Genetic diversity analysis of three Indian sheep breeds using microsatellite markers

R Arora and S Bhatia*

National Bureau of Animal Genetic Resources, P.O.Box 129, Karnal-132001, Haryana, India

ABSTRACT

Information is presented on the genetic diversity analysis involving within breed variability, genetic differentiation and genetic relationships among Jalauni, Marwari and Sonadi sheep breeds of Northwestern arid and semi arid zone of India, based on twenty microsatellite markers as proposed in FAO's MoDAD programme. The considerably high values obtained for allele and gene diversity showed that Jalauni, Marwari and Sonadi breeds possessed considerable amount of genetic diversity. A difference in inbreeding was elucidated by within population inbreeding estimate (F_{IS}), with Marwari exhibiting comparatively high level of inbreeding than Jalauni and Sonadi breeds. The present study thus elucidated the need for concerted genetic management efforts to be taken to combat harmful/adverse effects of inbreeding in the investigated breeds especially Marwari. The three investigated breeds also reflected absence of a recent genetic bottleneck. The pair wise genetic distance (DS), genetic differentiation (F_{ST}), gene differentiation (G_{ST}) and gene flow (Nm) between Jalauni, Marwari and Sonadi revealed Marwari and Sonadi to be the closest and highest degree of genetic differentiation was found between Jalauni and Sonadi. In addition, phylogeny analysis used to evaluate inter-breed genetic proximity also revealed substantial genetic differentiation between Jalauni and the other two sheep breeds of Northwestern arid and semi arid zone of India (Marwari and Sonadi). The generated molecular genetic information of breeds under consideration would provide a more precise insight for their conservation, genetic management and genetic improvement programmes.

Keywords: Indian sheep, microsatellites, diversity, genetic management, conservation

INTRODUCTION

India possesses diversified germplasm of sheep represented by several recognized breeds (40-43, Acharya, 1982; Bhatia and Arora, 2005) and several uncharacterized populations distributed across four agroecological regions of the country namely a) Northern temperate region, b) North-western arid and semi arid region, c) Southern peninsular region and d) Eastern region. These sheep thriving under zero/low input system have adapted to the local adverse conditions over the years. Such indigenous breeds represent a vast gene pool with untapped potential for our future commercial purposes as well as for maintenance of genetic diversity in this species. Safeguarding the genetic diversity is a major focus of conservationists as it represents the essential evolutionary potential for species to adapt to changing environment. Hence, there is an urgent need to characterize and document these indigenous ovine genetic resources, which contribute significantly to the Indian rural economy, before they are lost due to haphazard breeding and human overexploitation.

Over the past decade there has been a growing interest in using microsatellite markers for diversity analysis in livestock breeds. Microsatellites have advantage over other DNA markers as they combine high variability with codominant inheritance and can be easily typed (Litt and Luty, 1989). Although exotic breeds of sheep have been extensively evaluated for genetic diversity using microsatellite markers (Arranz et al. 2001; Saitbekova et al. 2001; Grigaliunaite et al. 2003; Paiva et al. 2005; Wafula et al. 2005) there are still limited reports on Indian Sheep breeds (Arora and Bhatia, 2004; 2006; Mukesh et al. 2006; Sodhi et al. 2003).

In the present study the genetic characterization of three native sheep breeds namely Jalauni, Marwari and Sonadi, well adapted to the North-western arid and semi arid region (Fig.1), was performed with FAO recommended microsatellite markers for ovines. Jalauni is one of the recognized sheep breeds of the Bundelkhand region, mostly prevalent in Jalaun, Jhansi and Lalitpur districts of Uttar Pradesh and the Tikamgarh and Datia districts of Madhya Pradesh. Jalauni sheep, well adapted to the local agro-climatic conditions of this region - maintained for mutton and wool are small to medium in size with straight nose line, white coloured body and light brown or black head in most animals. Jalauni sheep are reared by the local 'Pal' community which is comprised of small, marginal or landless, mostly illiterate farmers/ labourers. The breed is exbhiting declining status (Sahana et al. 2004) due to decrease in grazing area as a result of increased crop production in the breeding tract. Marwari (Boti), another unique breed of Rajasthan is black faced, small eared, small to medium sized, nomadic sheep known for its wool quality and hardiness. This breed is prevalent in Ajmer, Jodhpur, Udaipur, Jalore, Nagaur, Pali, Barmer districts of Rajasthan and the Jeoria region of Gujarat. These animals migrate to the States of Uttar

