Sheep Genetic Resources of India

KOLHAPURI SHEEP

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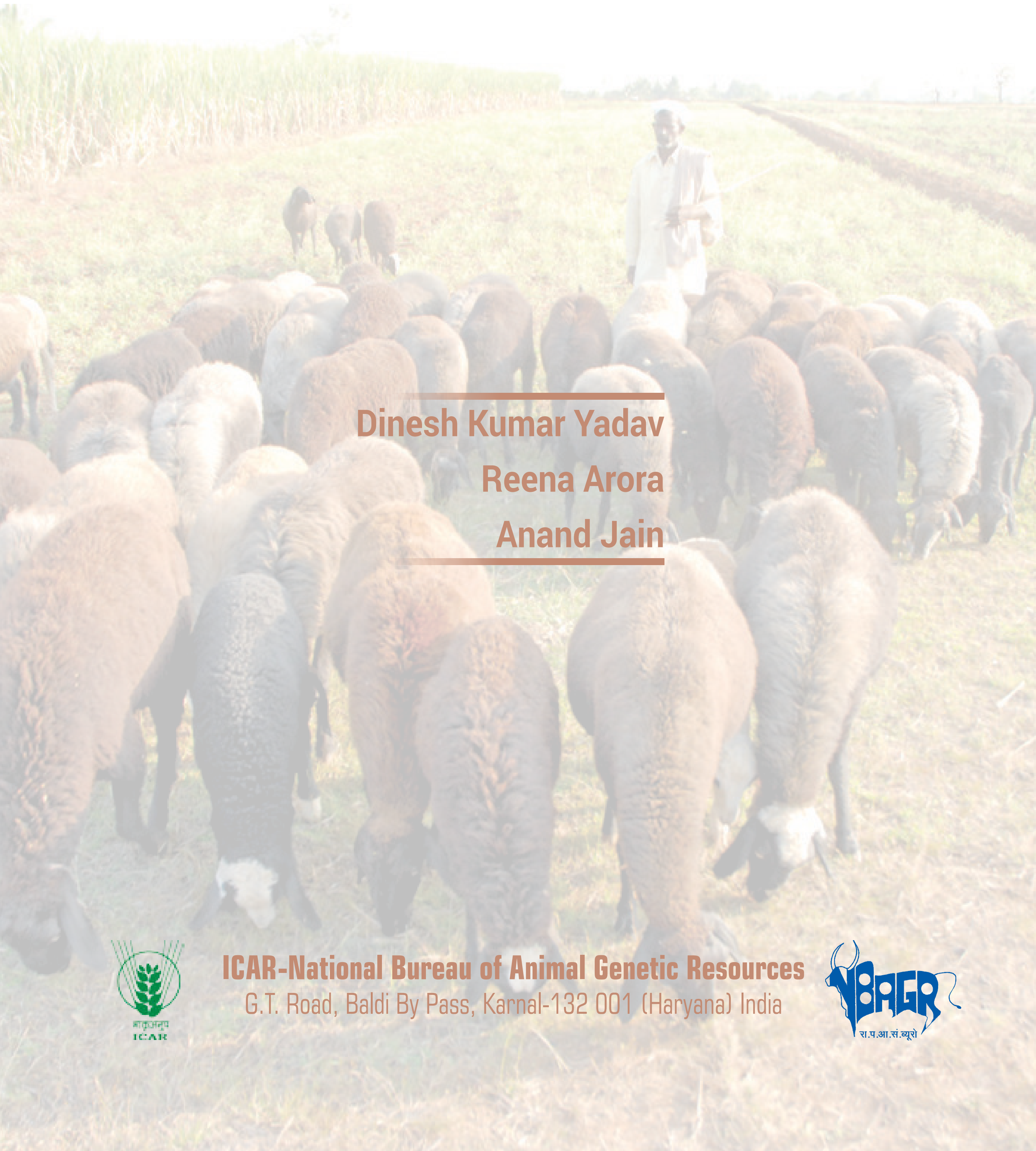

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Preface

Sheep biodiversity have been described using morphological data as well as molecular data. Breed characterization not only helps to physically identify, describe, and recognise a breed but also to classify livestock breeds into broad categories. Apart from 40 well recognized sheep breeds in India, there are some other populations like Kolhapuri, which are not characterized and registered as breeds. Kolhapuri is one of the five ecotypes of Deccani sheep (Lonand, Sangamneri, Solapuri, Madgyal and Kolhapuri) in Maharashtra. Despite the importance of Kolhapuri sheep in the lives of marginal and landless farmers, information on the population is still scarce. The objective of bringing out this monograph is to provide a landscape of morphometric and molecular genetic characterization of Kolhapuri sheep. The authors 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. Also, we draw attention to those responsible for designing appropriate breeding and selection schemes for indigenous sheep improvement and sustainable conservation.

