



Yalaga sheep - A microsatellite based genetic profile

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ABSTRACT

The genetic profile of Yalaga sheep of Karnataka was sketched based on genotypes at 25 microsatellite markers recommended for Indian sheep. A high level of genetic variation was discovered within the investigated breed as quantified from various genetic diversity estimates. Distinct alleles (240) were detected across the analyzed microsatellite loci. The mean number of observed alleles per microsatellite marker was 9.60 and number of effective alleles was 4.86. The average observed and expected heterozygosity values were 0.596 and 0.757, respectively. The mean polymorphism information content (PIC) value (0.725) reflected high level of genetic variability and utility of the markers used. The high F_{IS} value (0.215) may have been generated due to heterogeneity among the flocks sampled within the population caused by Wahlund effect. The information generated may be useful in guiding conservation management programs for sheep breeds in Karnataka.

Key words: Genetic variability, Karnataka, Microsatellite, Yalaga sheep

Use of commercial breeds of livestock for production gains over several decades, has led to stagnation or decline of the genetic diversity within species. Since India is one of the mega biodiversity hotspots, there is no dearth of genetic diversity in livestock species especially sheep. There are 40 registered sheep breeds in India, which have been characterised and documented. However, there are several local breeds with potential for improvement, which possess traits and genetic diversity that are still unidentified. Such breeds or populations need to be characterised and their genetic potential should be assessed for future utilisation. Microsatellite markers have been used extensively for the quantification of genetic diversity in livestock breeds all over the world (Arora and Bhatia 2004, Peter *et al.* 2007, Ginja *et al.* 2013, Montenegro 2015). Since microsatellites are neutral or non-coding, they are independent of natural selection. Hence, these markers are most suited for studies on conservation genetics. Information of genetic diversity of populations would enable their prioritization for guiding conservation management programs (Hanotte and Jianlin 2005).

Karnataka harbours some of the best ovine germplasm for mutton production and is the second largest producer of sheep meat in India. The 4 registered sheep breeds in Karnataka are Kenguri, Bellary, Hassan and Mandya (Acharya 1982). Besides these 4 breeds, another relatively

lesser known sheep population in Karnataka is the Yalaga, which also finds mention as a rare breed, in a Gazetteer compiled for the Government of India in 1877 (Rice 1877). The Yalaga sheep are long legged, long necked, thin bodied, with drooping hind quarters, short tail and well known for their high quality mutton (Singh 1967). Since sheep are able to subsist on meagre inputs, such lesser known sheep of economic value may provide alternate means of income for marginal farmers. Therefore, characterization of Yalaga sheep at the genetic level is pertinent as it is an important mutton breed on which negligible information is available. This study was therefore undertaken to quantify the genetic diversity of the Yalaga sheep population using microsatellite markers.

MATERIALS AND METHODS

Blood sampling and DNA extraction: Random blood samples of 52 Yalaga sheep were collected from Bagalkot district of Karnataka for microsatellite based genetic characterisation. Care was taken to avoid the possibility of mixing of blood with other populations or breeds by taking samples from animals exhibiting specific characteristics. Genomic DNA was isolated using the standard phenol chloroform extraction method (Sambrook *et al.* 1989).

Microsatellite analysis: Genetic diversity was assayed in Yalaga sheep using 25 microsatellite markers. Of the 25 microsatellite markers used in the study, 20 are recommended for ovines (Bradley *et al.* 1997), and the remaining markers (CSR247, HSC, INRA63, MAF214 and OarCP49) were taken from the panel of markers for parentage verification tested at the 2001/02ISAG

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comparison test (Di Stasio 2001). The forward primer for each marker was fluorescently labelled with one of the dyes - FAM, NED, VIC or PET. Amplifications of the loci were performed in 25 µl of final reaction volumes containing at least 100 ng of genomic DNA, 5 pM of each primer, 1.5 mM MgCl₂, 100 µM dNTPs, 0.5 U Taq polymerase and 1× buffer. A common touchdown PCR programme was used for amplification (Bradley *et al.* 1997). The 25 markers were divided into 5 multiplexes with 5 markers in each plex. Amplification was confirmed on 2% agarose gel, and the genotyping was carried out on an automated DNA sequencer using LIZ 500 as the internal size standard. Allele sizing was performed using GENEMAPPER software.