Address of corresponding author: *Dr. (Ms) S.Bhatia, Principal Scientist Animal Genetics Division, National Bureau of Animal Genetic Resources, P.O.Box 129, Karnal-132001, Haryana, India.
E-mail:bhatias@nbagr.ernet.in; bhatias11@yahoo.co.in

Pradesh, Madhya Pradesh and northern parts of Gujarat and Kathiawar in search of grazing lands (Acharya, 1982). The Marwari breed is also able to cope with extreme climatic conditions, low forage availability, resistance to diseases and endurance (http://www.pastoralpeoples.org/docs). This breed is kept mainly for its wool and in times of drought it is said to be preferred above all other breeds of the region. Sonadi, fairly well built, light brown faced sheep, also known as "Bhagli" is one of the triple purpose breeds (meat, wool and milk), found in Udaipur, Dungarpur and to some extent Chittorgarh districts of Rajasthan. This breed is mainly appreciated for its high milk and meat production qualities and it yields 250-500 gram of milk per day during lactation. In contrast to the Marwari breed, the Sonadi needs more fodder of better quality for sustenance (Geerlings, 2004). But in areas where there is no lack of grazing land and good access to grazing resources the Sonadi breed is still preferred. Moreover, this breed is under general apathy and neglect due to enhanced adoption of farmers to comparatively hardy and superior rams of Marwari breed.

The goal of this study was to assess the current genetic diversity, level of genetic differentiation and extent of genetic relationships using polymorphic microsatellite markers among these three important breeds of indigenous sheep from North-western arid and semi arid region of the country.

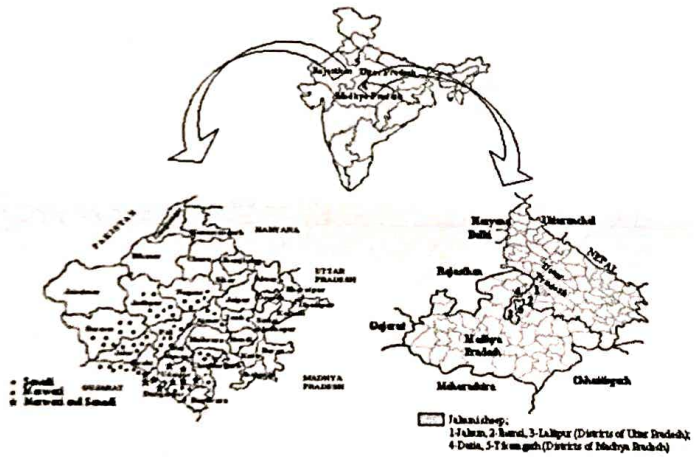

Figure 1. Geographical distribution of Marwari, Sonadi and Jalauni sheep breeds

MATERIALS AND METHODS

Samples: A total of 150 random blood samples were obtained from unrelated animals of Jalauni Marwari and Sonadi breeds of sheep from their respective breeding tracts. Genomic DNA was isolated from blood samples following standard procedures (Sambrook et al. 1989).

Microsatellite Genotyping: A set of twenty FAO suggested microsatellites for sheep genetic diversity studies was analyzed on all the individual samples (Table1). Amplifications for the loci were performed in a 25µl final reaction volume containing at least 100ng of genomic DNA, 50ng of each primer, 1.5mM MgCl2, 200µM dNTPs, 0.5U Taq polymerase and 1x buffer. The thermal touchdown profile for PCR was as follows: 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 and 20 cycles of 45 sec at 92° C, 1 min at 48° C. Amplification was confirmed on 2% agarose gel and the products were size separated on 6% denaturing polyacrylamide gel and visualized by silver staining (Bassam et al. 1991). Estimation of allele size was done by running a 10bp DNA molecular weight marker along with the PCR products.

