Short Communication

Indian Journal of Animal Sciences **83** (7): 768–771, July 2013/short communication

Genetic analysis of Osmanabadi goat breed*

Z I BHAT[1], S V KURALKAR[2], KRANTI KHARKAR[3] and PRAJAKTA KURALKAR[4]

Post Graduate Institute of Veterinary and Animal Sciences, Akola, Maharashtra 444 104 India

Received: 23 October 2012; Accepted: 27 February 2013

Key words: Allele, Goat, Heterozygosity, Microsatellite markers, Osmanabadi

The Osmanabadi goat (Accession number: INDIA_Goat_1100_ Osmanabadi _06017) is one of the meat type breeds of Maharashtra. Osmanabadi breed derives its name from its habitat Osmanabad district in Maharashtra. The breed is mainly concentrated and found in Latur and Solapur district of Maharashtra besides Osmanabad. This breed is predominant in scarcity zone and thrives under harsh climatic condition with low grade roughages, capable of walking long distance for grazing. The farmers of this region keep small flocks of Osmanabadi goats to meet out the family requirement of milk and finally sell the live animals for meat, this provides dependable income source to poorest poor population of rural areas. Osmanabadi is a medium sized breed with comparatively long body, long legs and most of the animals are black in colour. The breed shows an efficient reproductive performance. The meat quality is of lean type and preferred by most of the rural and urban population in Southern India. In view of the paramount importance of this breed in India, present research work was undertaken to characterize Osmanabadi goat at molecular level using microsatellite markers.

Experimental material for the present study comprised of 31 blood samples of Osmanabadi goat collected at random from unrelated animals from Nilanga and Deoni taluka of Latur Distrct, after questioning owners in detail in order to avoid close relationships. The samples of Osmanabadi goat were also collected from Rural Institute of Dairy Management, Amravati, Krishi Vigyan Kendra (KVK), Durgapur, KVK, Ghatkhed and Punyashlok Ahilya Devi Sheli Mendhi Farm, Pohara (Amravati), after verifying the pedigree record, samples were taken from unrelated animals.

The blood samples were collected from jugular vein in the vacutainer tubes having EDTA as blood anticoagulant. The samples were brought to the laboratory in an ice-cooled box, where they were kept under –20°C in a deep freezer until DNA isolation. Genomic DNA was isolated from the blood samples using a standard phenol : chloroform extraction method (Sambrook and Russell 2001). A battery of 25 heterologous polymorphic microsatellite markers used earlier (Sharma *et al.* 2008[a], Sharma *et al.* 2008[b]) were selected.

Polymerase chain reaction (PCR) was carried out on about 50–100 ng genomic DNA in a 25 µl reaction volume. The reaction mixture consisted of 200 µM each of dATP, dCTP, dGTP and dTTP, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.0 mM $MgCl_2$, 0.75 unit *Taq* DNA polymerase and 4 ng/µl of each primer using Mastercycler pro S PCR machine. The touchdown PCR protocol was used with initial denaturation of 95°C for 1 min, 3 cycles of 95°C for 45 sec and 60°C for 1 min, 3 cycles of 95°C for 45 sec and 57°C for 1 min, 3 cycles of 95°C for 45 sec and 54°C for 1 min, 3 cycles of 95°C for 45 sec and 51°C for 1 min, and 20 cycles of 95°C for 45 sec and 48°C for 1 min. At the end of the reaction, 5.0 µl of stop dye (95% formamide, 0.25% bromophenol blue and 0.25% xylene cyanol) was added and 6 µl of PCR products was loaded on a 2% agarose gel electrophoresis and visualized over UV light after ethidium bromide staining to detect the amplification.

The PCR products were resolved on 6% denaturing polyacrylamide gels, 100 bp ladder was used as a size standards for sizing PCR product. To visualize the PCR product, gels were stained using silver staining (Bassam *et al.* 1991) and dried between sheets of cellophone paper. The scoring of allele was carried out following gel documentation on a gel-doc system, where the image capture and analysis was done by Quantity oneTm software. While the scoring of the bands was aided by the software, the final allele calling was manually accomplished by the joint observation of two independent observers.

