Sheep Genetic Resources of India

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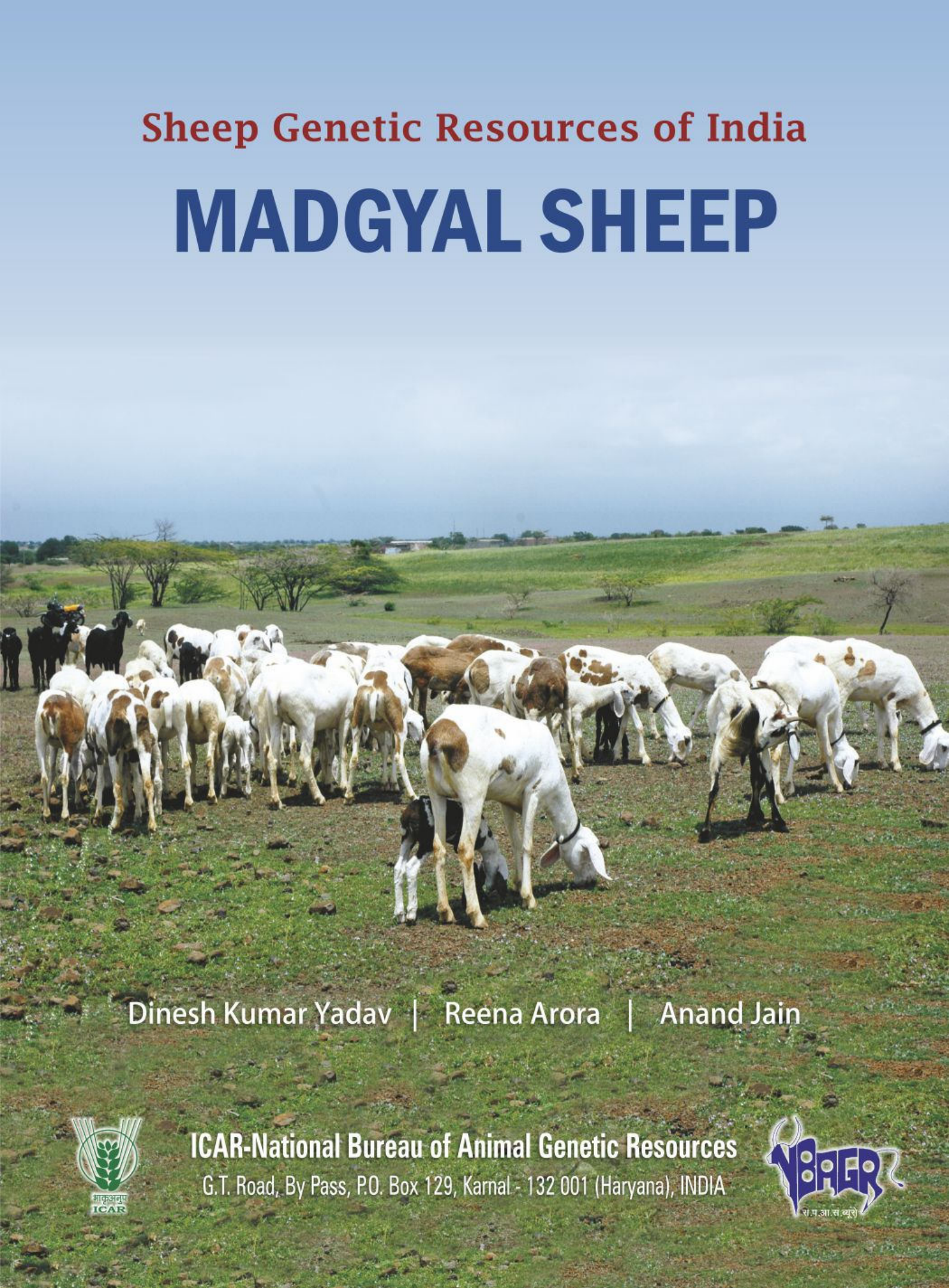

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Preface

Contributions to characterisation of local domestic animal populations are of major importance since breed is the operation unit for the assessment of livestock diversity all over the world. Madgyal is one of the five ecotypes of Deccani sheep (Kolhapuri, Lonand, Madgyal, Solapuri and Sangamneri) in Maharashtra with great significance because of excellent growth rate and heavy body size. Even though the Madgyal sheep are in high demand in the state, information on the population is still scarce. Apart from 40 well recognized sheep breeds in India, there are some other populations like Madgyal, which are not registered as breeds. The objective of bringing out this monograph is to provide a sketch of morphometric and genetic diversity of Madgyal sheep. Moreover, we also draw attention to those responsible for the importance of conserving the genetic diversity of the Madgyal sheep. We are thankful to Director, ICAR-NBAGR, Karnal and Government of Maharashtra for providing support in conducting survey on this important ovine genetic resource of the country. We hope that this bulletin will be of tremendous use in creating awareness among researchers, policy planners, breeders as well as students about this ovine germplasm of Maharashtra.

Authors

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Introduction

Sheep is an important livestock species reared primarily for meat and play significant socio-economic roles in the lives of rural dwellers. Maharashtra is one of the states in India where it is a traditional source of livelihood for communities in the drought prone areas of the state. The state lying in Southern peninsular agro-ecological zone enjoys tropical monsoon type climate and hosts five sheep ecotypes viz. Kolhapuri, Lonand, Madgyal, Solapuri and Sangamneri (Ghanekar, 1983; Gokhle, 2003; Karim and Prince, 2011) of popularly known Deccani sheep. The ecotypes exhibit distinct morphological characteristics (Yadav *et al.* 2014). These ovine genetic resources thrive in semi-arid areas and are reared mainly by the marginalized farmers and pastoralists who have maintained them since long under extensive grazing system. Breeding tract of Madgyal sheep lies in the Jath Taluka of Sangli district. Maintenance of true to breed elite rams and ewes to produce breeding stock for sale at best price is the unique feature of sheep husbandry in the Madgyal sheep breeding tract.

Characterization of breeds is presently considered by FAO as one of the strategic priorities to be undertaken in the development of a national plan for the management of Animal Genetic Resources (AnGR). Sheep breeds of India exhibit large variations in morphology and production. Distinct phenotypic characteristics have been defined for these breeds. Several studies have characterized the morphometric and genetic diversity of ovine genetic resources (Acharya 1982; Bohra *et al.* 1993; Sahana *et al.* 2001, 2004, Kumar *et al.* 2006; Singh *et al.* 2007; Yadav *et al.* 2009, 2010, 2011, 2011a, 2011b, 2013; Yadav and Arora 2014; Arora *et.al.* 2011b). Over the years, farming communities across the globe have directly or indirectly selected and improved indigenous sheep breeds to cope with the harsh and adverse conditions they dwell in. Complete characterization of these indigenous breeds is imperative as they are the only remaining sources of putative alleles of economic values that might be lost through multiple factors including unchecked increase, uncontrolled intermixing amongst them, infusion of exotic

germplasm due to haphazard crossbreeding, absence of planned strategies for their conservation and sheer negligence (Bhatia and Arora, 2005). In view of the socio-economic importance of Madgyal sheep, this monograph seeks to summarize their morphometric and molecular characterization under draught prone environment in Maharashtra. It attempts to define their morphometric standard and current genetic status.

