

Current status of microsatellite based genetic diversity in Indian sheep

Harikesh Singh Yadav¹, Avnish Kumar Bhatia², Reena Arora³, B. Prakash³
National Bureau of Animal Genetic Resources, Karnal-132001, Haryana

ABSTRACT

A comprehensive review of the genetic diversity of Indian sheep characterized genetically using microsatellite markers has been presented in this manuscript. FAO recommended microsatellite markers have been employed for the genetic characterization of Indian sheep breeds. Of the 39 registered breeds of Indian sheep (*Ovis aries*), 35 have been characterized using microsatellite markers, either by manual or automated methodologies. Four breeds (Gaddi, Bhakarwal, Poonchi and Tibetan) inhabit the disturbed regions of the country rendering collection of blood samples difficult, and consequently have not been hitherto characterized. The genetic diversity of several lesser known indigenous sheep populations has also been accomplished by microsatellite markers. Few descript and several lesser known sheep breeds/populations are yet to be studied at genetic level. These indigenous genetic resources need to be characterized using a uniform panel of markers and method of evaluation to enable comparison at the national level, in order to facilitate outlining of genetic management and conservation programs for these valuable sheep breeds/populations. This paper attempts to review the entire information to gain a comprehensive insight into the genetic diversity prevalent in our indigenous sheep breeds/populations. Limitations and future prospects of the use of microsatellite markers have also been discussed.

Key words: Indian sheep, genetic diversity, microsatellite markers

Present addresses: ¹ Phd Scholar, ² Senior Scientist, ³ Principal Scientist, NBAGR, Karnal, Haryana

INTRODUCTION

The sheep of India reflect as much diversity as represented by the varied cultures, religions and the people of the country. This is evident by the presence of 39 descript sheep breeds in addition to numerous undescribed ones found across the length and breadth of the country. These sheep breeds are distributed in the Northern temperate, North-western arid and semi arid (NWSA), Southern peninsular (SP) and Eastern agro-ecological regions (Acharya, 1982). The diversity of Indian sheep at morphological and production levels has been well documented by Acharya (1982). Recently, a total of 39 sheep breeds have been registered by the National Bureau of Animal Genetic Resources (NBAGR), Karnal, the nodal agency providing accreditation to Indian livestock breeds. To characterize and accord recognition to several less recognized sheep populations, NBAGR has already initiated surveys in the respective distribution areas, to collect detailed information for developing breed descriptors as well as diversity analysis at the

molecular genetic level.

The diversity of Indian sheep is apparent in the form of heaviest Muzzafarnagri breed, tallest Nellore, most prolific microsheat Garole, fast growing Madgyal, lustrous wool producing Magra, best carpet wool producing Nali and Chokla, butcher's sheep Bandur or Mandya, to cite a few. Some of these breeds are endangered or declining (Khan, 2001), thereby accelerating the need for their characterization and conservation/restoration /evaluation. Though the status of the non-descript/lesser known sheep populations is not yet known, their importance cannot be undermined as they represent 75% of the total Indian sheep population (Bhatia and Arora, 2010) and contribute substantially to both wool and meat industry of the country. Most of these ovine genetic resources thrive in extreme climatic conditions and are reared mainly by the marginalized farmers and pastoralists who have developed and maintained these breeds for centuries as a source of their livelihood. There are very few organized sheep herds/farms in

(GenAlex, MICROSAT, Genepop etc).

Northern Temperate Region

The Northern temperate region comprises Jammu & Kashmir, Himachal Pradesh and hilly regions of Uttaranchal states of India. The important sheep breeds of this region are Rampur Bushair, Gaddi, Gurej, Karnah, Bhakarwal, Poonchi, Kashmir Merino and Changthangi. Most of the breeds of this region are considered endangered (Khan, 2001). Breeds like Bhakarwal, Gaddi, Kashmir Merino and Poonchi are yet to be characterized at the genetic level. Rampur Bushair, Gurej, Karnah and Changthangi have been characterized using neutral markers. Genetic variability estimates for the different sheep breeds of this region are depicted in Table 1. Microsatellite marker based analyses revealed substantial genetic diversity (expected heterozygosity values ≥ 0.6) in majority of these breeds despite their declining status. India and sheep are rapidly on the decline due to increase in agricultural mechanization and industrialization which results in loss of grazing land, non availability of proven rams, absence of any planned strategies for their management and conservation, shift in profession and lack of awareness about the importance of maintaining indigenous genetic diversity, and scarce information on the production potential of these breeds.