Statistical analysis: Genotyping was done manually from the silver stained gels. Allele frequencies were determined by direct counting. The genetic variability for each breed was estimated as allele diversity, observed heterozygosity and gene diversity using POPGENE software programme (Yeh et al. 1999). The recent bottleneck effect was inferred for the three populations using mode shift analysis under the assumption of two-phase microsatellite mutation model (TPM), implemented in the programme BOTTLENECK ver 1.2.02 (Cornuet and Luikart, 1996). The computer programme FSTAT ver. 2.9.3.2 (Goudet et al. 1995) was used to obtain estimates of inbreeding coefficients and population subdivision based on unbiased F-statistics according to Weir and Cockerham (1984). The F_{ST} values among pairs of breeds were calculated by using the GENEPOP program (Raymond and Rousset, 1995), which was also used to quantify the effects of migration on the genetic structure and gene flow was estimated between each pair of breeds. The genetic relationships between the studied breeds were inferred from Nei's genetic distance (Ds, Nei 1972) and the distances between breeds were used for tree construction based on unweighted pair group methods with arithmetic averages (UPGMA) method using the PHYLIP ver. 3.6 software package (Felsenstein, 1993).

RESULTS AND DISCUSSION

Breed variability, breed relationships and extent of genetic relationships among the investigated breeds were assessed from genotypic data of 150 animals using twenty microsatellite markers. All the microsatellite loci amplified well and were found to be effective in detecting polymorphism with reasonable amount of genetic variation apparent in each of the investigated breeds from the allele frequency data. The number of observed alleles per locus varied from 2 (Jalauni -BM8125, Marwari –BM6506) to 11 (Marwari- CSSM31). Mean number of observed alleles (allele diversity) in Jalauni, Marwari and Sonadi sheep was 5.85, 6.25 and 5.75, respectively. The number of effective alleles per locus varied from 1.30 (Jalauni-OarCP20, Sonadi- CSSM47) to 6.7 (Sonadi-OarHH47). The mean effective number of alleles was highest in Marwari (4.02) followed by Sonadi (3.71) and Jalauni (3.69) breeds. Earlier studies on several Indian sheep breeds from different parts of the country viz., Karnah, Muzzafarnagri, Magra, Nali, Chokla, Bellary, Chhotanagpuri (Arora and Bhatia 2004; 2006; Sodhi et al. 2006; Kumar et al. 2007; Gupta et al 2007; Bhatia et al. 2008), and a few exotic breeds (Paiva et al. 2005; Wafula et al. 2005) reported allele diversity estimates which are comparable with those obtained in this study for the three breeds (Table 2).

Table 1. Microsatellite markers, chromosomal location, allele size range and their primer sequences