Statistical analysis of data: Allele frequencies, observed number of alleles (N_a), observed heterozygosity (H_o) and expected heterozygosity (H_e) were calculated using the GenAlex program (Peakall and Smouse 2006). Polymorphism information content (PIC) was calculated according to Botstein *et al.* (1980). The genetic bottleneck effect was inferred for the populations using mode shift analysis under the assumption of the two-phase microsatellite mutation model, implemented in the program Bottleneck version 1.2.02 (Piry *et al.* 1999).

RESULTS AND DISCUSSION

Marker diversity: The 25 microsatellite markers used in the investigation covered 19 ovine chromosomes. All the microsatellite loci amplified well and were effective in detecting polymorphism with reasonable amount of genetic variation apparent from the allele frequency data as well as more than 3 alleles/marker. Distinct alleles (240) were detected across the analysed microsatellite loci. The polymorphic information content is a parameter indicative of the degree of informativeness of a marker. Following the criteria of Botstein *et al.* (1980), in the present study, all the markers except BM6506 were highly informative (PIC >0.5). The most polymorphic locus was OarHH35 (PIC=0.875) and the least polymorphic was BM6506 (PIC=0.393). High level of genetic variability and utility of the set of markers used in biodiversity evaluation of native Indian sheep was reflected by the fact that 96% of markers were highly polymorphic (PIC ≥ 0.5, Botstein *et al.* 1980).

Intra-breed genetic diversity: The genetic diversity measures for Yalaga sheep are reported in Table 1. The number of observed alleles (N_a) varied between 3 (BM6506) and 15 (HSC) with an average of 9.60. The effective number of alleles (N_e) was less than the observed values ranging from 1.99 (BM6506) to 8.76 (OarHH35) with mean of 4.86. A high degree of intra-breed genetic variation was observed within Yalaga sheep in terms of allele diversity. Previous studies on other Indian ovine breeds also reported substantial level of allele diversity (Arora *et al.* 2011a, b, c). These values were higher than those reported for Bellary, Mandya, Kenguri and Hassan sheep of Karnataka (Table 2). A direct comparison cannot be made as these studies were based on silver staining and manual recording of

Table 1. Genetic diversity measures for Yalaga sheep across 25 microsatellite markers

Locus	N_a	N_e	H_o	H_e	F_{IS}
BM0757	8	3.243	0.521	0.692	0.247
BM0827	7	5.062	0.795	0.802	0.009
BM1314	11	3.055	0.298	0.673	0.557
BM6506	3	1.992	0.426	0.498	0.145
BM6526	14	7.681	0.447	0.870	0.486
BM8125	6	2.854	0.580	0.650	0.107
CSRD247	12	7.379	0.706	0.864	0.183
CSSM31	12	7.425	0.809	0.865	0.066
CSSM47	12	4.617	0.605	0.783	0.228
HSC	15	6.245	0.682	0.840	0.188
INRA63	14	4.700	0.553	0.787	0.297
MAF214	7	2.330	0.304	0.571	0.467
OarAE129	7	3.343	0.105	0.701	0.850
OarCP20	7	2.314	0.694	0.568	-0.222
OarCP34	9	4.728	0.559	0.788	0.291
OarCP49	10	6.793	0.880	0.853	-0.032
OarFCB128	10	7.257	0.740	0.862	0.142
OarFCB48	11	6.046	0.761	0.835	0.088
OarHH35	11	8.767	0.590	0.886	0.334
OarHH41	6	3.176	0.646	0.685	0.057
OarHH47	11	5.501	0.720	0.818	0.120
OarHH64	8	3.653	0.409	0.726	0.437
OarJMP08	10	5.273	0.711	0.810	0.122
OarJMP29	11	4.437	0.720	0.775	0.070
OarVH72	8	3.752	0.633	0.733	0.137
	9.6	4.865	0.596	0.757	0.215

