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Genetic Diversity of Magra Sheep from India Using Microsatellite Analysis

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ABSTRACT : Genetic diversity of Magra - a lustrous carpet wool breed of India, was investigated by means of 25 ovine microsatellite markers proposed by the Food and Agriculture Organization and the International Society for Animal Genetics (FAO-ISAG). All used microsatellites amplified well and exhibited polymorphisms. A wide range of genetic variability was observed as allele number from 3 (BM6506, OarCP20) to 10 (CSSM31), observed heterozygosity from 0.200 (BM6506) to 0.947 (OarHH35), expected heterozygosity from 0.368 (CSSM47) to 0.864 (BM1314) and Polymorphism Information Content (PIC) from 0.347 (CSSM47) to 0.849 (BM1314). This supported the utility of these microsatellite loci in the measurement of genetic diversity indices in Indian sheep too. Various average genetic variability measures viz., allele diversity (5.7), observed heterozygosity (0.597), expected heterozygosity (0.694) and mean PIC (0.648) values showed high genetic variability despite accumulated inbreeding as reflected by the high average inbreeding coefficient (F_{IS} = 0.159) due to the unequal sex ratio of the breeding animals. (**Key Words :** Magra, Indian Indigenous Sheep, Microsatellite, Genetic Diversity)

INTRODUCTION

Sheep is keystone species with over forty indigenous breeds in India (Acharya, 1982; Khan, 2001; Bhatia et al., 2004) reared for a) apparel wool, b) carpet wool, c) meat and carpet wool and d) meat play an important role in the biodiversity and livelihood of a large proportion of small and landless laborers. According to the Food and Agriculture Organization (FAO, United Nations) and World Watch list (2000) there exist sixty breeds of sheep in India. The list includes both well recognized and lesser known breeds along with some wild species. Due to indiscriminate crossbreeding, great amount of intermixing and habitat destruction more than 50% of India's sheep breeds are currently under threat (Press Information Bureau, Government of India, http://pib.nic.in/focus/fojan99/fo200199.html; Bhatia and Arora, 2005). This has led conservationists to recognize the significance of maintaining biodiversity in this important species of small ruminants. Not as widely recognized but potentially just as critical for ensuring long term survival, is the preservation of the sheep species' genetic structure at the breed/population level. Over the past decade, researchers have placed increased emphasis on the role that genetics plays in ovine conservation and have used genetic information to preserve variation within sheep species.

The potential of "microsatellites," molecular markers for genetic analysis of various livestock breeds has been illustrated by several workers (Kemp et al., 1995; Mukesh et al., 2004; Sun et al., 2004; Yang et al., 2004). Following the guidelines proposed by FAO (1996) under the global project for the measurement of domestic animal diversity (MoDAD), several programmes are in progress at the National Bureau of Animal Genetic Resources (NBAGR), Karnal, to genetically characterize indigenous sheep breeds by using microsatellite markers, which permit a highly precise dissection of the genetic structure.

Magra, a unique breed of sheep, formerly known as Bikaneri sheep is the only lustrous carpet wool producing breed of India (Figure 1). This important breed is found in the north western arid borders of Rajasthan and animals true to breed type are found only in the eastern and southern parts of Bikaner district. Magra sheep have been identified to be suitable for the desert and thrive happily on hard gravelly soil. It is peculiar to note that the breed can sustain if watered twice a week without any adverse effect on bodyweight. The breed is under constant threat due to much breeding with other breeds in the vicinity and there is serious need for its conservation. The present study was undertaken as part of the institute's ongoing programme on molecular genetic characterization of indigenous breeds of

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Figure 1. Magra ram.

sheep to (i) examine the effectiveness of the FAO recommended microsatellite markers for the measurement of genetic diversity and (ii) assess within breed genetic variability to establish genetic structure at the molecular level in Magra sheep.

MATERIAL AND METHODS

Sample collection and DNA extraction

During the period under study, genomic DNA from 48 blood samples of unrelated Magra sheep randomly collected across their breeding tract in line with MoDAD recommendations (FAO, 1993), was isolated by using the standard phenol-chloroform extraction method.