	Microsatellite Marker	Chromosomal Location	Allele size range (bp)	Primer Sequences (5'-3')
1	BM757	9	178-200	F-TGGAAACAATGTAAACCTGGG R-TTGAGCCACCAAGGAACC
2	BM827	3	210-216	F-GGGCTGGTCGTATGCTGAG R-GTTGGACTTGCTGAAGTGACC
3	BM1314	22	149-179	F-TTCCTCCTCTTCTCTCCAAAC R-ATCTCAAACGCCAGTGTGG
4	BM6506	1	193-203	F-GCACGTGGTAAAGAGATGGC R-AGCAACTTGAGCATGGCAC
5	BM6526	26	142-172	F-CATGCCAAACAATATCCAGC R-TGAAGGTAGAGAGCAAGCAGC
6	BM8125	17	106-132	F-CTCTATCTGTGGAAAAGGTGGG R-GGGGGTTAGACTTCAACATACG
7	CSSM31	23	130-170	F-CCAAGTTTAGTACTTGTAAGTAGA R-GACTCTCTAGCACTTTATCTGTGT
8	CSSM47	2	132-172	F-TCTCTGTCTCTATCACTATATGGC R-CTGGGCACCTGAAACTATCATCAT
9	HUJ616	13	118-144	F-TTCAAACTACACATTGACAGGG R-GGACCTTTGGCAATGGAAGG
10	OMHC1	20	183-217	F-ATCTGGTGGGCTACAGTCCATG R-GCAATGCTTTCTAAATTCTGAGGAA
11	OarAE129	5	138-164	F-AATCCAGTGTGTGAAAGACTAATCCAG R-GTAGATCAAGATATAGAATATTTTTCAACACC
12	OarCP20	21	71-75	F-GATCCCCTGGAGGAGGAAACGG R-GGCATTTCATGGCTTTAGCAGG
13	OarCP34	3	116-128	F-GCTGAACAATGTGATATGTTCAGG R-GGGACAATACTGTCTTAGATGCTGC
14	OarFCB48	17	146-166	F-GAGTTAGTACAAGGATGACAAGAGGCAC R-GACTCTAGAGGATCGCAAAGAACCAG
15	OarFCB128	2	108-134	F-CAGCTGAGCAACTAAGACATACATGCG R-ATTAAAGCATCTTCTCTTTATTTCCTCGC
16	OarHH35	4	87-135	F-AATTGCATTCAGTATCTTTAACATCTGGC R-ATGAAAATATAAAGAGAATGAACCACACGG
17	OarHH41	10	118-136	F-TCCACAGGCTTAAATCTATATAGCAACC R-CCAGCTAAAGATAAAAGATGATGTGGGAG
18	OarHH47	18	136-154	F-TTTATTGACAAACTCTCTTCCTAACTCCACC R-GTAGTTATTTAAAAAAATATCATACCTCTTAAGG
19	OarHH64	4	120-134	F-CGTTCCCTCACTATGGAAAGTTATATATGC R-CACTCTATTGTAAGAATTTGAATGAGAGC
20	OarJMP8	6	121-133	F-CGGGATGATCTTCTGTCCAAATATGC R-CATTTGCTTTGGCTTCAGAACCAGAG

The number of alleles observed at a locus is an indication of genetic variability at that locus having direct impact on differentiation of breeds within a species. The allele size range of the loci was in agreement with that of loci investigated earlier in other Indian sheep breeds (Arora and Bhatia 2004; 2006; Sodhi et al 2006). The observed heterozygosity per locus ranged from 0.227 (Sonadi-OarAE129) to 1.000 (Marwadi-OarHH35). The expected heterozygosity per locus varied from 0.248 (Jalauni-OarCP20) to 0.852 (Sonadi-OarHH47). The highest average observed heterozygosity was seen in Sonadi (0.611) followed by Marwari (0.598) and Jalauni (0.570). The gene diversity (mean expected heterozygosity) estimate however were observed to be largest in Marwari (0.689) and lowest in Sonadi (0.670), with Jalauni (0.673) exhibiting a trend almost similar to Sonadi. The estimates of observed heterozygosity averages and gene diversity obtained in the present study were in close agreement with those reported in other domestic and exotic sheep breeds investigated earlier (Table 2). The gene diversity values were, however, much higher than wild Mouflon sheep (He 0.45) probably due to close captive relatedness in the wild sheep flock (Saitbekova et al. 2001). Observed heterozygosity was less than expected though not significantly different (using ANOVA test, $p>0.05$) when averaged over all loci, which indicated random mating in these investigated sheep breeds.

Table 2. Genetic diversity estimates in different sheep breeds

Sheep breeds	Na	Ho	He	Reference
Indian				
Northern temperate region				
Karnah	6.83	0.533	0.680	Gupta et al 2007
Gurez	7.08	0.450	0.670	Gupta et al 2007
Northwestern arid and semiarid region				
Muzzafarnagri	5.04	0.652	0.697	Arora and Bhatia2004
Magra	5.70	0.597	0.694	Arora and Bhatia2006
Nali	5.52	--	0.651	Sodhi et al 2006
Chokla	5.32	--	0.657	Sodhi et al 2006
Southern peninsular region				
Bellary	6.55	0.510	0.680	Kumar et al 2007
Hassan.	7.40	0.530	0.690	Sharma et al 2006
Eastern region				
Chhotanagpuri	5.64	0.510	0.680	Bhatia et al 2008
Bonpala	4.0	0.529	0.554	Pandey et al 2007
Exotic				
African (Djallonke)	6.37	0.650	0.670	Wafula et al 2005
Brazilian (Rabo Largo)	6.30	0.630	0.650	Paiva et al 2005.
Spanish (Latxa)	7.70	0.710	--	Arranz et al 1998
Swiss	--	--	0.630	Saitbekova et al 2001