Genetic diversity plays a very important role in survival and adaptability of a species. It is required to meet current production needs in various environments, to allow sustained genetic improvement, and to facilitate rapid adaptation to changing breeding objectives (Notter, 1999). A major drawback in formulation and implementation of conservation, breeding and management policies for Indian sheep breeds is the lack of information regarding their current genetic status. Over the past decade microsatellite markers have proven to be useful in genetic diversity studies in several livestock species including sheep (Cinkulov et al., 2008; Alvarez et al., 2009). The awareness of the importance of this diversity at the phenotypic level has led to the assessment of diversity at the genetic level as well. It is imperative to understand the genetic diversity in order to prioritize these breeds for conservation. Genetic characterization enables the prioritization of breeds for conservation. The amount of genetic divergence between populations is regarded

as a major criterion for deciding their uniqueness and therefore prioritizing their conservation (Eding et al., 2002).

Microsatellite markers have been liberally used globally to measure genetic diversity, gene flow, migration and effective population size in livestock breeds (Kantanen et al., 2000; Peter et al., 2007, Cinkulov et al., 2008). Co-ancestry and kinship between breeds has also been determined through the use of neutral microsatellite markers. Past genetic bottlenecks have been detected in several livestock breeds using microsatellites. These markers are also being used for assigning individuals to the population of origin as well as for parentage verification. They are the best suited markers for differentiating closely related breeds.

A plethora of information has been generated on the genetic characterization of Indian sheep during the last decade, using neutral microsatellite markers. The aim of this review is to summarize the use of microsatellite markers in Indian sheep and to discuss the limitations and benefits of these molecular markers. This compilation is therefore, an attempt to review and document this crucial information for gaining a comprehensive insight into the genetic diversity prevalent in our sheep breeds/populations.

Status in Indian sheep breeds

Recognizing the importance of breed characterization (Barker et al., 1993), FAO in 1995 proposed details of a Global Project for Research on Animal Genetic Resources. Following a brainstorming session organized by NBAGR, Karnal at NBPGR, New Delhi, in August 1997 (Annual Report, NBAGR, 1997-98), attended by eminent scientists, it was recommended that microsatellite markers be used for genetic characterization of livestock breeds of India. As a consequence, characterization of farm animal genetic resources by neutral microsatellite markers was initiated at NBAGR. The microsatellites are one of the best markers in present scenario of genetics and population studies. They have been widely used in characterization of the ovine species. Microsatellite characterization of Indian sheep breeds was performed on the guidelines of MoDAD (FAO, 1996). Sampling as well as the markers used was recommended by FAO (1996). These initial studies were based on silver staining techniques for allele

Table 1. Details of animals, markers, methodology and genetic diversity indices across sheep breeds of India