Data Collection and Analysis

Purposive sampling was used to determine the distribution area of the Madgyal sheep. Villages having Madgyal sheep were identified for data recording based on information of the officials of Maharashtra Sheep and Goat Development Corporation, Pune, and the shepherds. Interview method and questionnaire were used for data collection on socio-economic parameters, reproduction traits, disease prevalence, mortality, management practices and current breed merit. The age was determined by dentition and the animals having two or more permanent teeth were included in the study. A dial spring balance of 100 kg × 500 gm. (capacity 100 kg; least count 500 gm.) was used to record body weight in kilogram (kg). Body dimensions were measured using a steel tape of 5 m length of class II accuracy with records taken to the nearest centimeter (cm) holding the animal in normal standing position. The traits measured were body length (BL), height at withers (HW), chest girth (CG), paunch girth (PG), ear length (EL) and tail length (TL). The sheep studied from different flocks were reared under extensive management system. Sixty one sheep flocks were assessed in the breeding tract of Madgyal sheep. Data were recorded on above mentioned morphometric traits of 255 adult sheep (37 rams and 218 ewes). Body weight of 117 lambs was also recorded. Statistical analysis was carried out using JMP software of SAS (2012).

For genetic characterization, blood samples were randomly collected from 100 Madgyal sheep across its distribution tract in line with MoDAD recommendations (FAO, 1996). Blood sampling was coordinated with owners and veterinary officers. Samples were taken from distinct flocks exhibiting specific Madgyal characteristics. Genomic DNA was isolated and purified using the standard phenol chloroform

extraction protocol. Genetic variation was assayed using 25 microsatellite markers, out of which 20 have been recommended for ovines by Bradley *et al.* (1997), and the remaining markers (CSRD247, HSC, INRA63, MAF214 and OarCP49) were taken from the panel of markers for parentage verification tested at the 2001/02 ISAG comparison test (Di Stasio 2001). The forward primer for each marker was fluorescently labelled with either FAM, NED, VIC or PET dye. 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 $MgCl_2$, 200µM dNTPs, 0.5 U Taq polymerase and 1x buffer. A common touchdown PCR programme was used for amplification (Bradley *et al.* 1997). The 25 markers were divided into five multiplexes with five markers in each plex. Amplification was confirmed on 2% agarose gel, and the genotyping was carried out on an ABI 3100 automated DNA sequencer using LIZ 500 as the internal size standard. Allele sizing was performed using GENEMAPPER software. 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 2005). 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 (Cornuet and Luikart 1996).

Habitat and Population Status

Madgyal is an improved sheep whose origins are obscure to say the least. Some shepherds say they are the result of the selective breeding around 100 years ago by local sheep breeders at Madgyal and the adjoining village Sanmadi. The breed got its name after the Madgyal village in Jath taluka in Sangli district. Madgyal sheep are typically adapted to the Jath taluka which hosts pure specimens. Abachiwari, Gholeshwar, Kunikonur, Madgyal, Pandozari, Sanamadi and Sonyal are prominent villages having typical Madgyal sheep. These sheep show potential of rapid weight gain, thus are founding ground in adjoining districts of Sangli. Their performance is affected in other areas. If crossed with other ecotypes, the performance is lesser

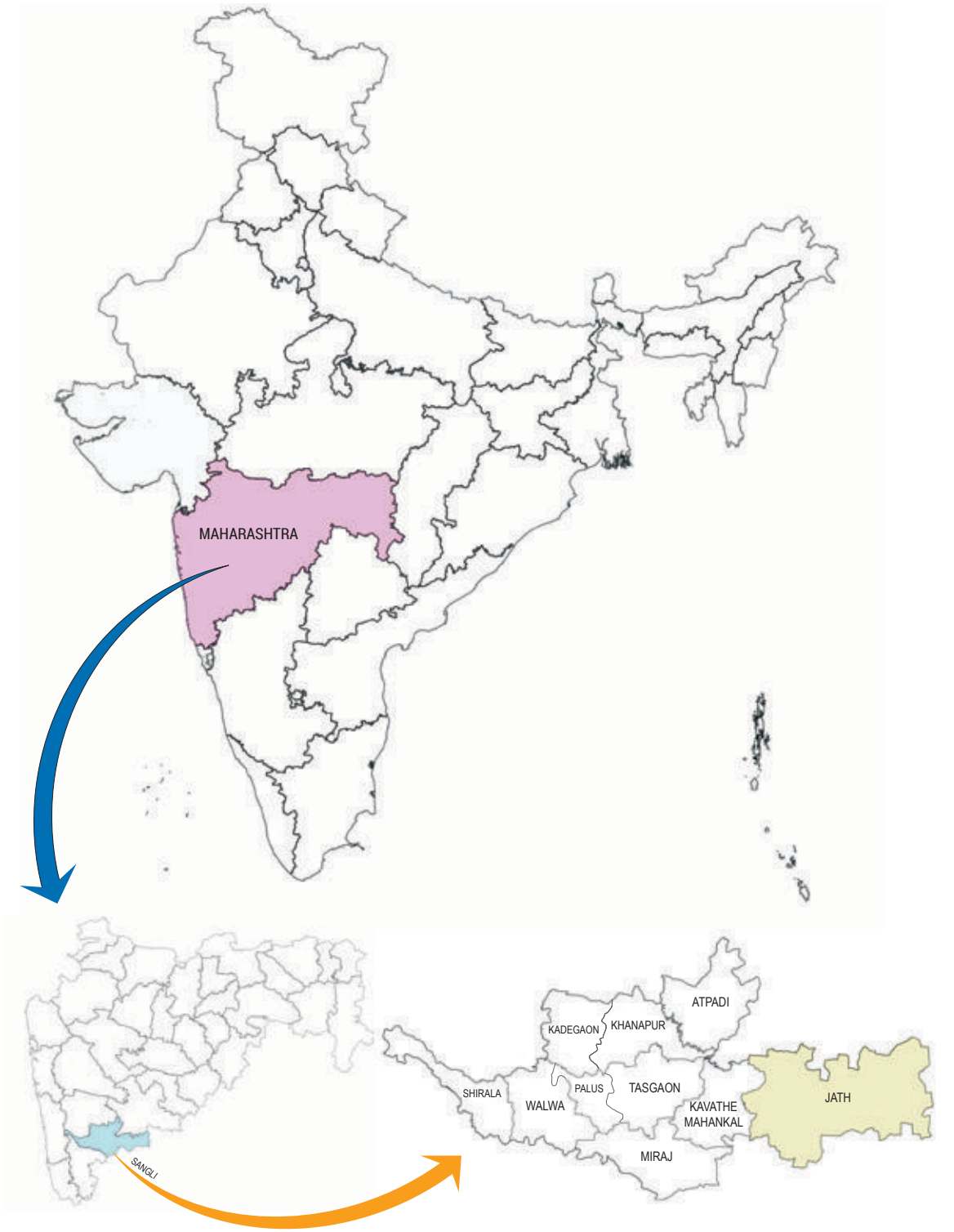


Figure 1: Distribution Area of Madgyal Sheep

than pure bred animals. They are not suited to long migration. In this area most of the flocks are pure and small sized. In the average flock size of 33.4 sheep, 30.2 exhibited Madgyal characteristics, a significant level of breed purity in the distribution area as compared to other breeds/populations. Figure 1 shows the habitat of Madgyal sheep. According to the 19th Livestock Census (2012), there are 0.157 million sheep in the Sangli district (Table 1). The sheep population is maximum in Jath taluka (0.054 million) and minimum in Khanapur taluka (543 only).