Genotyping

The genomic DNA was used for the amplification of twenty five FAO proposed microsatellite loci (Table 1). Polymerase Chain Reaction (PCR) was carried out in 25 µl reaction volume containing ~100 ng of template DNA, 50 ng of each primer, 200 µM of each dNTP, 0.5 units of Taq DNA Polymerase and 1.5 mM $MgCl_2$ using PTC-100 thermocycler (MJ Research Inc., MA, USA). A common "Touchdown" PCR programme used for the amplification of all the twenty five markers 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 and 20 cycles of 45 sec at 92°C, 1 min at 48°C (FAO 1996). The PCR products were separated on a 6% denaturing polyacrylamide gel and the resolved bands of DNA (alleles) were visualized by a silver

Table 1. Genetic variability measures in Magra sheep across 25 microsatellite markers

Locus	Chr. no.	Allele size range (bp)	No. of alleles		No. of genotypes	Heterozygosity		PIC	F_{IS}
			Observed (na)	Effective (ne)		Observed (Ho)	Expected (He)		
BM757	9	178-198	4	2.6	7	0.608	0.617	0.562	0.036
BM827	3	210-222	5	3.5	11	0.607	0.719	0.682	0.174*
BM1314	22	147-175	9	7.3	18	0.882	0.864	0.849	-0.006
BM6506	1	185-193	3	1.9	4	0.200	0.486	0.389	0.605*
BM6526	26	146-172	7	6.0	14	0.652	0.833	0.811	0.239*
BM8125	17	107-135	6	2.7	9	0.388	0.632	0.561	0.397*
CSSM31	23	130-170	10	4.9	14	0.863	0.797	0.775	-0.006
CSSM47	2	138-182	4	1.5	6	0.324	0.368	0.347	0.133
HUJ616	13	122-158	5	2.6	10	0.487	0.619	0.559	0.226*
OMHC1	20	189-213	7	4.5	13	0.703	0.781	0.753	0.119*
OarAE129	5	140-164	6	4.2	9	0.771	0.744	0.705	0.005
OarCP20	21	71-81	3	1.7	4	0.400	0.422	0.354	0.068
OarCP34	3	110-128	4	3.2	7	0.400	0.690	0.629	0.432*
OarFCB48	17	138-166	9	5.3	17	0.777	0.812	0.790	0.062
OarFCB128	2	108-134	4	3.6	9	0.727	0.728	0.677	0.025
OarHH35	4	128-160	8	6.4	10	0.947	0.844	0.825	-0.095
OarHH41	10	96-120	6	3.7	13	0.514	0.731	0.690	0.310*
OarHH47	18	122-152	7	3.9	12	0.310	0.749	0.717	0.597*
OarHH64	4	116-132	5	3.4	9	0.562	0.707	0.656	0.220*
OarJMP8	6	119-131	6	4.9	17	0.611	0.798	0.717	0.248*
OarJMP29	24	130-150	6	4.1	11	0.852	0.760	0.727	-0.107
OarVH72	25	113-137	7	3.9	15	0.657	0.744	0.715	0.129*
RM4	15	135-143	5	3.2	11	0.437	0.693	0.651	0.383*
TGLA137	5	119-161	4	2.5	7	0.600	0.601	0.530	0.017
TGLA377	2	86-122	4	2.5	7	0.657	0.601	0.530	-0.079
Mean			5.7	3.8		0.597	0.694	0.648	0.159*

* $p<0.05$.

staining procedure. The allelic size range was estimated by using a 10 bp sequencing ladder (Gibco BRL, life technologies, TM) as the standard molecular weight marker. The genotypes of individual animal at 25 microsatellite loci were scored visually.

Statistical analysis

The allele number for each locus was scored manually from the silver stained gels. The allele frequencies, observed number of alleles, effective number of alleles, observed and expected heterozygosity, within breed heterozygotes deficiency (F_{IS}) and past population genetic bottleneck were computed by using the appropriate software packages viz. POPGENE 3.2 (Yeh et al., 1999), FSTAT ver 2.9.3.2 (Goudet, 1995), and BOTTLENECK (Piry et al., 1999) The Polymorphism information content (PIC) was calculated as per Botstein et al. (1980).