*Part of M.V. Sc. thesis submitted by first author to the Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra.

Present address: [1]At-Kharbrari, Post-Bhgam Yaripura, Tah-Kulgaon, Anantnag (Jammu and Kashmir). [2]Head and Associate Professor (svkuralkar@rediffmail.com), [3]Assistant Professor (drkranti1@gmail.com), M. B. Veterinary College, Dungarpur (Rajasthan). [4]Assistant Professor, Department of Veterinary Physiology (pskuralkar@rediffmail.com).

For the 25 microsatellites loci analysed, observed and effective number of alleles (Kimura and Crow 1964); observed and expected heterozygosity estimates were calculated after Levene (1949) and Nei (1973), as implemented in the POPGENE software (Yeh *et al*. 1999) to determine genetic variation within the breed. The tests for deviation from Hardy–Weinberg equilibrium were also derived using the exact tests of POPGENE. Polymorphism information content values (PIC) for all the loci was estimated according to Botstein *et al.* (1980). Heterozygote deficiency, gene diversity and allelic richness was calculated with FSTAT (Goudet 1995).

Bottleneck hypothesis (Cornuet and Luikart 1996) was investigated using BOTTLENECK 1.2.01. The BOTTLENECK tests for the departure from mutation drift equilibrium were based on heterozygosity (not heterozygotes), excess or deficiency. The BOTTLENECK test compares heterozygosity expected at Hardy–Weinberg equilibrium (H_E) with the heterozygosity expected at mutation drift equilibrium (H_{eq}) in the same sample, that has the same size and the same number of alleles. All the three models of mutation were used to calculate H_{eq}: the strict 1 step-wise mutation model (Ohta and Kimura 1973), the infinite allele model (Kimura and Crow 1964) and the 2-phase model (Di Rienzo *et al*. 1994).

The number of alleles observed over a range of loci in different population is considered to be a reasonable indicator of genetic variation within the population (MacHugh *et al*. 1997). The observed number of alleles varied from 5 (RM088) to 21 (OarAE129) with a mean of 12.36±0.822. The effective number of alleles varied from 2.324 (ILSTS065) to 12.903 (OarAE129) with a mean 8.277±0.626. The mean observed and effective number of alleles were higher in the present study than that reported by Kumar *et al*. (2009) in Gohilwari, Verma *et al.* (2010) in Sangamneri, Vijh *et al.* (2010) in Black Bengal and Chegu, Mishra *et al*. (2010) in Changthangi and Dixit *et al.* (2011) in Kanniaddu goat breed. A total of 309 alleles were observed for 25 loci and the allele size ranged from 86 bp (OarJMP29) to 239 bp (ILSTS022). Our values are comparable with the values obtained for Kanniaddu goat breed (Dixit *et al.* 2011).

The observed heterozygosity ranged from 0.074 (ILSTS002) to 1.0 (RM004) with an average of 0.707±0.062 (Table 1) whereas, expected heterozygosity ranged from 0.579 (ILSTS065) to 0.940 (OarAE129) with an overall mean of 0.867±0.017. Vijh *et al.* (2010) reported similar estimate of observed and expected heterozygosity in Black Bengal and Chegu goat, while, lower estimate were reported in Barbari (Sharma *et al.* 2008[a]), Beetal (Sharma *et al.* 2008b), Malabari (Verma *et al.* 2009), Gohilwari (Kumar *et al*. 2009), Changthangi (Mishra *et al*. 2010) and Kanniaddu (Dixit *et al*. 2011) goat breeds. Genetic variation observed in Osmanabadi goat breed may be due to their large effective population size and low selection pressure.

