences between observed and expected heterozygosities using ANOVA test ($p>0.05$) suggested random mating in Kheri.

PIC values varied from 0.079 (OarCP20) to 0.806 (BM1314) with mean of 0.595 per locus. Based on the PIC values, nearly 76% of the markers were observed to be highly informative (PIC>0.50) and 16% were reasonably informative (0.50>PIC>0.25) while only 8% were less informative (PIC<0.25), which further indicated high utility of used set of markers for genetic diversity analysis.

Table 1—Allele size range, number of alleles (observed-no, effective- ne), heterozygosity (observed-Ho and Expected -He), heterozygosity deficit (F_{IS}) and PIC for each of the 25 microsatellite loci for Kheri sheep

Locus	Allele size range (bp)	No. of alleles		Heterozygosity		F_{IS}	PIC
		Observed (na)	Effective (ne)	Observed (Ho)	Expected (He)		
BM757	178-200	5	4.3	0.739	0.771	0.064	0.733
BM827	210-216	5	3.7	0.522	0.731	0.306*	0.682
BM1314	149-179	8	5.7	0.818	0.828	0.034	0.806
BM6506	193-203	2	1.9	0.409	0.499	0.203*	0.374
BM6526	142-172	3	2.2	0.571	0.550	-0.015	0.479
BM8125	106-132	5	2.2	0.565	0.563	0.019	0.531
CSSM31	130-170	10	4.9	0.695	0.799	0.152*	0.783
CSSM47	132-172	2	1.2	0.217	0.194	0.583*	0.175
HUJ616	118-144	5	2.9	0.727	0.666	-0.068	0.620
OMHC1	183-217	5	2.6	0.318	0.619	0.503*	0.579
OarAE129	138-164	4	2.0	0.217	0.518	0.595*	0.449
OarCP20	71-75	2	1.0	0.087	0.083	-0.023	0.079
OarCP34	116-128	6	3.2	0.727	0.693	-0.026	0.654
OarFCB48	146-166	7	5.2	0.913	0.809	-0.107	0.782
OarFCB128	108-134	5	3.5	0.695	0.722	0.059	0.677
OarHH35	87-135	8	5.2	1.000	0.811	-0.212	0.787
OarHH41	118-136	7	3.7	0.478	0.732	0.366*	0.703
OarHH47	136-154	5	3.9	0.696	0.748	0.092	0.710
OarHH64	120-134	6	3.7	0.652	0.733	0.132	0.698
OarJMP8	121-133	6	4.2	0.737	0.765	0.063	0.735
OarJMP29	128-158	10	4.5	0.696	0.781	0.131	0.752
OarVH72	114-140	4	2.7	0.435	0.639	0.339*	0.568
RM4	137-145	3	2.0	0.217	0.506	0.585*	0.427
TGLA137	138-164	4	2.6	0.739	0.626	-0.158	0.577
TGLA377	70-80	4	2.4	0.695	0.598	-0.141	0.532
Mean		5.3	3.3	0.582	0.651	0.128*	0.595

* $p<0.05$

The high average estimates of allele diversity (mean number of observed alleles per locus, 5.3) and gene diversity (mean expected heterozygosity, 0.651) displayed by panel of 25 microsatellites again implied the presence of substantial amount of genetic variability in the investigated sheep. High average PIC values (0.595) also supported suitability of used set of markers in population assignment and genome mapping studies in addition to genetic diversity analysis in Indian sheep too. The high values of various diversity measures obtained in the present study for Kheri were in accordance with that of other domestic sheep breeds[3,12,13]. In contrast, low gene diversity has been reported in wild Mouflon and Bighorn sheep herds with values of 0.45 and 0.43, respectively[3,14]. A high within population inbreeding estimate, which revealed heterozygotes deficit in Kheri, has been obtained from this work. The mean Fis estimate (0.128) was significantly different ($p<0.05$) from zero. Significant heterozygotes deficiencies have also been reported in goat[15] and cattle[10]. Possible explanation could be the presence of null alleles; locus may be under selection; breeding among related animals or population subdivision [16,17]. However, distinguishing among these is generally difficult. Due to non-availability of pedigreed animals with the farmers for analysis, it was not possible to demonstrate the presence of null alleles (usually caused by a mutation in the primer binding site leading to an allele that will not amplify). The selection influence could not be proved because production data were not available. The deficit of heterozygotes may partly be explained by Wahlund effects at our level of sampling (i.e. sampling at random from the whole population). The main factor that possibly has provoked this lack of heterozygotes might be attributed to consanguinity that has resulted in accumulation of inbreeding in the breeding groups.

Efforts were made for the estimation of genetic bottleneck by using three tests viz., Sign rank test, Standardized differences test and Wilcoxin test in each of three models of mutations namely IAM (Infinite Allele Model), SMM (Stepwise Mutation Model) and TPM (Two Phase Model). SMM, which

Table 2 —Test for null hypothesis under three microsatellite evolution models

	Models of microsatellite evolution		
	IAM	TPM	SMM
Sign test			
Expected no. of loci with heterozygosity excess	14.23	14.50	14.69
Observed no. of loci with heterozygosity excess	23*	18	14
Standardized differences test			
T_2 values	3.887*	2.131*	-0.517
Wilcoxon rank test			
Probability of het. excess	0.00000*	0.00306*	0.65424

*Bottleneck (rejection of null hypothesis of mutation drift equilibrium)

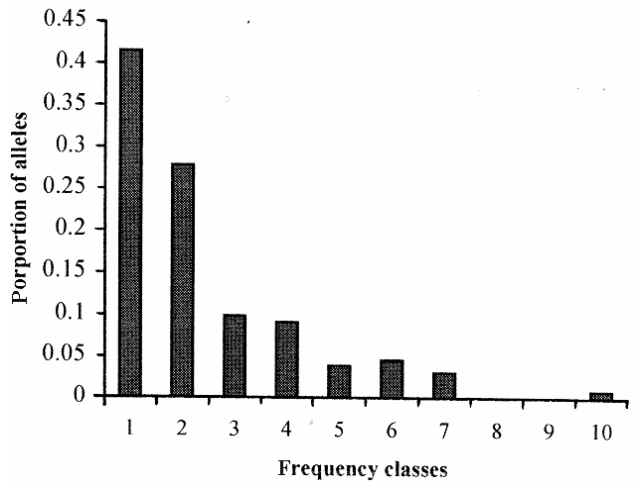

Fig. 1—Frequency histogram depicting normal L-shaped form indicating no mode shift in Kheri sheep.

is the most suited model for evolution of microsatellites, revealed absence of significant heterozygotes excess for Kheri accepting the null hypothesis of mutation drift equilibrium (Table 2). Another powerful test of qualitative graphical method based on mode-shift distortion[18] was utilized to visualize the allele frequency spectra as a check for genetic bottleneck. No Mode shift was detected in the frequency distribution of alleles and a normal L-shaped form was observed (Fig.1). These finding further suggested absence of any recent reduction in the effective population size and non- bottlenecked Kheri population under mutation drift equilibrium.

In conclusion, the information generated from the present study on the genetic diversity of Kheri sheep will be valuable in formulating effective management and conservation programmes/policies for this indigenous sheep population.

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