Table 1: Sheep population of Sangli district of Maharashtra*

S. No.	Tehsil/Taluka	Exotic/crossbred	Indigenous	Total
1	Atpadi	0	42566	42566
2	Jath	0	53945	53945
3	Kadegaon	1138	4759	5897
4	Kavathemahankal	0	21865	21865
5	Khanapur	19	524	543
6	Miraj	441	8654	9095
7	Palus	307	1494	1801
8	Shirala	111	1258	1369
9	Tasgaon	1371	5102	6473
10	Walwa	809	13027	13836
Total		4196	153194	157390

*Source-19th Livestock Census (2012) of Maharashtra.

Agro-climatic Conditions

Sangli district is situated in the Southern part of Maharashtra and is a part of Deccan plateau. It is one of the most fertile and highly developed districts in Maharashtra. It lies in the Warna and Krishna river basins and houses many sugar factories. River Krishna is a main source of water supply for drinking, industries and irrigation. Warna is the largest tributary of Krishna in the district. The Krishna valley of the district is the most fertile. Sangli is also known as the 'Turmeric City' of Maharashtra due to production and trade of the turmeric spice. It lies at an altitude of 610 m MSL. Geographical location of the district is between 16°45' and 17°33' North latitude and 73°41' and 75°41' East longitude. It falls under the rain shadow region of Sahyadri Mountain. Temperature ranges from 14 to 42 degree Celsius and average annual rainfall is 650 mm. Temperature increases from west to east

and rainfall decreases from west to east. The rainfall is purely seasonal. Most of the rain occurs in monsoon season (June to September). There is an extreme regional variation in the average annual rainfall in the district. The Chandoli (Shirala) region receives average annual rainfall of 4000 mm whereas in Atpadi and Jath tehsils, the average annual rainfall is around 500 mm.

Soil is very important resource because it acts as medium for the cultivation of crops. Sangli district is endowed with coarse shallow, medium black and deep black soil types. The vegetation cover also varies from the tropical monsoon forest in the western parts to scrubs and poor grasses in the eastern parts. Topographically the region has large diversified surface. The central part of the district is endowed with fertile black soils and is cultivable area. The eastern part, including Jath and Kavathe Mahankal tehsils, is drought prone area and has insignificant irrigation facilities. The northern part is rain shadow area. Agriculture in Sangli district is mostly of the intensive subsistence type with an emphasis on the production of food grains. Some commercial crops are also cultivated. Main crops of the district are rice, jowar, bajra, groundnut, turmeric, soyabean, sugarcane, wheat, grape and pomegranate. Here, vegetation is sparse and the trees are of dry deciduous and thorny bush types. The main species are Babul, Khair, Tarwad, Neem, Chinch, Jambhul, Bor (*ziziphus jujuba*) and Mango. Jath tehsil is rocky and grows only inferior type of grasses with sparse tree growth. (Source: Agricultural Statistical Information, Maharashtra; Maharashtra State Gazetteer; Internet)

Morphometric Characteristics

Madgyal are unique sheep of Maharashtra popular for mutton. They are large sized animals, predominantly white with brown patches/spots. Few white as well as brown animals are also seen. The coat colour varies from white with brown patches to light brown with white patches. Head, face, belly and legs are devoid of wool. They have prominent brown ring around the eyes. Backline is straight. Both sexes are polled and have typical roman nose. The ears are long, leaflike and drooping, the tail is medium and thin, and fleece is extremely coarse, hairy and open. Wattles are observed in a number of animals. Legs are long, strong and thin with gray hooves. Udder is round with cylindrical teats and pointed tip.

Body Biometry

Average body weight and body biometry of adult Madgyal sheep are presented in Table 2. Average body weight of Madgyal lambs are shown in Table 3. The average body weights of ewes and rams were 44.7±0.48 and 57.5±1.50 kg respectively. In adult females; the body length, height at wither, chest girth, paunch girth, ear length and tail length were 77.1±0.22, 77.7±0.22, 81.7±0.28, 81.9±0.29, 20.0±0.07, 19.4±0.10 cm. respectively; and in adult males the corresponding values were 83.9±0.66, 83.7±0.61, 87.3±0.80, 87.8±0.77, 20.1±0.23, 21.0±0.36 cm. respectively. The body biometry reflects that Madgyal sheep are large in size with medium tail. The body weights in 3 to 6 months age-group are important from marketing point of view, and those between 12 and 15 months are important from breeding point of view. Up to 3 months of age, male and female lambs attained the body weight of 33 and 25 kg respectively. Two-tooth animals weighed 40.5 kg in females and 54.6 kg in males. Substantial sexual dimorphism was observed in all seven morphometric traits (Table 2). Males were 29 % heavier than females. Coefficient of variation of the morphometric traits varied from 4.3 to 17.2%. Coefficient of variation was slightly higher in males as compared to females. The difference may be ascribed to the smaller sample size in males.

Table 2: Mean, standard error, coefficient of variation, range and sexual dimorphism of the morphological traits in Madgyal sheep

Morphometric Trait	Female (218)			Male (37)			Sexual dimorphism (m/f)
	Mean ± SE	Coefficient of variation	Range	Mean ± SE	Coefficient of variation	Range	
BW	44.7±0.48	16.0	23-65	57.5±1.50	17.2	38-84	1.29
BL	77.1±0.22	4.3	68-89	83.9±0.66	4.8	77-92	1.09
HW	77.7±0.22	4.3	70-86	83.7±0.61	4.5	78-94	1.08
CG	81.7±0.28	5.1	69-94	87.3±0.80	5.5	77-101	1.07
PG	81.9±0.29	5.2	71-96	87.8±0.77	5.4	76-99	1.07
EL	20.0±0.07	5.6	17-23	20.1±0.23	6.9	18-24	1.00
TL	19.4±0.10	8.0	16-24	21.0±0.36	10.4	16-25	1.08

BW, body weight; BL, body length; HW, height at wither; CG, chest girth; PG, paunch girth; EL, ear length; TL, tail length

Table 3: Average body weight of Madgyal lambs

Age (month)	Female		Male	
	Average ± SE	Range	Average ± SE	Range
0-3	12.4±1.40 (24)	3.5-25	14.5±1.43 (32)	3.5-33
3-6	29.3±0.81 (21)	21-37	35.3±1.35 (10)	30-41
6-12	36.1±1.14 (14)	29-42	48.5±1.62 (16)	38-58

Socio-economic Status of the Shepherds/ Sheep Farmers

Madgyal sheep are mostly reared by shepherds / farmers belonging to Dhangar and Ramoshi communities. Some Marathas also keep Madgyal sheep to supplement their income. The Dhangar (also known as Dhangad and Dhanpal) are a shepherd caste of people primarily located in Maharashtra. The word 'Dhangar' may be associated with a term for 'cattle wealth' or be derived from the hills in which they lived (Sanskrit 'dhang') (Singh, 2006). In Maharashtra, the Dhangars are classified as a Nomadic Tribe (NT). The Ramoshi (alternately Berad) is an Indian community found largely in Maharashtra, Madhya Pradesh and Karnataka. They are historically associated with great empires, important historical conflicts and agriculture. They are also nicknamed as Bedar or fearless for their braveness by the Mughals. They are classified as Vimukt Jati (VJ)/Denotified Tribes by the Maharashtra government.

Sixty one sheep farmers belonging to Dhangar, Ramoshi and Maratha communities that reared Madgyal sheep were interviewed. Most of the families belonged to low-income group; 13% were landless. The average family size was 7.3 with 2.7 males, 2.2 females and 2.4 children. The overall literacy was 57%. The literacy among male, female and children was 53.3%, 37.8% and 79.2% respectively. Besides rearing sheep, 85%, 95%, 85%, and 79% also maintained cattle, goat, buffalo and chicken respectively, whereas 73% maintained both cattle and buffalo.