N_a , observed alleles; N_e , effective number of alleles; H_o , observed heterozygosity; H_e , expected heterozygosity; F_{IS} , heterozygote deficiency.

genotypes. However, the observed number of alleles in Yalaga was comparable to that reported for Madras Red (9.8, Selvam *et al.* 2009) and Ramnad White (9.667, Raja *et al.* 2012). The effective number of alleles (mean=4.86) though lower than the allele diversity in Yalaga was also in conformity with other indigenous breeds investigated earlier (Arora *et al.* 2011a). The average observed heterozygosity was less than the expected. The intra-population observed heterozygosity ranged from 0.105 (OarAR129) to 0.880 (OarCP49). The expected heterozygosity per locus varied from 0.498 (BM6506) to 0.886 (OarHH35) in Yalaga sheep.

The mean average observed (H_o) and expected heterozygosity (H_e) values were 0.596 and 0.757, respectively. The estimates of heterozygosity for Yalaga sheep were similar to those of the registered breeds of Karnataka (Table 2). The gene diversity (H_e) values, reflecting a considerable level of genetic variability in Yalaga sheep, were in accordance to earlier studies in several indigenous sheep breeds (Prasanna *et al.* 2009, Arora *et al.* 2010, Arora *et al.* 2011a). Significant deviations from Hardy Weinberg equilibrium (HWE) observed at most of the loci ($P < 0.001$) in the investigated population might be due to heterozygote deficit.

A high level of F_{IS} (0.215) revealed heterozygote deficiency in Yalaga sheep population. Significant

Table 2. Comparative genetic diversity estimates of sheep breeds of Karnataka

Breed	Sample No. used	No. of markers	Genotyping method	No. of alleles		Heterozygosity		PIC	F _{IS}	Reference
				N _a	N _e	H _o	H _e			
Bellary	50	20	Silver staining	6.650	3.660	0.510	0.680	0.660	0.237	Jain <i>et al.</i> 2005a
Deccani	40	25	Automated DNA sequencer	7.440	3.810	0.593	0.678	0.637	0.132	Arora <i>et al.</i> 2010
Hassan	50	18	Silver staining	7.400	3.694	0.533	0.686	0.644	0.207	Jain <i>et al.</i> 2006a
Kenguri	50	20	Silver staining	6.550	4.140	0.520	0.730	0.700	0.293	Jain <i>et al.</i> 2006b
Mandya	50	19	Silver staining	5.740	3.330	0.550	0.650	0.630	0.161	Jain <i>et al.</i> 2005b
Yalaga	52	25	Automated DNA sequencer	9.600	4.865	0.596	0.757	0.725	0.215	Present study

Na, observed alleles; Ne, effective number of alleles; Ho, observed heterozygosity; He, expected heterozygosity; PIC, polymorphism information index; F_{IS}, heterozygote deficiency.

heterozygote deficiencies were also reported in Indian sheep (Radha *et al.* 2011, Arora *et al.* 2011b). Possible explanations could be presence of null alleles; locus may be under selection or population subdivision. However, distinguishing among these is generally difficult. It was not possible to demonstrate the presence of null alleles due to non-availability of pedigreed animals with the farmers for analysis. The highly positive F_{IS} value was highly positive which may be due to population substructure, as few samples were collected from several flocks. The high F_{IS} value may have been generated due to heterogeneity among the flocks sampled within the breed caused by Wahlund effect (Hedrick 2013).