RESULTS AND DISCUSSION

Loci variation

The various variability measures estimated across 25 loci in Magra sheep are shown in Table 1. All the microsatellites amplified well and were polymorphic in nature (Crawford et al., 1995). A total of 144 distinct alleles were detected across the analyzed microsatellite loci. The investigated 25 ovine microsatellites represented 19 autosomal chromosomes in sheep (Table 1). These microsatellite loci also exhibited high level of genetic variability as revealed by a wide range of alleles which varied from 3 (BM6506, OarCP20) to 10 (CSSM31). The effective number of alleles per locus ranged from 1.5 (CSSM47) to 7.3 (BM1314). These findings are in agreement with those of Garole, Nali, Chokla and Muzzafarnagri breeds of Indian sheep investigated earlier by the authors (Sodhi et al., 2003; Sodhi et al., 2004; Arora and Bhatia, 2004). The level of variation depicted by the number of alleles at each locus serves as a measure of genetic variability having direct impact on differentiation of breeds within a species (Buchanan et al., 1994). The allele size range observed in the studied population was in agreement with those of Swiss and Indian sheep breeds (Saitbekova et al., 2001; Sodhi et al., 2003). The number of genotypes per locus varied from 4 (BM6506, OarCP20) to 18 (BM1314). The high genotypic values could be attributed to the high number of alleles in Magra sheep, which also suggested the existence of heterozygous genotypes in this population.

The allele frequency data (not presented) revealed considerable variation in the distribution of allele frequencies between loci BM8125 (0.013) to CSSM47 (0.783) as observed earlier among exotic sheep breeds (Buchanan et al., 1994; Arranz et al., 1998). The low frequency of the most common alleles (<95%) at each investigated locus further supported the polymorphic nature of the used microsatellites and their utility in the measurement of diversity indices based on genetic polymorphism studies.

The observed and expected heterozygosity values of the microsatellite loci ranged from 0.200 (BM6506) to 0.882 (BM1314) and 0.368 (CSSM47) to 0.864 (BM1314) respectively. The use of microsatellites with a wide range of heterozygosity reduces the risk of overestimating genetic variability, which might occur with microsatellites exhibiting only high heterozygosity.

The PIC values varied from 0.347 (CSSM47) to 0.849 (BM1314). The fairly high PIC values (>0.5) for the majority of markers employed (88%), supported their utility in biodiversity evaluation (Kemp et al., 1995) of native Indian sheep too.

Intra breed genetic variation

The intra breed genetic variation is indicated by the overall allele diversity (mean number of observed alleles over a range of loci), observed (Ho) and expected heterozygosity (He) averages, mean polymorphism information content (PIC) value and within population inbreeding estimate (F_{IS}) (Table 1).

The overall allele diversity, considered to be a reasonable indicator of genetic variation within the population, displayed high genetic variation (5.7) in Magra sheep. The observed (0.597) and expected heterozygosity (0.694) averages of Magra were relatively similar to those of other domestic sheep breeds investigated earlier (Arranz et al., 1998; Saitbekova et al., 2001; Arora and Bhatia 2004) but were much higher than wild Mouflon sheep's expected heterozygosity (0.45) probably due to close captive relatedness within the wild sheep flock (Saitbekova et al., 2001). The mean observed heterozygosity values, though lower than the expected values, did not exhibit significant differences when tested using ANOVA ($p>0.05$), which suggested random mating in Magra. The high value of expected heterozygosity indicated that the population had retained the presence of several alleles although at low frequencies. This implied a substantial amount of genetic variability in Magra that might be used in planning breeding strategies particularly in populations of small sizes.

This population also showed a high within population inbreeding estimate (F_{IS} = 0.159), which revealed heterozygote deficiency in Magra, in comparison to Muzzafarnagri sheep investigated earlier by the authors. The average F_{IS} values for this breed and for most of the loci were significantly different from zero ($p<0.05$). Significant heterozygote deficiencies have also been reported in cattle (Mukesh et al., 2004). The high heterozygote deficiencies could be due to any one or more