Flock Size and Breed Purity

In 61 flocks, the average flock size was 33.4 with 25.1 ewes, 1.5 rams and 6.8 lambs. Most of the farmers kept one or two rams. The percentage of Madgyal sheep in the surveyed flocks was 90.6. Comparatively higher price has tempted the farmers to breed the ewes with Madgyal rams. State government has also supported the farmers by providing Madgyal rams. The survey indicated that demand of Madgyal sheep has acted a driving force in maintaining high breed purity in Madgyal flocks. The major income from rearing Madgyal sheep is earned from the sale of lambs at 3-4 months of age. Madgyal male lamb of this age fetches a market price ₹4000-6000 whereas female counterparts realize ₹4000-5500 to its owner. Madgyal lambs give better market price as compared to their counterparts in the surrounding districts.

Management Practices

Madgyal sheep are primarily maintained on grazing. Flocks are grazed in the open for 8-10 hours daily. The distance travelled from paddock to pastoral area for grazing varies from 3-10 km/day. In pastoral areas, sheep normally graze within a radius of about 3 km of a watering point. Water is provided 2-3 times a day depending upon availability of water source and weather conditions. The sheep flocks are migratory. Shepherds remain on temporary migration for 6-8 months (November-June). Migration is restricted mostly within the state in the adjoining districts. Shepherds migrate after celebrating Deepawali at home and return after the arrival of monsoon rains. Majority of farmers provide traditional housing especially during night. Generally it is open, thatched, fenced and adjacent to owner's house. While on migration the flocks are housed in open fields with temporary fencing of either ropes or iron wires. The boundaries of the enclosures are made of tree branches/bushes/rope or wire netting. The shepherds have a strong synergistic relationship with farmers along the traditional migratory routes and the penning of sheep flocks in farmer fields contributes to maintaining and improving soil fertility. Supplements viz. groundnut cake, jowar, maize and wheat bran (daliya) are provided to lambs regularly. Some farmers provide supplements to rams also. The lambs are kept in the pens for about 15-30 days after birth and thereafter join the flock for grazing. Lambs are cared for by women, children and elder persons at home. In addition to suckling mother's milk, most of the farmers feed the lambs on buffalo milk. Half litre of buffalo milk is fed to lambs twice a day (morning and evening) till the age of two months. Thereafter, maize and groundnut cake are fed. Rams are also given groundnut, jowar and maize.

Matki (*Vigna aconitifolia*), a drought resistant legume, commonly grown in arid and semi-arid regions of India. It grows up to a height of one feet. Madgyal sheep are left to graze on the Matki field, a typical management practice observed in Madgyal sheep only. Babool leaves (a source of protein) are specially fed to lambs. Pneumonia, bluetongue (BT), numbness, bloat or tympany, enterotoxemia (ET), foot-and-mouth disease (FMD), ecthyma, haemorrhagic septicemia (HS) and peste des petits ruminants (PPR) are common sheep diseases. Preventing disease reduces economic losses and improves animal welfare. Vaccination against FMD, ET, HS and PPR is done by government agencies. Dipping and deworming are also performed by the farmers themselves. Mortality is between 5-10 %. Still births and abortions are also reported.

Breeding and Reproduction

In sheep husbandry, lamb production is the primary objective. The reproductive rate, the number of live lambs born per ewe exposed for breeding, is therefore important in such a system. Optimal reproductive rates are essential to profitable sheep production. Management affects the reproductive rates. Shepherds keep breeding rams with ewes. Maintaining more than 90% of breed purity in the Madgyal sheep flocks is a remarkable management feat of the shepherds. August-November is the main lambing season and March-April, the minor. Age and weight at puberty in females are around 8-10 months and 38 kg. Age at first lambing is 13-15 months. In farmers' flocks, lambing percentage on the basis of ewes available is 95-98%. Lambing interval is 7-8 months. Litter size is single, but about 10-15% of ewes gives birth to twins. Age at first breeding in males is 8-10 month and weight

at two teeth age is around 55 kg. Breeding life of a ram is 6-7 years. Breeding rams are raised by the farmers at their flocks. Some farmers exchange the rams to avoid inbreeding. Rams are selected on the basis of body size and conformation. Body weight, height, typical Roman nose and large scrotum are the preferred traits for selection of rams. Sometimes rams are purchased from the market. Lambs of ewes which give more milk are selected for future stock. Daily milk yield is 300-500 ml and lactation length is 90-100 days.

Wool Production

Madgyal sheep are mainly reared for mutton purpose, as the earnings from their coarse and short wool are of little market value. These are shorn twice a year in December and August. Wool yield per year is around 250-350 grams and it is utilized for making local products viz. Ghoghri (blanket) and Jena (carpet).

Lamb Marketing

Most sheep operations derive the majority of the income from the sale of lambs. As a result, lamb prices have a large influence on profitability and viability of the sheep husbandry. Lamb prices vary by year, season, body condition and sex of lambs, and marketing method. In India demand for mutton is increasing due to increasing human population, urbanisation and paying capacity. Due to gap between demand and supply of mutton, the sale prices of lambs are also increasing every year. For trading of Madgyal sheep a weekly market is held on every Friday at Madgyal village. Lambs are marketed between the age of 3 and 4 months. These are usually sold in groups and butchers and traders purchase them on visual appraisal. The traded sheep are marketed to terminal markets to Pune, Solapur, Hubli, Bengaluru and Belgaon. A sheep owner's income depends largely on the number of saleable lambs produced per ewe per year. A male lamb of 3-4 month age realises a market price of ₹4000-6000 whereas its female counterparts bring ₹4000-5500 to the owner. The main reason for the existence of a ewe is the production of lambs. Sheep farmers generally keep the ewe lambs for replacement lest opt for distress sale to meet out emergency monetary requirements. Two year old breeding ram is higher priced (₹15000-20000) than a corresponding ewe (₹10000-12000). Old aged ram (6-7 years) procures a price of ₹9000-10000 depending upon the body condition. Selected true to breed ewes and rams having typical Roman nose are priced between ₹25000-30000 and ₹30000-60000 respectively.

Socio-economic and Cultural Values

Madgyal sheep act a mobile bank and meet out about 80% of economic needs of the shepherds. Emergency financial needs are generally met by borrowings from relatives, friends and money lenders. Lambs are sold at appropriate age and weight avoiding distress sale. Sheep mutton is not the regular source of protein for the family. It is taken occasionally, mainly during the cultural ceremonies. Shepherds undertake a Yatra (holy trip) in honour of Biroba (a deity of shepherds) in October. A ram or male lamb is slaughtered in the Biroba and Bhiwaya temple in a year by every shepherd for a feast. Custom of paying sheep as social penalties is not prevalent among the shepherd communities. Birudev festival is celebrated in Karnataka and Maharashtra for two days. Around 10000 sheep are sacrificed. A single family sometimes sacrifices 5-10 sheep.

On the occasion of Deepawali festival, sheep are bathed in Hanuman or family deity temple. They are coloured. Sheep owner runs and the flock follow him. Suddenly crackers are burst. Sheep stop and jump about 4-5 ft. high. Assembled crowd enjoy the spectacle. Owners assemble their sheep flocks at a common place. They raise a banana leaves gate (around 3×5 ft.). The owners wear colourful clothes and cross the gate at random. The owners' flock follow him showing affinity to him/her. Jumping of sheep over a turban is yet another way of testing affinity between the owner and the sheep. The crowd create obstacles by waving clothes. Still, sheep jump on the turban of the owner; a belief among the crowd that the owner cares his/her sheep well.