Region/ Breed	Sample size	No. of markers	Genotyping method	No. of alleles		Heterozygosity		PIC	FIS	References
				Obs.	Eff.	Obs.	Exp.			
North temperate region										
Changthangi	38	25	DS	8.760	4.539	0.691	0.716	-	0.047	Sharma et al., 2010
Gurej	50	24	SS	7.080	3.680	0.450	0.670	-	0.238	Gupta et al., 2007 ^c
Karnah	50	24	SS	6.830	3.600	0.533	0.680	-	0.192	Gupta et al., 2007
Rampur Bushair	47	20	SS	6.000	3.471	0.515	0.675	0.618	0.227	
North-western Arid and Semi Arid region										
Chokla	50	25	SS	5.320	3.271	-	0.657	0.605	0.299	Sodhi et al., 2006
Jaisalmeri	50	25	SS	7.080	3.640	0.493	0.686	0.642	0.269	Gupta et al., 2007 ^a
Jalauni	50	25	SS	5.920	3.710	0.580	0.680	0.640	0.120	Arora et al., 2008
Magra	48	25	SS	5.700	3.800	0.597	0.694	0.648	0.159	Arora and Bhatia, 2006
Malpura	-	19	-	6.684	3.926	-	0.706	0.672	0.365	Gupta et al., 2007 ^b
Marwari	48	25	SS	6.240	3.940	0.584	0.683	0.650	-	Bhatia and Arora, 2007
Muzzafarnagri	34	25	SS	5.040	3.640	0.652	0.697	0.636	0.058	Arora and Bhatia, 2004
Nali	50	25	SS	5.520	3.338	-	0.651	0.613	0.397	Sodhi et al., 2006
Patanwadi	50	20	DS	8.250	4.000	0.600	0.700	0.660	-	Jyotsana et al., 2010
Pugal	34	22	SS	4.680	3.07	0.648	0.640	-	-0.30	Mishra et al., 2009
Sonadi	48	25	SS	5.880	3.940	0.639	0.677	0.630	-	Bhatia and Arora, 2007
Southern peninsular region										
Bellary	50	20	SS	6.650	3.660	0.510	0.680	0.660	0.253	Jain et al., 2005 ^a Kumar et al., 2007
Coimbatore	-	27	-	6.889	4.932	0.740	0.810	-	0.066	Kumarasamy et al., 2009
Deccani	40	25	DS	7.440	3.810	0.593	0.678	0.637	0.132	Arora et al., 2010 ^a
Hassan	50	18	SS	7.400	3.694	0.533	0.686	0.644	0.218	Jain et al., 2006 ^a Sharma et al., 2006
Kenguri	50	20	SS	6.550	4.140	0.520	0.730	0.700	-	Jain et al., 2006 ^b
Kilakarsal	50	25	DS	7.600	3.880	0.618	0.725	0.831	0.147	Radha et al., 2011
Madras Red	50	10	SS	9.800	-	0.972	0.785	0.809	-	Selvam et al., 2009
Mandya	50	19	SS	5.740	3.330	0.550	0.650	0.630	-	Jain et al., 2005 ^b
Mecheri	48	17	SS	5.000	3.610	0.669	0.706	0.660	0.004	Prema et al., 2008
Nellore	36	25	DS	7.920	-	0.658	0.727	-	0.095	Arora et al., 2010 ^a

SS= Silver staining; DS = DNA sequencer

discrimination. But later with the popularization and reduction in cost of fluorescent dye based capillary methods the automated procedures were adopted. Of the 39 registered breeds of sheep 35 have been characterized using microsatellite markers by various workers. The remaining four breeds (Gaddi, Bhakarwal, Poonchi and Tibetan) mainly inhabit the disturbed areas of the country. These studies are not directly comparable due to different set of microsatellite markers and allele discrimination methods used.

Intra-breed genetic diversity: The genetic diversity within breeds/populations is estimated in terms of observed and effective number of alleles, average allele number per locus (allele diversity), observed heterozygosity and expected heterozygosity (gene diversity). Various software programmes are available for estimating these values from genotype data

North Western Arid and Semi Arid Region

The important breeds of sheep in the NWASA region are Chokla, Magra, Nali, Pugal, Marwari, Malpura, Sonadi, Jaisalmeri, Patanwadi, Munjal, Muzaffarnagri and Jalauni. Most of the sheep breeds of this region produce good carpet grade wool. Rajasthan alone accounts for 16% of the total sheep population and 40% of the total carpet-wool production of the country (Suresh et al., 2008). All the registered sheep breeds of this region have been well characterized using microsatellite markers. Table 1 gives the average values of the diversity indices for sheep breeds of NWASA region. PIC values for the markers used were > 0.5 , suggesting that the markers are highly informative (Botstein, 1980). Although there is no uniformity in the total number of markers/samples used, the overall values of allele diversity (average allele diversity) reflect considerable genetic variation in all of these breeds. Similarly the observed heterozygosity and gene diversity estimates reveal substantial diversity in each of the breeds.