The average means for various genetic diversity measures further suggested that all the sampled breeds contained high level of genetic variability. This study also reflected comparatively higher variability in Marwari than the Jalauni and Sonadi breeds which further substantiated the presence of several alleles in this Rajasthani breed.

Population inbreeding estimate (F_{IS}), which indicates heterozygote deficiency, was observed to be 11% in Jalauni, 17% in Marwari and 9% in Sonadi breed. The lack of heterozygotes in the three investigated breeds may be attributed to segregation of non amplifying (null) alleles, Wahlund effect (population structure) or inbreeding. In the present study it was not possible to estimate the extent of null alleles as no pedigree records were available for analysis and blood samples were taken from unrelated animals. However, in view of absence of pedigreed data under field conditions, the effect of relatedness of few samples otherwise deemed unrelated during collection may not be denied. Although the main cause for high genetic homogeneity or lack of heterozygotes in these Indian sheep breeds might be ascribed to inbreeding (overall positive FIS value), the possibility of Wahlund effect (population substructure) may also not be ruled out due to pooling samples (within breed) from different breeding flocks i.e. different villages in the same area. Concerted genetic management efforts which involve production of potential breeding rams and their frequent exchange between farmers' flocks are suggested in

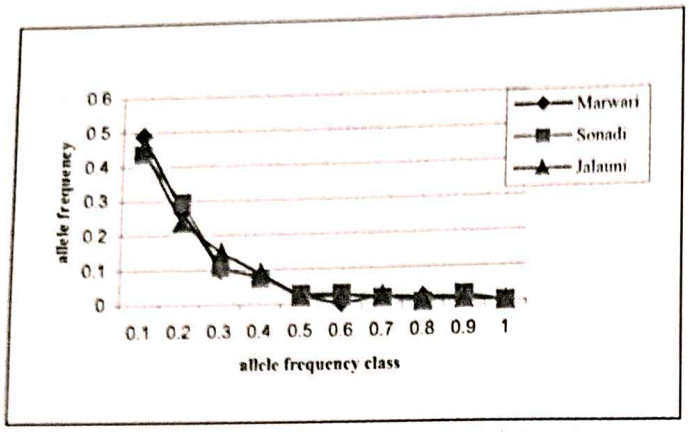

Figure 2. Normal L-shaped curve depicting absence of bottleneck

investigated sheep breeds, especially Marwari, to combat harmful/adverse effects of inbreeding in this native ovine germplasm.

Efforts were made to study recent genetic bottleneck effect (up to 40-80 generations) in the investigated breeds by subjecting the microsatellite data to Mode shift test (Luikart et al. 1998). The occurrence of a normal L-shaped curve in all the three breeds (Fig.2) revealed that these breeds have not experienced a genetic bottleneck and confirmed the absence of a recent reduction in the effective population size i.e. number of breeding individuals during several generations despite declining status of Jalauni sheep. The absence of genetic bottleneck in the experimental populations was consistent with the results of similar studies on other indigenous sheep breeds (Sodhi et al. 2003; Arora and Bhatia 2004; 2006; Mukesh et al. 2006).