Genetic Diversity

Genotypic data from 25 microsatellites (Table 4) was used to assess the genetic diversity of Madgyal sheep. Parameters of genetic variability are included in Table 5. A total of 298 alleles were detected across the 25 analysed microsatellite loci. All the loci were observed to be polymorphic and also exhibited high level of genetic variability as revealed by wide range of alleles,

which varied from 7 (BM6506) to 19 (BM6526) with an average of 11.92 (Fig 2). The effective number of alleles ranged between 1.859 (CSSM47) to 13.737 (BM6526). The estimates of allele diversity values were comparable with those reported for other Indian sheep breeds analysed (Jyotsana *et al*. 2010; Arora *et al*. 2010; Arora *et al*. 2011a, b, c).

Table 4. Primer sequences, type of repeat, size range, location and accession numbers of the used microsatellites.

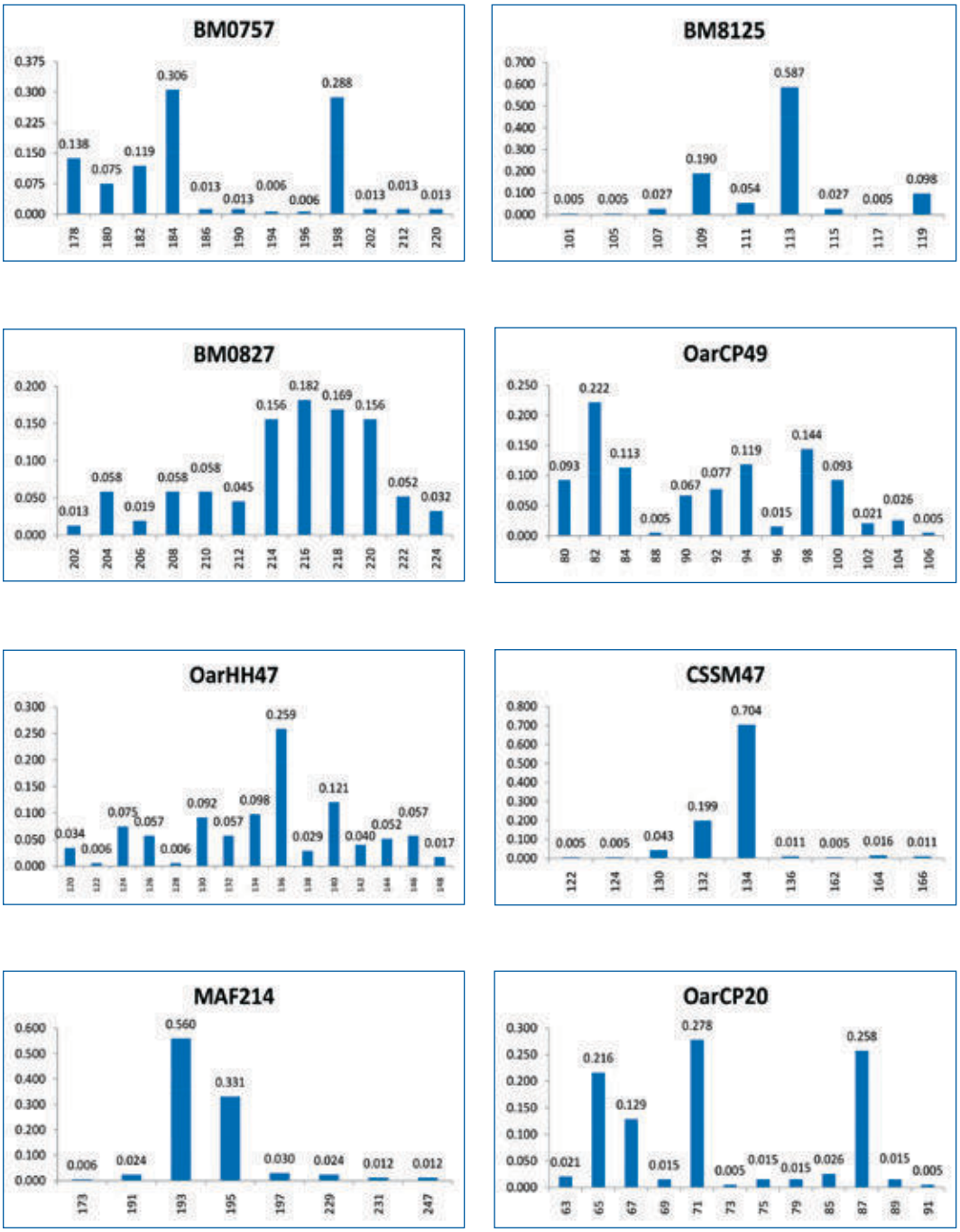
Locus	Primer sequence	Type of repeat	Size range (bp)	Chr. no.	GenBank Acc. no.
BM757	tgg aaa caa tgt aaa cct ggg ttg agc cac caa gga acc	$(GT)_{17}$	178-198	9	G18473
BM827	ggg ctg gtc gta tgc tga g gtt gga ctt gct gaa gtg acc	-	214-224	3	U06763
BM1314	ttc ctc ctc ttc tct cca aac atc tca aac gcc agt gtg g	-	141-161	22	G18433
BM6506	gca cgt ggt aaa gag atg gc agc aac ttg agc atg gca c	-	189-199	1	G18455
BM6526	cat gcc aaa caa tat cca gc tga agg tag aga gca agc agc	-	140-170	26	G18454
BM8125	ctc tat ctg tgg aaa agg tgg g ggg ggt tag act tca aca tac g	-	105-121	17	G18475
CSRD247	gga ctt gcc aga act ctg caa t cac tgt ggt ttg tat tag tca gg	$(AC)_n$	203-237	14	EU009450
CSSM31	cca agt tta gta ctt gta agt aga gac tct cta gca ctt tat ctg tgt	$AAAA(CA)_7TA(CA)_{25}$	162-182	23	U03838
CSSM47	tct ctg tct cta tca cta tat ggc ctg ggc acc tga aac tat cat cat	$(TG)_{12}TATGTA(TG)_4$	120-160	2	U03821
HSC	ctg cca atg cag aga cac aag a gtc tgt ctc ctg tct tgt cat c	-	267-285	20	M90759
INRA63	gac cac aaa ggg att tgc aca agc aaa cca cag aaa tgc ttg gaa g	$(AC)_{13}$	165-203	14	X71507
MAF214	aat gca gga gat ctg agg cag gga cg ggg tga tct tag gga ggt ttt gga gg	-	187-231	16	M88160
OarAE129	aat cca gtg tgt gaa aga cta atc cag gta gat caa gat ata gaa tat ttt tca aca cc	$(AC)_{14}$	141-169	5	L11051
OarCP20	gat ccc ctg gag gag gaa acg g ggc att tca tgg ctt tag cag g	$(AC)_{14}$	67-79	21	U15695
OarCP34	gct gaa caa tgt gat atg ttc agg ggg aca ata ctg tct tag atg ctg c	$(AC)_{17}TTGC\ GTGT\ (CA)_4$	108-122	3	U15699
OarCP49	cag aca cgg ctt agc aac taa acg c gtg ggg atg aat att cct tca taa gg	$(AC)_{17}$	80-110	17	U15702
OarFCB48	gag tta gta caa gga tga caa gag gca c gac tct aga gga tcg caa aga acc ag	$(TG)_{11}CA(TG)_3$	142-164	17	M82875
OarFCB128	cag ctg agc aac taa gac ata cat gcg att aaa gca tct tct ctt tat ttc ctc gc	$(GT)_6GC(GT)_{15}$	97-123	2	L01532
OarHH35	aat tgc att cag tat ctt taa cat ctg gc atg aaa ata taa aga gaa tga acc aca cgg	$(TG)_{17}$	111-139	4	L12554
OarHH41	tcc aca ggc tta aat cta tat agc aac c cca gct aaa gat aaa aga tga tgt ggg ag	$(AC)_{23}$	118-140	10	L12555
OarHH47	ttt att gac aaa ctc tct tcc taa ctc cac c gta gtt att taa aaa aat atc ata cct ctt aag g	$(AC)_{32}$	124-146	18	L12557
OarHH64	cgt tcc ctc act atg gaa agt tat ata tgc cac tct att gta aga att tga atg aga gc	$(TG)_{17}$	120-134	4	L12558
OarJMP8	cgg gat gat ctt ctg tcc aaa tat gc cat ttg ctt tgg ctt cag aac cag ag	$(GT)_n$	115-129	6	U35059
OarJMP29	gta tac acg tgg aca ccg ctt tgt ac gaa gtg gca aga ttc aga ggg gaa g	$(CA)_{21}$	86-144	24	U30893
OarVH72	ctc tag agg atc tgg aat gca aag ctc ggc ctc tca agg ggc aag agc agg	$(AC)_{14}$	121-133	25	L12548