Southern Peninsular Region

The Southern peninsular region comprises the states of Maharashtra, Andhra Pradesh, Karnataka, Tamil Nadu, Kerala and other adjacent territories. Important breeds of sheep of this region are Deccani, Bellary, Nellore, Madras Red, Mandya, Tiruchy Black, Mecheri, Ramnad White, Nilgiri, Coimbatore, Kilakarsal, Kenguri, Hassan and Vembur. All the descript sheep breeds of this region

have been investigated at molecular level using microsatellite markers by various workers (Table 1). In spite of different methods of allele discrimination as well as number of markers used, breeds of this region too, exhibited heterozygosity values above 0.6 reflecting substantial genetic variation in them.

Eastern Region

The sheep breeds of the Eastern region are Shahabadi and Chottanagpuri in Bihar, Jharkhand and part of West Bengal, Garole sheep in West Bengal, Ganjam and Balangir in Orissa, Bonpala in Sikkim and Tibetan sheep in parts of Arunachal Pradesh and Sikkim. Except for Tibetan all the descript breeds of this region have been characterized using microsatellite markers. Reported mean estimates of parameters for genetic variability of sheep of the Eastern region are given in Table 1. Except for Balangir, all the breeds show values of observed heterozygosity higher than 0.5. However, the use of varying number of markers across the studies does not allow direct comparison between breeds of this as well as other regions of the country.

Lesser known sheep populations

Attempts have also been made to genetically characterize some important but lesser known populations of sheep (Table 1). Munjal (Yadav et al., 2011), Kheri (Bhatia and Arora, 2008), Dumba (Jyotsana et al 2010) and Madgyal (Arora et al., 2010a) sheep have been investigated using neutral microsatellite markers. These populations too exhibit sufficient genetic diversity.

Deficit of heterozygotes (FIS): Most of the sheep breeds/populations exhibited a positive FIS value indicating population structure (Wahlund effect) or inbreeding. Segregation of non amplifying (null) alleles is unlikely to affect this value as it is derived from ≥ 10 microsatellite markers. 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. Further, in view of absence of pedigreed records data under field conditions, the effect of relatedness of few samples otherwise deemed unrelated during collection may not be denied.

The negative values of FIS in some breeds like Pugal,

Chhotanagpuri and Nilgiri indicated that the mates were less related in comparison to the average relationship of the population. Therefore, it could be concluded that there was no inbreeding in these populations. This observation was supported by the high levels of observed heterozygosities reported in these populations.

Kinship analysis: Microsatellite markers are useful tools for estimating co-ancestry between individuals in the absence of known pedigree (Toro et al., 2002). These markers have been used to estimate shared ancestry between horses (Marletta et al., 2006), goats (Traore et al., 2009), pigs (Toro et al., 2002) as well as sheep (Legaz et al., 2008; Angelo et al., 2009; Alvarez et al., 2008; 2012). Further, the knowledge of co-ancestries enables the optimization of genetic management in conservation programmes (Toro et al., 2011). Therefore, the use of microsatellite markers was extended to evaluate the relationship of Marwari, Malpura, Jaisalmeri and Patanwadi sheep breeds with Kheri sheep, as these breeds are believed to be the ancestral founders of Kheri sheep and there is frequent intermixing in these breeds due to their closer breeding tracts and uncontrolled breeding across breeds. The results revealed high molecular co-ancestry and least kinship distance between Kheri and Malpura sheep, whereas Marwari sheep appeared distinct (Yadav et al., in press). The molecular co-ancestry and genetic distance analyses suggested that Kheri sheep share more genetic similarity with Malpura than with Marwari, Jaisalmeri or Patanwadi sheep breeds. The genetic distances, phylogenetic tree and principal coordinate analyses reflected that Malpura sheep breed, which appeared closely related to Kheri sheep, may have been a significant contributor towards the formation of Kheri sheep, while the participation of other breeds like Marwari, Jaisalmeri and Patanwadi was less apparent and may have occurred at an early stage of its evolution.