A population undergoing a demographic decline would have reduced level of gene diversity (Cornuet and Luikart 1996). Since Yalaga is reported as a rare breed (Rice 1877), the bottleneck analysis was performed. The microsatellite data was subjected to a test based on mode-shift distortion under the TPM (two phase mutation model) (Luikart *et al.* 1998) implemented in the Bottleneck software (Cornuet and Luikart 1996). No mode shift was detected in the frequency

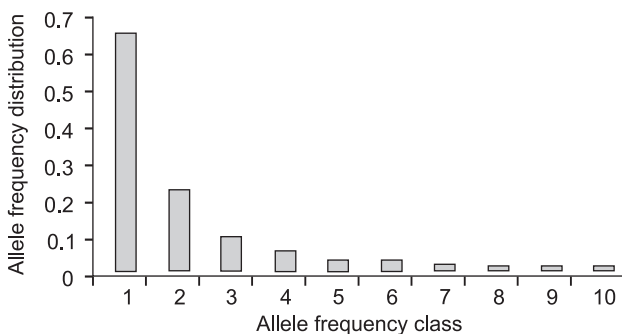


Fig. 1. Mode shift analysis depicting absence of genetic bottleneck in Yalaga sheep.

distribution of alleles and a normal L-shaped form was observed (Fig. 1). These findings suggested absence of any recent reduction in the effective population size and non-bottlenecked Yalaga sheep population, as also evident from recent survey conducted by ICAR-NBAGR.

The presence of overall substantial genetic variability in Yalaga sheep suggested that this sheep population has no danger of loss of any genetic variation at nuclear level at present. The genetic characterisation of indigenous breeds is climbing a rung to reach the possibilities of utilizing the natural gene pool in future breeding strategies as well as in preserving biodiversity.

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REFERENCES

- Acharya R M. 1982. *Sheep and Goat Breeds of India*. Animal Production and Health Paper 30, Food and Agriculture Organization (FAO) of the United Nations, Rome. Pp. 1–190.
- Arora R and Bhatia S. 2004. Genetic structure of Muzzafarnagri sheep based on microsatellite analysis. *Small Ruminant Research* **54**: 227–30.
- Arora R, Bhatia S and Mishra B P. 2010. Genetic structure analysis of Deccani sheep-A major breed from Maharashtra. *Indian Veterinary Journal* **87**(11): 1109–11.
- Arora R J, Bhatia S, Mishra B P, Jain A and Prakash B. 2011a. Diversity analysis of sheep breeds from Southern peninsular