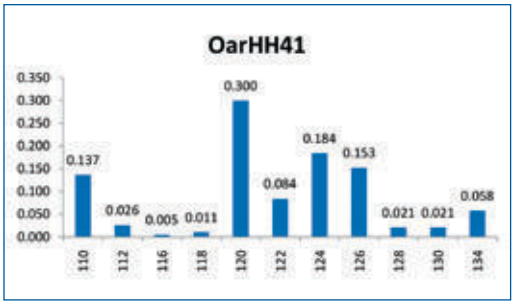
Table 5. Genetic diversity indices across 25 microsatellite markers in Madgyal sheep

Locus	N_a	N_e	I	H_o	H_e
BM0757	12	4.631	1.778	0.600	0.784
BM0827	12	7.890	2.226	0.403	0.873
BM1314	14	2.152	1.377	0.433	0.535
BM6506	7	1.873	0.971	0.161	0.466
BM6526	19	13.737	2.766	0.542	0.927
BM8125	9	2.533	1.295	0.533	0.605
CSRD247	14	5.372	1.951	0.526	0.814
CSSM31	14	6.560	2.112	0.520	0.848
CSSM47	9	1.859	0.952	0.247	0.462
HSC	13	8.199	2.254	0.731	0.878
INRA63	18	6.154	2.192	0.565	0.838
MAF214	8	2.347	1.113	0.386	0.574
OarAE129	10	2.001	1.095	0.143	0.500
OarCP20	12	4.774	1.787	0.918	0.791
OarCP34	9	4.678	1.737	0.550	0.786
OarCP49	13	7.940	2.226	0.969	0.874
OarFCB128	11	4.378	1.742	0.582	0.772
OarFCB48	14	8.332	2.275	0.456	0.880
OarHH35	9	2.510	1.351	0.472	0.602
OarHH41	11	5.614	1.939	0.853	0.822
OarHH47	15	8.223	2.368	0.471	0.878
OarHH64	12	7.190	2.184	0.254	0.861
OarJMP08	13	7.558	2.203	0.273	0.868
OarJMP29	12	5.608	2.005	0.652	0.822
OarVH72	8	4.212	1.626	0.415	0.763
Mean	11.92	5.453	1.821	0.506	0.753

N_a: Observed number of alleles; N_e: Effective number of alleles; I: Shannon information index; H_o: Observed heterozygosity; H_e: Expected heterozygosity

Figure 2: Allele Frequency Distribution at 25 Microsatellite Loci in Madgyal Sheep (x-axis: allele size in base pair; y-axis: allele frequency)