Phylogenetic analysis: Efforts have also been made for the comparison of the genetic diversity of Indian sheep breeds/populations as well as estimation of genetic relationships between them (Mukesh et al., 2006; Jyotsana et al., 2010; Bhatia and Arora, 2007; Arora and Bhatia, 2008, 2009; Arora et al., 2011a, 2011b). A comprehensive insight into the prevailing genetic status, relationships and structure of 17 Indian sheep

breeds was given by Arora et al. (2011c). The genetic basis which underpinned the diversity of 17 Indian sheep breeds from different agro-ecological regions was explored using 25 microsatellite markers. Genetic distances and principal component analysis revealed that the breeds were related as per their geographical proximity and suggested stratification of the breeds in subpopulations. Genetic management strategies like availability of proven rams and effective breeding policies were recommended in order to prevent intermixing between neighbouring breeds. The findings also indicated that the set of 25 microsatellite markers used could successfully identify population substructure within individual Indian sheep breeds. These studies have been used to define priorities for conservation and sustainable use of sheep genetic resources.

Microsatellite Database: A database on genetic characterization of animal genetic resources has been developed at the National Bureau of Animal Genetic Resources, Karnal, India. It stores microsatellite markers-based genetic diversity values of indigenous breeds of animal species including sheep, as reported in published literature. Genetic diversity parameters included in the database are effective and observed number of alleles, observed and expected heterozygosity, assignment accuracy, FIT, FST, PIC values, etc. It has the capability to store information on breeds, microsatellite loci, software and methods of analysis, and genetic diversity within and across breeds along with references.

Microsatellite markers for conservation of breeds: Diversity analysis using microsatellite markers allows the estimation of the overall magnitude of genetic diversity within each of the breeds since the priority breeds for conservation should be the ones with the largest within breed diversity (Gibson et al., 2007). The relationships, expressed as genetic distances among breeds, within sheep species would increase the cost-effectiveness of the conservation efforts. In addition, the microsatellite based analysis of Indian sheep breeds would allow interpretation of gene flow among them as well as give some indication of levels of inbreeding in each breed. A substantial decrease in population size might create high levels of inbreeding which would result in inbreeding depression, thereby increasing the risk of breed extinction. Identification of

most diverse and distinctive i.e. “genetically unique” breeds /populations for higher priority in conservation programmes and the estimation of the relative contribution of each breed to the total ovine genetic diversity can also be done.

Genetic diversity measures provide additional basic information for the design and interpretation of breeding programmes, by identification of the most heterozygous individuals in the population. Such studies enhance the global information system on domestic animal diversity based on molecular data sets and consequently the development of more effective and efficient conservation programmes for the animal genetic resources.

Limitations: The data generated from diversity analysis would allow the most diverse breeds to be selected and provides quantitative diversity indicators for prioritization of Indian sheep breeds /populations for effective conservation policies and breeding plans, in terms of allele and gene diversity, inbreeding and admixture. However, microsatellite markers have been associated with problems of null alleles as well as homoplasy. Further, use of different (number and set of) microsatellite markers by various workers makes comparison between breeds/populations difficult.

Future Prospects: SNP markers are gradually replacing microsatellite markers for diversity analysis within species. SNPs have been successfully used to determine the genetic diversity and population structure of sheep (Kijas et al., 2009) but majority of the Indian sheep are yet to be analyzed. However, SNPs are not without limitations of ascertainment bias (Schlotterer, 2004). In addition, there are limitations with existing genetic programs and computer applications to be able to process the huge amounts of data generated in genome wide SNP studies (Decker et al., 2009). Further, diversity analyses using SNPs/microarrays involves high costs. Therefore, despite some limitations of sampling methods, number of markers used and type of analyses, microsatellite based studies remain viable for analysis of biodiversity, potential conservation and sustainable utilization of ovine genetic resources particularly the indigenous breeds. Few descript and several lesser known sheep populations are yet to be analyzed at the genetic level. These indigenous genetic resources need to be characterized using a uniform panel of markers to enable comparison of all the breeds simultaneously at the national level. Although the

information generated from microsatellite data facilitates in outlining genetic management and conservation programs for these valuable sheep breeds/populations, additional information on population trends, economic importance and specific adaptive features needs to be taken into consideration while prioritizing breeds for conservation. Finally, it is high time to develop resource flocks for identification of QTLs, Whole genome association studies (WGAS) and understanding of the functional genomics which will eventually accelerate the improvement of our valuable ovine genetic resources.

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