The Shannon's information index (I) represents the relative abundance of genetic information of a specific locus to the total information available of the loci. The index (I) value ranged from 1.202 (ILSTS065) to 2.724 (OarHH64) with an average of 2.194±0.087. The polymorphic information index (PIC) values ranged from 0.604 (RM088) to 0.915 (ILSTS008) with an average of 0.836±0.019. PIC estimated in the present study are comparable with the findings of Aggarwal *et al.* (2007) in Mehsana (0.72) and Vijh *et al.* (2010) in Black Bengal (0.80) and Chegu goat (0.80). In contrast, lower PIC values were reported by Kumar *et al*. (2009) in Gohilwari, Verma *et al.* (2010) in Sangamneri, and Dixit *et al.* (2011) in Kanniaddu goat breed than the present study.

The F statistics showed that 13 out of 25 loci had positive F_{is} values, which indicated significant heterozygote deficiency at these loci (Table 1) and showed deviation from Hardy Weinberg equilibrium. The negative values of F_{is} indicated out breeding i.e. mating of individuals who are less related than the average relationship of the population. Among the positive values the F_{is} varied from 0.049 (OARFCB304) to 0.909 (ILSTS002) with a mean of 0.187. Significant heterozygote deficiencies have also been reported in Indian goats *viz.,* Mehsana (Aggarwal *et al*. 2007), Barbari (Sharma *et al.* 2008[a]), Beetal goat (Sharma *et al.* 2008b), Malabari goat (Verma *et al.* 2009), Mishra *et al.* (2010) in Changthangi and Dixit *et al.* (2011) in Kanniaddu goat. The fairly high value of F_{is} (0.187) indicated that some of the loci in this breed were homozygous presumably resulting from the mating between relatives and consequent genetic drift.

Mutation drift equilibrium: The sign, standardized differences and Wilcoxon tests under bottleneck hypothesis showed mutation-drift-equilibrium in the population for most of the loci studied by allele frequency method. However, based on heterozygosity, standardized differences test indicated significant departure of the population from the mutation drift equilibrium in the entire model, sign test indicated significant departure in 2 phase and single step mutation models whereas Wilcoxon test indicated mutation drift equilibrium in all models. The sign test revealed substantial heterozygosity deficiency under SMM model in both methods. The standardized difference test also showed significant heterozygosity deficiency in Osmanabadi goat population under SMM model in frequency methods and under all model in heterozygosity method. In Wilcoxon rank test probability values for one tail for H excess (He) in all the 3 models in both the methods probability values are more than 0.05.

Mode shift analysis: Allele frequency spectra were visualized to be L shaped through the qualitative graphical method of Cornuet and Luikart (1996). The microsatellite alleles were organized into 10 frequency classes, which permit checking whether the scattering followed the normal

Table 1. Measures of genetic variation in Osmanabadi goat at studied microsatellites loci