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0.050
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0.005
0.011
0.300
0.084
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0.021
0.021
0.058
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112
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118
120
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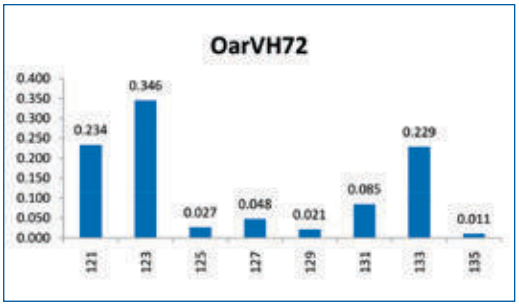
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0.300
0.250
0.200
0.150
0.100
0.050
0.000
0.234
0.346
0.027
0.048
0.021
0.085
0.229
0.011
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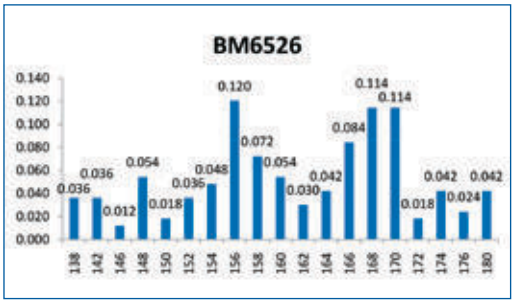
123
125
127
129
131
133
135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0.020
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0.036
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0.054
0.018
0.036
0.048
0.120
0.072
0.054
0.030
0.042
0.084
0.114
0.114
0.018
0.042
0.024
0.042
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150
152
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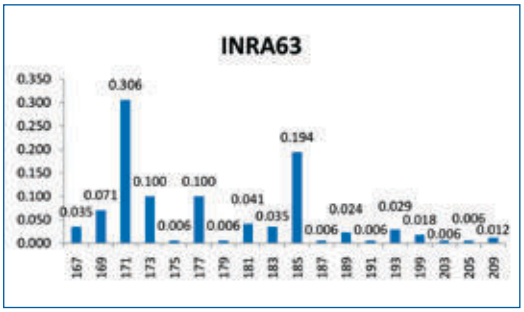
176
180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0.100
0.006
0.100
0.006
0.041
0.035
0.194
0.006
0.024
0.006
0.029
0.018
0.006
0.006
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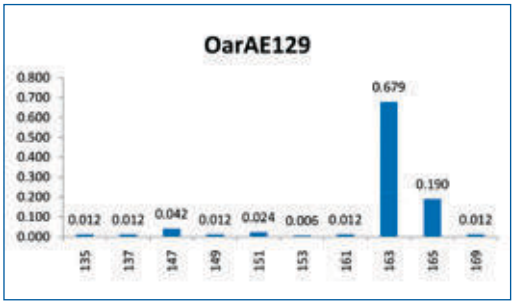
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0.042
0.012
0.024
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0.012
0.679
0.190
0.012
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153
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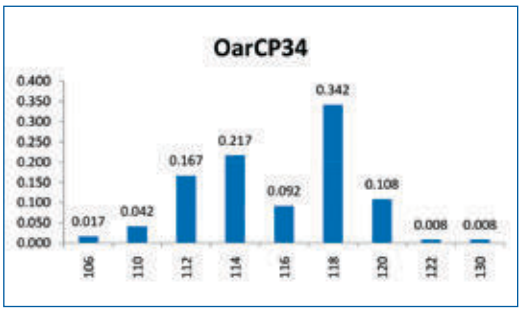
163
165
169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0.000
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0.217
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0.008
0.008
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110
112
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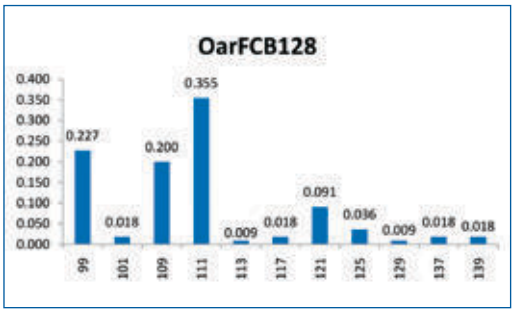
118
120
122
130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0.200
0.355
0.009
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0.018
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101
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111
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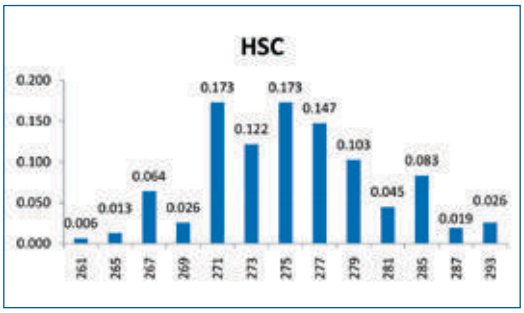
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0.013
0.064
0.026
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0.122
0.173
0.147
0.103
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0.019
0.026
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273
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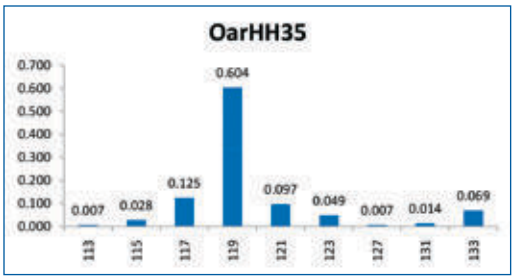
287
293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0.049
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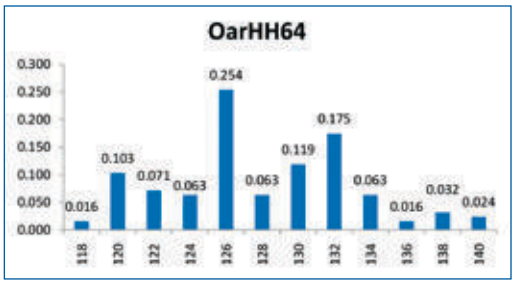
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0.063
0.119
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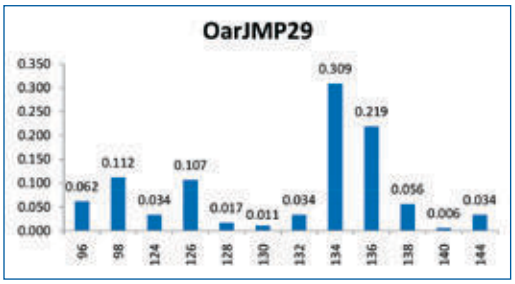
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0.034
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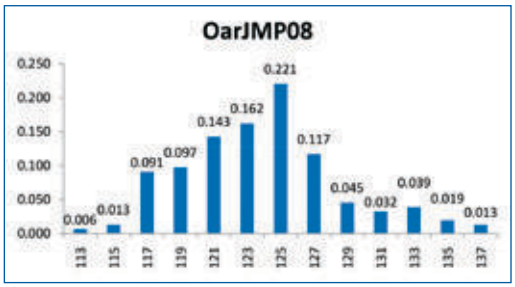
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0.162
0.221
0.117
0.045
0.032
0.039
0.019
0.013
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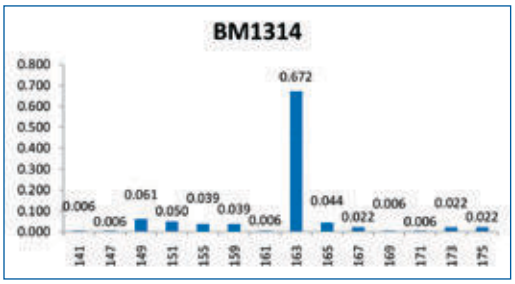
137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0.039
0.039
0.006
0.672
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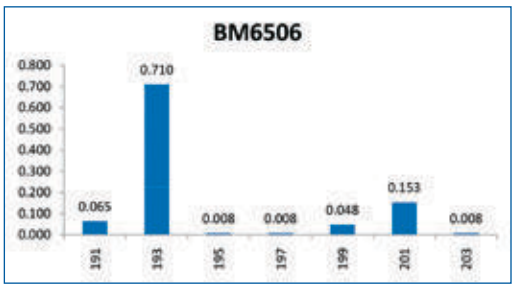
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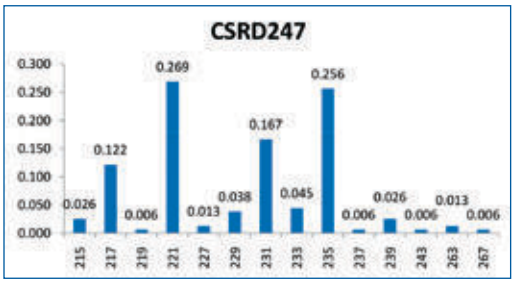
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0.050
0.000
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0.006
0.269
0.013
0.038
0.167
0.045
0.256
0.006
0.026
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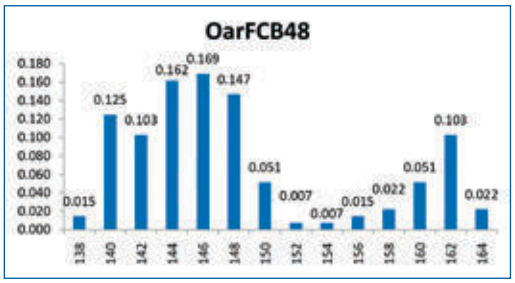
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0.060
0.040
0.020
0.000
0.015
0.125
0.103
0.162
0.169
0.147
0.051
0.007
0.007
0.015
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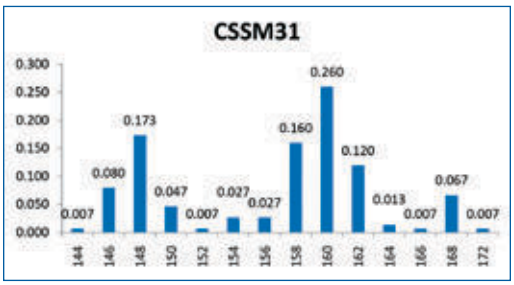
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0.027
0.027
0.160
0.260
0.120
0.013
0.007
0.067
0.007
144
146
148
150
152
154
156
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160
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164
166
168
172

Polymorphic Information Content

The PIC values varied from 0.420 (CSSM47) to 0.923 (BM6526) (Fig 3) and Shannon's information index (I) from 0.952 (CSSM47) to 2.766 (BM6526) (Table 5). Fairly high mean informative estimates of PIC (0.729) and Shannon's information index (I=1.821) supported the utility of used set of microsatellites in biodiversity evaluation of native Indian sheep.