BREED DIFFERENTIATION

Based on pairwise Fst estimate, genetic differentiation values ranged from 6.16% for Marwari – Sonadi breed pair to 14.8% for Jalauni – Sonadi breed pair. The G_{ST} values of breed differentiation were also similar to the F_{ST} estimates and ranged from 6.2% (Marwari-Sonadi pair) to 14.9% (Jalauni-Sonadi pair). The present values of genetic differentiation are lower than those reported for Korean and Chinese domestic goats (F_{ST} =20.2%, Kim et al. 2002), Swiss goat breeds (G_{ST} =17%, Saitbekova et al. 1999), Sub Saharan African goats (F_{ST} =15.7%, Chenyambuga, 2004) and Swiss sheep breeds (G_{ST} =18%, Saitbekova et al. 2001). The F_{ST} estimates obtained in this study suggested low to moderate level of genetic differentiation between the three Indian sheep populations of North-western arid and semi arid region (rates up to 15%, Hartl and Clark, 1997) in comparison to high genetic distinctness reported earlier among sheep breeds from two different geographical locations of the country (F_{ST} =18. 3% and G_{ST} =16.5%, Mukesh et al. 2006). In this study moderate variability between sheep breeds is most likely attributed to genetic drift/admixture between the investigated breeds (Saitbekova et al. 1999). Since no herd books are established till date and hence even today admixture of neighboring breeds is a common phenomenon.

The highest degree of genetic differentiation found between Jalauni - Sonadi was further supported by relatively low level of gene flow between these two breeds. Considerably higher level of gene flow observed between Marwari and Sonadi (3.6), in comparison to Jalauni – Marwari (Nm=2.1) or Jalauni - Sonadi (Nm=1.7) breed pairs, further substantiated genetic closeness of these two sheep breeds. Similar to present findings, Kim et al. (2002) also reported high genetic similarity between Chinese and Saanen goats (Nm =3.18) and Mukesh et al. (2006) in brown faced Indian sheep breeds of the same region (Nm =3.8). Gene flow (Nm) >1.0 is enough to attenuate the genetic differentiation between investigated breeds exhibiting moderate variability (Trexler, 1988). The high level of genetic exchanges between Marwari and Sonadi may be attributed to the highly migratory nature of Marwari breed and cross breeding with Sonadi or other nearby breeds in better years in view of higher productive potential of Sonadi under years with better foliage availability (Geerlings, 2004). Moreover, more and more Sonadi sheep breeders are adopting Marwari rams for breeding their native ewes since Marwari rams are not only hardy but are also superior to Sonadi as regards to wool production traits (Mehta et al. 1995).

Nei's (1972) genetic distance (DS), another parameter for estimating the genetic differentiation/ relationships among breeds, revealed a close relationship between Marwari and Sonadi breeds (0.261). While the distances between Jalauni-Sonadi (0.468) and Jalauni-Marwari (0.412) were comparatively greater which further supported highest degree of divergence between these breed pairs and substantial genetic similarity between Marwari and Sonadi breeds. Theses values were of the same order as those reported earlier for closely related sheep breeds (Arranz et al. 1998; Sodhi et al. 2006; Mukesh et al. 2006).

PHYLOGENETIC RELATIONSHIP

Phylogenetic tree constructed for genetic distances to elucidate relationships among these breeds showed that Marwari and Sonadi breeds clustered together whereas Jalauni appeared separated from both the breeds. These results suggested a marked differentiation of Jalauni from Marwari and Sonadi breeds, which appeared to be closely related to each other. The grouping of Marwari and Sonadi in our study is in agreement with behaviour and geographical propinquity of the studied breeds rather than the morphological differences between them, since morphological variations between populations are not taken into account by neutral markers such as microsatellites (Hartl and Clark, 1997). The results suggested that black faced Marwari and light brown faced Sonadi are closely related to each other than black/light brown faced Jalauni breed of same region. The close relationship between Marwari - good fleece breed and Sonadi - milk, meat, inferior carpet wool breed may be explained on the basis of highly migratory nature of Marwari. Following a trans-human system

of management in Marwari it has left greatest impact on other breeds, especially those with very coarse and hairy fleece like Malpura and Sonadi. Nevertheless, high genetic similarity between these two sheep breeds may also be ascribed to their occurrence as an admixture in some states of North-western arid and semi arid agro-ecological region viz., Rajasthan (Udaipur) and Gujarat (North Gujarat). The genetic distinctness of Jalauni from Marwari and Sonadi may perhaps be ascribed to its being isolated from other sheep breed (Sahana et al 2004).

The study contributes to the knowledge of genetic diversity of three important Indian sheep breeds that might have implications in future breed improvement, management and conservation programmes.

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