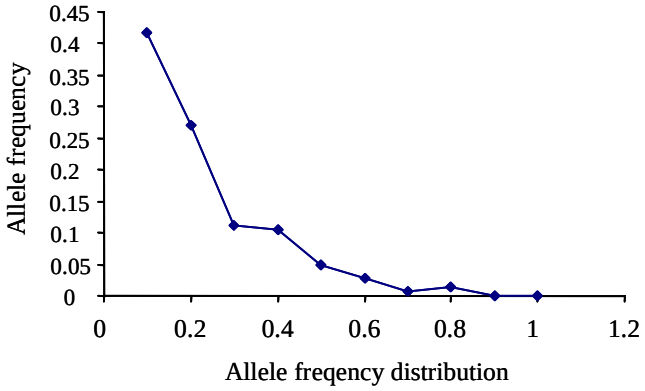

Figure 2. Normal L-Shaped curve depicting no Mode-shift in Magra sheep.

of the following: segregation of non amplifying (null) alleles, Wahlund effect (population substructure) or inbreeding. Moreover, due to uncontrolled mating in Indian sheep populations at the farmers' level, a breeding group most likely comprises a dominant male and some number of females, many males may aggregate in the vicinity of the oestrous females, but the dominant male generally excludes subordinate males, and presumably sire most of the offspring. With phylopatric females, breeding groups will become genetically differentiated (Chesser, 1991) leading to Wahlund effects at the level of sampling. In addition the breeding groups will be expected to be inbred, with the unequal sex ratio of breeding animals causing inbreeding to accumulate. These practices may explain the possibility of heterozygote deficiency in Magra to Wahlund effects due to pooling samples (within breed) from different breeding flocks i.e. different villages in the same area.

The mean PIC value of 0.648 further reflected the high level of polymorphisms of the used set of microsatellites and heterogeneity in Magra. The high estimates of PIC further substantiated the suitability of the used set of markers to applications such as parentage control, linkage-mapping programmes in addition to genetic polymorphism studies in Indian sheep.

The similar tendencies of the three variables viz., mean number of alleles, mean heterozygosity and mean PIC estimates observed in Magra showed it to be under mutation drift equilibrium (Hanslik et al., 2000). These measurements, however, behave differently when a population bottleneck is followed by a rapid population expansion (Kimmel et al., 1998). Efforts made to study recent bottleneck effect (up to 40-80 generations) in the investigated breed by using the Mode shift test (Luikart et al., 1998) revealed a normal L-shaped curve (Figure 2). This finding clearly suggested the absence of a recent reduction in the effective population size or a genetic bottleneck and further supported Magra as being a non-bottlenecked population under mutation drift equilibrium.

The present results support the usefulness of the battery of FAO recommended ovine microsatellite markers to assess the genetic variability of Indian sheep and further contribute to the knowledge of the genetic structure of Magra sheep. Further, extension of this approach under our Institute's ongoing programme in ovines by analysis of microsatellites to study the genetic variability within/between breeds and the relationships among different breeds of Indian sheep could contribute to the establishment of their own conservation programmes.

REFERENCES

Acharya, R. M. 1982. Sheep and goat breeds of India. FAO Animal production and Health Paper. 30. FAO of United Nations, Rome, Italy.

Arranz, J. J., Y. Bayon and F. San Primitivo. 1998. Genetic relationships among Spanish sheep using microsatellites. Anim. Genet. 29:435-440.

Arranz, J. J., Y. Bayon and F. San Primitivo. 2001. Genetic variation at microsatellite loci in Spanish sheep. Small Rumin. Res. 39:3-10.

Arora, R. and S. Bhatia. 2004. Genetic structure of Muzzafarnagri sheep based on microsatellite analysis. Small Ruminant Res. 54:227-230.

Bhatia, S. and R. Arora. 2005. Biodiversity and conservation of Indian sheep genetic resources-An overview. Asian-Aust. J. Anim. Sci. 18(10):1387-1402.

Botstein, D., R. L. White, M. Skolnick and R. W. Davis. 1980. Construction of genetic linkage maps in man using restriction fragment length polymorphisms. Am. J. Human Genet. 32:314-331.

Buchanan, F. C., L. J. Adams, R. P. Littlejohn, J. F. Madox and A. M. Crawford. 1994. Determination of evolutionary relationships among sheep breeds using microsatellites. Genomics 22:397-403.

Chesser, R. K. 1991. Gene diversity and female philopatry. Genet. 127:437-447.