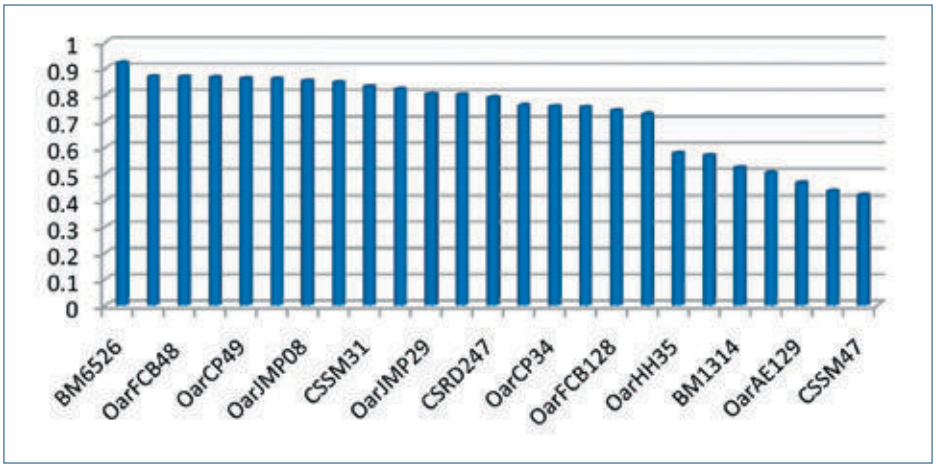

Figure 3: Polymorphism Information Content of 25 microsatellite markers in Madgyal sheep

Absence of significant deviations from HWE across all the loci within the population suggested no evidence of linkage between loci. Subsequent analyses were, therefore, carried out on the basis that HWE prevailed in the investigated population (Marshall *et al*. 1999).

Observed heterozygosity ranged from 0.143 (OarAE129) to 0.969 (OarCP49) and expected heterozygosity from 0.462 (CSSM47) to 0.927 (BM6526) across the 25 microsatellite loci. The results show that Madgyal sheep possesses a high level of gene diversity (mean expected heterozygosity), indicating that the population has retained the presence of several alleles although at a small frequency. The average observed (0.506) and expected heterozygosity (0.753) of Madgyal sheep were relatively similar to other domestic sheep breeds investigated earlier (Jyotsana *et al*. 2010; Arora *et al*. 2011a, b).

Within Population Inbreeding Estimate (F_{IS})

The F_{IS} estimate (0.334) revealed a deficit of heterozygotes in Madgyal sheep. Significant heterozygotes deficiencies have also been reported in several earlier investigated Indian sheep breeds (Radha *et al*. 2011; Arora *et al*. 2011b). The main cause for high genetic homogeneity or lack of heterozygotes in Madgyal sheep breed might be attributed to Wahlund effect (population substructure) due to collection of samples from different breeding flocks from different villages in the same area. Further, the relatedness of few samples otherwise deemed unrelated during collection may not be denied due to non-availability of pedigreed data under field conditions.

Bottleneck Analysis

A quantitative graphical test based on mode-shift distortion under the TPM (Two Phase Mutation Model) (Luikart *et al*. 1998) was utilized to visualize the allele frequency spectra as a check for genetic bottleneck. No mode shift was detected in the frequency distribution of alleles and a normal L-shaped form was observed (Fig 4). These finding further suggested absence of any recent reduction in the effective population size and non-bottlenecked Madgyal sheep population under mutation drift equilibrium.

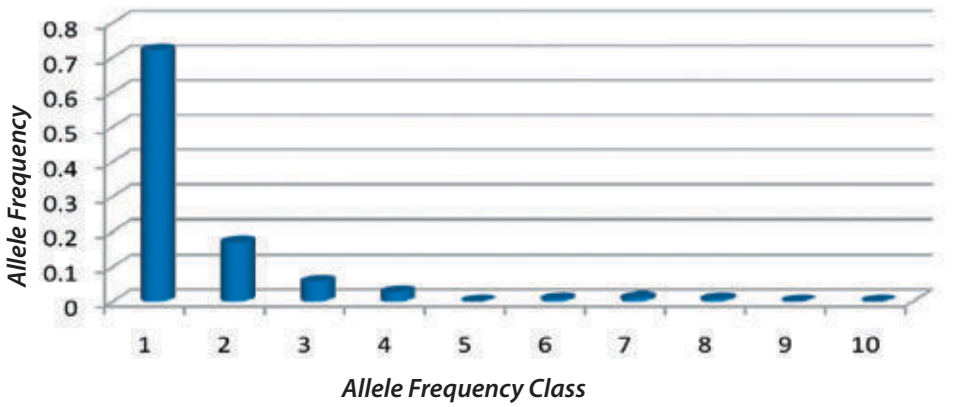

Figure 4: Allele frequency distribution in Madgyal sheep revealing absence of genetic bottleneck

Conclusion

Morphological structure of livestock species has been defined using body measurements which together with body weight describe an individual or population holistically. This study defines the morphometric standard of Madgyal sheep. The studied population showed marked sexual dimorphism. Rams were 30% heavier than ewes. The average values of BW, BL, HW and CG in Madgyal rams were comparable to Munjal rams (Yadav and Arora 2014). Coefficients of variation for all traits ranged from 4.9 to 16.3% and were similar to those obtained by Yadav and Arora (2014) in Munjal and Muzaffarnagri sheep. The flocks are of small size but maintain their integrity in terms of breed purity. The study presents the current status of breeding tract and population, morphological characterization, production and reproduction traits of Madgyal sheep. Estimation of genetic variability in Madgyal sheep is a step towards the on-going characterization of lesser known sheep populations. The holistic approach on phenotypic characterization as well as genetic diversity analysis will provide an insight for its conservation and improvement programmes. Madgyal sheep have promising quality of rapid weight gain, well adapted to harsh climatic conditions and are most beneficial for mutton production. Thus, they are of value to the Indian sheep industry. With disease resistance, good lamb survivability, good mothering instincts and good flocking, Madgyal sheep have a lot to offer shepherds and mutton industry in Maharashtra. Concrete and concerted efforts are needed for multiplication of the population of Madgyal sheep. The following recommendations may be advantageous in horizontal and vertical proliferation of its population.

Recommendations

1. Madgyal sheep show potential for rapid weight gain, therefore, this should be promoted to increase the meat production of the state. Issues like profitability of Madgyal crosses *vis-a-vis* pure Madgyal and adaptation outside the breeding tract (Jath taluka) need to be studied by setting appropriate hypotheses. Accordingly, policy decisions, whether Madgyal

should be promoted to increase the meat production of the state within its breeding tract or beyond also, need to be taken.

2. In Madgyal sheep average flock size is small, though flock purity is not an issue. Increase in flock size seems to be a utilitarian proposition. Therefore, policy initiatives should be taken for its vertical proliferation (increasing flock size at shepherd's level). It should require marshalling of resources through planning and policy interventions. Packaging of many plan components in one could be one of the alternatives.

3. A breed society could provide crucial support for recognition of a breed at national level. 'Madgyal Sheep Breeder Society' should therefore be formed and the population should be registered as a breed. Collectively, these interventions would ensure the horizontal proliferation (increased number of people adopting sheep husbandry). Once the benefits and resources start pouring in, it would act as a catalyst for vertical proliferation of the population.

4. The system of unorganized marketing of lambs and adult animals (selling animals purely on the basis of visual appraisal of their age, form and weight, and male animals getting higher prices than females) is disadvantageous to the shepherds. It is typically non-standardized, unregulated and ad hoc transactions oriented wherein a butcher earns more by slaughtering sheep and selling mutton than a shepherd/farmer who rears lambs about 6 months by sweating. This imbalance needs course correction by building equitable livestock markets.

Acknowledgements

Completing a successful characterization and classification project on sheep ecotypes of Maharashtra is really a great pleasure to us. The project required huge amount of work and dedication in the field. Still, implementation would not have been possible if we did not have a support of many individuals and organizations. Therefore, we would like to extend our sincere gratitude to all of them. We are thankful to Director, ICAR-National Bureau of Animal Genetic Resources, Karnal

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