Locus	Haploid Sampled	Size (range) of alleles bp	Observed number of alleles	Effective number of alleles	Observed heterozygosity	Expected heterozygosity	Nei expected heterozygosity	Shannon's information index (I)	PIC value	Heterozygote deficiency (F_{is})
	N		Na	Ne	Ho	He	Nei			
ETH225	28	150–170	7	4.392	0.821	0.786	0.772	1.638	0.737	–0.045
ILSTS002	27	130–140	6	4.658	0.074	0.800	0.785	1.653	0.755	0.909
ILSTS005	30	177–203	14	10.976	0.967	0.924	0.909	2.514	0.902	–0.047
ILSTS008	31	162–194	17	12.562	0.871	0.935	0.920	2.642	0.915	0.070
ILSTS019	31	143–169	12	5.324	0.903	0.825	0.812	2.004	0.792	–0.096
ILSTS022	29	195–239	18	12.277	0.310	0.935	0.919	2.664	0.913	0.672
ILSTS029	30	154–180	8	4.592	0.967	0.795	0.782	1.696	0.749	–0.220
ILSTS030	29	164–188	12	8.995	0.931	0.904	0.889	2.319	0.879	–0.030
ILSTS033	26	161–189	13	9.657	0.808	0.914	0.896	2.390	0.887	0.118
ILSTS034	31	154–180	13	8.940	0.935	0.903	0.888	2.348	0.878	–0.037
ILSTS044	31	161–195	16	8.580	0.613	0.898	0.883	2.405	0.873	0.321
ILSTS049	29	166–194	14	9.397	0.931	0.909	0.894	2.393	0.884	–0.024
ILSTS058	31	116–146	12	8.580	0.968	0.898	0.883	2.288	0.873	–0.079
ILSTS059	26	116–140	10	8.667	0.308	0.902	0.885	2.205	0.873	0.663
ILSTS065	31	118–132	6	2.324	0.355	0.579	0.570	1.202	0.542	0.391
ILSTS082	27	102–134	14	10.721	0.926	0.924	0.907	2.489	0.899	–0.002
ILSTS087	30	144–176	15	10.405	0.967	0.919	0.904	2.501	0.896	–0.053
OarAE129	27	141–187	21	12.903	0.889	0.940	0.922	2.774	0.917	0.055
OarHH64	27	129–151	10	6.284	0.519	0.857	0.841	1.999	0.821	0.399
OarJMP29	30	86–120	17	11.921	0.200	0.932	0.916	2.664	0.910	0.788
OMHC1	27	180–216	13	8.191	0.519	0.894	0.878	2.286	0.866	0.425
RM004	30	116–130	8	6.338	1.000	0.856	0.842	1.941	0.823	–0.171
OarFCB48	30	148–174	12	5.294	0.900	0.825	0.811	1.985	0.790	–0.093
RM088	29	117–131	5	3.009	0.103	0.679	0.668	1.224	0.604	0.850
OARFCB304	27	177–207	16	11.951	0.889	0.934	0.916	2.620	0.910	0.049
Mean	28.960		12.360	8.277	0.707	0.867	0.852	2.194	0.836	0.187
SE	0.349		0.822	0.626	0.062	0.017	0.017	0.087	0.019	0.069

L shaped form, where alleles with low frequencies (0.01–0.1) are the most numerous. The L shaped curve thus obtained indicated that Osmanabadi population has not suffered genetic bottleneck in the recent past (Fig 1).

In conclusion, study showed that all the microsatellite markers used were highly polymorphic and informative for molecular characterization of Osmanabadi goat. There was substantial genetic variability across studied loci in Osmanabadi goats as reflected from the heterozygocity and number of allele per locus. Therefore, appropriate breeding strategies should be designed under field conditions for its conservation and improvement.

SUMMARY

A battery of 25 selected micro-satellite markers was used to study the genetic variability in Osmanabadi breed. The observed number of alleles varied from 5 (RM088) to 21 (OarAE129) with a mean of 12.36±0.822. The effective number of alleles varied from 2.324 (ILSTS065) to 12.903 (OarAE129) with a mean 8.277±0.626. The observed heterozygosity ranged from 0.074 (ILSTS002) to 1.0 (RM004) with an average of 0.707±0.062 whereas, expected heterozygosity ranged from 0.579 (ILSTS065) to 0.940 (OarAE129) with an overall mean of 0.867±0.017. All the studied loci showed the polymorphic information content value greater than 0.5 The F statistics showed that 13 out of 25 loci had positive F_{is} values, among the positive values the F_{is} varied from 0.049 (OARFCB304) to 0.909 (ILSTS002) with a mean of 0.187. The L shaped curve obtained indicated that Osmanabadi population has not undergone any recent bottleneck. The study indicated that Osmanabadi goats exhibited substantial amount of genetic variation as reflected from the heterozygosity and number of alleles per locus.

ACKNOWLEDGEMENTS

Authors thank the Department of Science & Technology, New Delhi, for providing financial support for the present study under the project of Fast Track Scheme for Young Scientists. Thanks are also expressed to the University

Authority of Maharashtra Animal and Fishery Sciences University, Nagpur for providing the necessary facilities.