Crawford, A. M., K. G. Dodds, A. J. Ede, C. A. Pierson, G. W. Montgomery, H. G. Garmonsway and A. E. Beattie. 1995. An autosomal genetic linkage map of the sheep genome. Genet. 140:703-724.

FAO. 1993. An integrated global programme to establish the genetic relationships among the breeds of each domestic animal species.

FAO. 1996. Global project for the Maintenance of Domestic Animal Genetic Diversity (MoDAD). http://www.fao.org/dad-is/

FAO. 2000. World Watch List for Domestic Animal Diversity. 3rd edition. (Ed. Beate D. Scherf) FAO of United Nations, Rome, Italy.

Goudet, J. 1995. FSTAT (vers. 2.9.3): a computer program to calculate F-statistics. J. Hered. 86:485-486.

Hanslik, S., V. Harr, G. Brem and C. Schlotterer. 2000. Microsatellite analysis reveals substantial genetic differentiation between contemporary New World and Old World Holstein Friesian populations. Anim. Genet. 31:31-38.

Kemp, S. J., O. Hishida, J. Wambugu, A. Rink, M. L. Longeri, R. Z. Ma, Y. Da, H. A. Lewin, W. Barendse and A. J. Teale. 1995. A panel of polymorphic bovine, ovine and Caprine microsatellite markers. Anim. Genet. 26:299-306.

Khan, B. U. 2001.Conservation and management of sheep genetic resources of India. In: Proceedings of Summer School on Recent advances in Characterization and Conservation of Animal Genetic Resources. 16th July-6th Aug. NBAGR, Karnal. pp. 162-176.

Kimmel, M., R. Chakraborty, J. P. King, M. Bamshad, W. S. Watkins and L. B. Jorde. 1998. Signatures of population expansion in microsatellite repeat data. Genet. 148:1921-1930.

Luikart, G., F. W. Allendorf, J. M. Cornuet and W. B. Sherwin. 1998. Distortion of allele frequency distributions provides a test for recent population bottleneck. J. Hered. 89:238-247.

Mukesh, M., M. Sodhi, S. Bhatia and B. P. Mishra. 2004. Genetic diversity of Indian native cattle breeds as analysed with 20 microsatellite loci. J. Anim. Breed. Genet. 121:416-424.

Piry, S., G. Luikart and J. M. Cornuet. 1999. BOTTLENECK a computer programme for detecting reductions in the effective size using allele frequencies. J. Hered. 90:502-503.

Press Information Bureau. Government of India. Focus; List of threatened breeds of India. pp. 1-2. http://pib.nic.in/focus/fojan99/fo200199.html.

Saitbekova-Stahlberger, N., J. Schlapfer, G. Dolf and C. Gaillord. 2001. Genetic relationships in Swiss sheep breeds based on microsatellite analysis. J. Anim. Breed. Genet. 118:379-387.

Sodhi, M., M. Mukesh, R. Arora, M. S. Tantia and S. Bhatia. 2003. Genetic structure of Garole- a unique Indian microsheep assessed using microsatellite markers. Indian J. Dairy Sci. 56(3):167-173.

Sodhi, M., M. Mukesh and S. Bhatia. 2004. Microsatellite based genetic characterization of three breeds of Indian sheep. In Proceedings of the 91st Indian Science Congress, 3-7 January 2004. Chandigarh. India

Sun, W., H. Chang, Z. J. Ren, Z. P. Yang, R. Q. Geng, S. X. Lu, L Du and K. Tsunoda. 2004. Genetic differentiation between sheep and goats based on microsatellite DNA. Asian-Aust. J. Anim. Sci. 17(5):583-587.

Yang, Z. P., H. Chang, W. Sun, R. Q. Gen, Y. J. Mao and K. Tsunoda. 2004. A comparison of two kinds of markers applied in analysis of genetic diversity in sheep and goat populations. Asian-Aust. J. Anim. Sci. 17(7):892-896.

Yeh, F. C., T. Boyle, Y. Rongcai, Z. Ye and J. M. Xian. 1999. POPGENE version 1.31. A Microsoft window based freeware for population genetic analysis. University of Alberta, Edmonton.