- and Eastern regions of India. *Tropical Animal Health and Production* **43**(2): 401–08.
- Arora R, Bhatia S, Yadav D K and Mishra BP. 2011b. Current genetic profile of sheep breeds/populations from Northwestern semi arid zone of India. *Livestock Science* **135**: 193–98.
- Arora R, Bhatia S, Mishra B P and Joshi B K. 2011c. Population structure in Indian sheep ascertained using microsatellite information. *Animal Genetics* **42**(3): 242–50.
- Botstein D, White R L, Skolnick M and Davis R. 1980. Construction of genetic linkage maps in man using restriction fragment length polymorphisms. *American Journal of Human Genetics* **32**: 314–31.
- Bradley D G, Fries R, Bumstead N, Nicholas F W, Cothran E G, Ollivier L and Crawford A M. 1997. Report of an advisory group of the International Society of Animal Genetics as a contribution to assist FAO prepare for the MODAD Project, *DADIS. FAO*. Pp. 1–15.
- 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.
- Di Stasio L. 2001. Applied Genetics in Sheep and Goats. Panels of markers for parentage verification tested at the 2001/02 ISAG comparison test. www.isag.org.uk/pdf/2005_Panels_Markers_Sheep_Goats.pdf.
- Ginja C, Gama L T, Cortes Ó, Delgado J V, Dunner S, García D, Land V, Martín-Burriel I, Martínez-Martínez A, Cecilia M, Penedo T, Rodellar C, Zaragoza P, Cañon J and BioBovis Consortium. 2013. Analysis of conservation priorities of Iberoamerican cattle based on autosomal microsatellite markers. *Genetics Selection Evolution* **45**: 35.
- Hanotte O and Jianlin H. 2005. *Genetic characterization of livestock populations and its use in conservation decision-making*. Book of proceedings of the international workshop of the role of biotechnology for the characterization and conservation of crop, forestry, animal and fishery genetic resources held in Turin, Italy, 5–7 March 2005. Pp. 131–36.
- Hedrick P W. 2013. High inbreeding in sheep or erroneous estimation? *Journal of Heredity* **104**: 298–99.
- Jain A, Kulkarni V S, Govindaiah M G, Sadana D K, Aswathnarayan T, Pandey A K, Kumar D, Sharma R and Singh G. 2005a. *Sheep Genetic Resources of India: Bellary*. Monograph, NBAGR, Karnal.
- Jain A, Sadana D K, Govindaiah M G, Kulkarni V S, Aswathnarayan T, Pandey A K, Kumar D, Sharma R and Singh G. 2005b. *Sheep Genetic Resources of India: Mandya*. Monograph, NBAGR, Karnal.
- Jain A, Govindaiah M G, Sadana D K, Kulkarni V S, Aswathnarayan T, Pandey A K, Sharma R, Kumar D and Ahlawat S P S. 2006a. *Sheep Genetic Resources of India: Hassan*. Monograph 34, NBAGR, Karnal.
- Jain A, Kulkarni V S, Govindaiah M G, Sadana D K, Aswathnarayan T, Pandey A K, Kumar D, Sharma R and Ahlawat S P S. 2006b. *Sheep Genetic Resources of India: Kenguri*. Monograph 33, NBAGR, Karnal.
- Luikart G, Allendorf F W, Cornuet J M and Sherwin W B. 1998. Distortion of allele frequency distributions provides a test for recent population bottleneck. *Journal of Heredity* **89**: 238–47.
- Montenegro M, Llambí S, Castro G, Barlocco N, Vadell A, Landi V, Delgado J V and Martínez A. 2015. Genetic characterization of Uruguayan Pampa Rocha pigs with microsatellite markers. *Genetics and Molecular Biology* **38**(1): 48–54.
- Peakall R and Smouse P E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**: 288–95.
- Peter C, Bruford M, Perez T, Dalamitra S, Hewitt G, Erhardt G and the ECONOGENE Consortium. 2007. Genetic diversity and subdivision of 57 European and Middle-Eastern sheep breeds. *Animal Genetics* **38**: 37–44.
- Piry S, Luikart G and Cornuet J M. 1999. BOTTLENECK a computer programme for detecting reductions in the effective size using allele frequencies. *Journal of Heredity* **90**: 502–03.
- 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 Journal of Science and Technology* **2**: 55–58.
- Radha P, Sivaselvam S N, Kumarasamy P and Kumanan K. 2011. Genetic diversity and bottleneck analysis of Kilakarsal sheep by microsatellite markers. *Indian Journal of Biotechnology* **10**: 52–55.
- Raja K N, Jain A, Singh G, Kumar L, Yadav H K and Arora R. 2012. Ramnad white sheep – phenotypic and genetic characterization. *Indian Journal of Animal Sciences* **82**(9): 1082–86.
- Rice B L. 1877. Mysore and Coorg: A Gazetteer compiled for the Government of India, Volume 1, Bangalore, Mysore government Press. (Digitized by Google) pp. 171–72.
- Sambrook J, Fritsch E F and Maniatis T. 1989. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Press, New York, 2nd edn.
- 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. *Livestock Research for Rural Development* **21** Article #50. <http://www.lrrd.org/lrrd21/4/selv21050.htm>
- Singh A R. 1967. 'Selection and breeding programs for improving Indian sheep.' M.Sc. Thesis, Kansas State University, Manhattan, Kansas.