REFERENCES

Aggarwal R A K, Dixit S P, Verma N K, Ahlawat S P S, Kumar Y, Kumar S, Chander R and Singh K. 2007. Population genetics analysis of Mehsana goat based on microsatellite markers. *Current Science* **92** (8): 1133–37.

Bassam B J, Coetano-Anolles G and Gresshoff P M. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. *Analytical Biochemistry* **196**: 80–83.

Botstein D, White R L, Skolnick M and Davis R W. 1980. Construction of a genetic linkage map in man using restriction fregment length polymorphism. *American Journal of Human Genetics* **32**: 324–31.

Cornuet J M and Luikart G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**: 2001–14.

Dixit S P, Aggarwal R A K, Verma N K, Vyas M K, Rana J, Sharma A, and Chander R. 2011. Genetic variability and bottleneck analyses of Kanniadu goat breed based on microsatellite markers. *Indian Journal of Animal Sciences* **81**: 40–43.

Di Rienzo A, Peterson A C, Garca J C, Valdes A M and Slatkin M. 1994. Mutational proceses of simple sequence repeats in human populations. *Proceedings of National Academy of Sciences*, *USA* **91**: 3168–70.

Goudet J. 1995. FSTAT (version1.2): A programme to calculate F-statistics. *Journal of Heredity* **86**: 485–86.

Kumar D, Dixit S P, Verma N K, Singh D K, Pande A, Kumar S, Chander R and Singh L B. 2009. Genetic diversity analysis of the Gohilwari breed of Indian goat (*Capra hircus*) using microsatellite markers. *American Journal of Animal and Veterinary Sciences* **4** (3): 49–57.

Kimura M and Crow J W. 1964. The number of alleles that can be maintained in a finite population. *Genetics* **49**: 725–38.

Levene H. 1949. On a matching problem arising in genetics. *Annals of Mathmetic Statistics* **20**: 91–94.

MacHugh D E, Loftus R T, Cunningham P and Bradley D G. 1997. Microsatellite DNA variation and the evolution, domestication and phylogeography of Taurine and Zebu cattle (*Bos taurus* and *Bos indicus). Genetics*. **146**: 1071–86.

Mishra P, Verma N K, Aggarwal R A K and Dixit S P. 2010. Breed characteristics and genetic variability in Changthangi goats. *Indian Journal of Animal Sciences* **80** (12): 1203–09.

Nei M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of National Academy of Sciences USA* **70**: 3321– 23.

Ohta T, and Kimura M. 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. *Genetic Research Cambridge* **22**: 201–04

Sambrook, J and Russell D W.2001. *Molecular Cloning: A Laboratory Manual.* 3rd edn. Cold spring Harbour Cold Spring Laboratory Press NY.

Sharma R, Kumar D, Pandey A K, Dixit S P and Ahlawat S P S. 2008[a]. Genetic variability and bottleneck analysis of Barbari goat population using microsatellite markers. *Indian Journal of Animal Sciences* **78** (4): 391–96.

Sharma R, Kumar D, Pandey A K, Dixit S P and Ahlawat S P S. 2008[b]. Genetic variability in Beetal goat breed assessed by microsatellite markers. *Indian Journal of Animal Sciences* **78** (5): 505–509.

Verma N K, Dixit S P, Dangi P S, Aggarwal R A K, Kumar S and Joshi B K. 2009. Malabari goats: Characterization, management, performance and genetic variability**.** *Indian Journal of Animal Sciences* **79** (8): 813–18.

Verma N K, Dixit S P, Aggarwal R A K, Dangi P S and Joshi B K. 2010. Phenotypic and genetic characterization of Sangamneri goat breed. *Indian Journal of Animal Sciences* **80** (11): 1109–14.

Vijh R K, Tantia M S, Bhel R and Bina Mishra. 2010. Genetic architecture of Black Bengal and Chegu goats. *Indian Journal of Animal Sciences* **80** (12): 1134–37.

Yeh F C, Boyle T, Rongcai Y, Ye Z and Xian J M. 1999. POPGENE version 3.1(http: //www.ualberta.ca/-fyeh/fyeh.)