Authors

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Introduction

One of the Strategic Priority Areas of the *Global Plan of Action* is the characterization, inventorization and monitoring of trends in AnGR diversity in order to properly assess the value of breeds and to guide decision making in livestock development and breeding programmes (FAO, 2007). In India, sheep breeds most often correspond to local populations that differ gradually according to geographical separation. These exhibit large variations in morphology and production (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, 2014; Yadav and Arora 2014). There are five sheep ecotypes viz. Madgyal, Lonand, Kolhapuri, Solapuri and Sangamneri (Ghanekar, 1983; Gokhle, 2003; Karim and Prince, 2011) of Deccani sheep in Maharashtra. The ecotypes exhibit distinct morphological characteristics (Yadav *et al.* 2014), thrive in less favoured rural areas and are reared mainly by the marginalized farmers and pastoralists who have maintained them under extensive grazing system. Kolhapuri ecotype is found in Kolhapur district and is one of the most important means of livelihood security for landless and marginal rural populace. This monograph presents its morphometric and molecular genetic characterization parameters. It attempts to define the morphometric standard and current genetic status which would not only have impact on management of these animals but also reduce misidentification by sheep traders and help in the conservation of genetic resources.

Data Collection and Analysis

Interview method and questionnaire were used for data collection on socio-economic parameters, reproduction traits, disease prevalence, mortality, management practices and current breed merit. Purposive sampling was used to determine the distribution area. Villages having Kolhapuri sheep were identified for data recording based on information of the officials of Maharashtra Sheep and Goat Development Corporation, Pune, and the shepherds. 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 sheep studied from different flocks were reared under extensive management system. The traits measured were body length (BL), height at withers (HW), chest girth (CG), paunch girth (PG), ear length (EL) and tail length (TL). Seventy sheep flocks were assessed in the breeding tract of Kolhapuri sheep. Data were recorded on above mentioned morphometric traits of 313 adult sheep (60 rams and 253 ewes). Body weight of 245 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 Kolhapuri 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 Kolhapuri characteristics. Genomic DNA was isolated and purified using the standard phenol chloroform extraction protocol. Genetic variation was assayed using 25 microsatellite markers 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

The habitat or distribution of a breed is the geographical area within which that breed can be found. Kolhapuri sheep are distributed in Kagal, Karvir and Hatkanangle tehsils of Maharashtra (Fig 1). Kolhapuri sheep, also known as 'Konkani' got its name after Kolhapur. Kolhapur, according to a legend, was settled by 'Kolhasur', a demon who was later killed by 'Mahalakshmi' to relieve the local populace. Honouring the demon's dying wish, the city was named after him. According to the 19th Livestock Census (2012), there were 0.104 million sheep in Kolhapur district (Table 1). Out of 12 taluka, 7 were having <4000 sheep. In the rest 5, the sheep population was maximum in Hatkanangle taluka (0.03 million) and minimum in Karvir (0.01 million).

Table 1: Sheep population of Kolhapur district of Maharashtra*

S. No.	Tehsil/Taluka	Exotic/crossbred	Indigenous	Total
1	Ajra	4	25	29
2	Bavda	10	2	12
3	Bhudargad	7	3002	3009
4	Chandgad	2	659	661
5	Gadhinglaj	2183	13058	15241
6	Hatkanangle	1132	29090	30222
7	Kagal	274	23732	24006
8	Karvir	58	11147	11205
9	Panhala	829	2725	3554
10	Radhanagari	1	594	595
11	Shahuwadi	96	2645	2741
12	Shirol	3	12852	12855
Total		4599	99531	104130

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

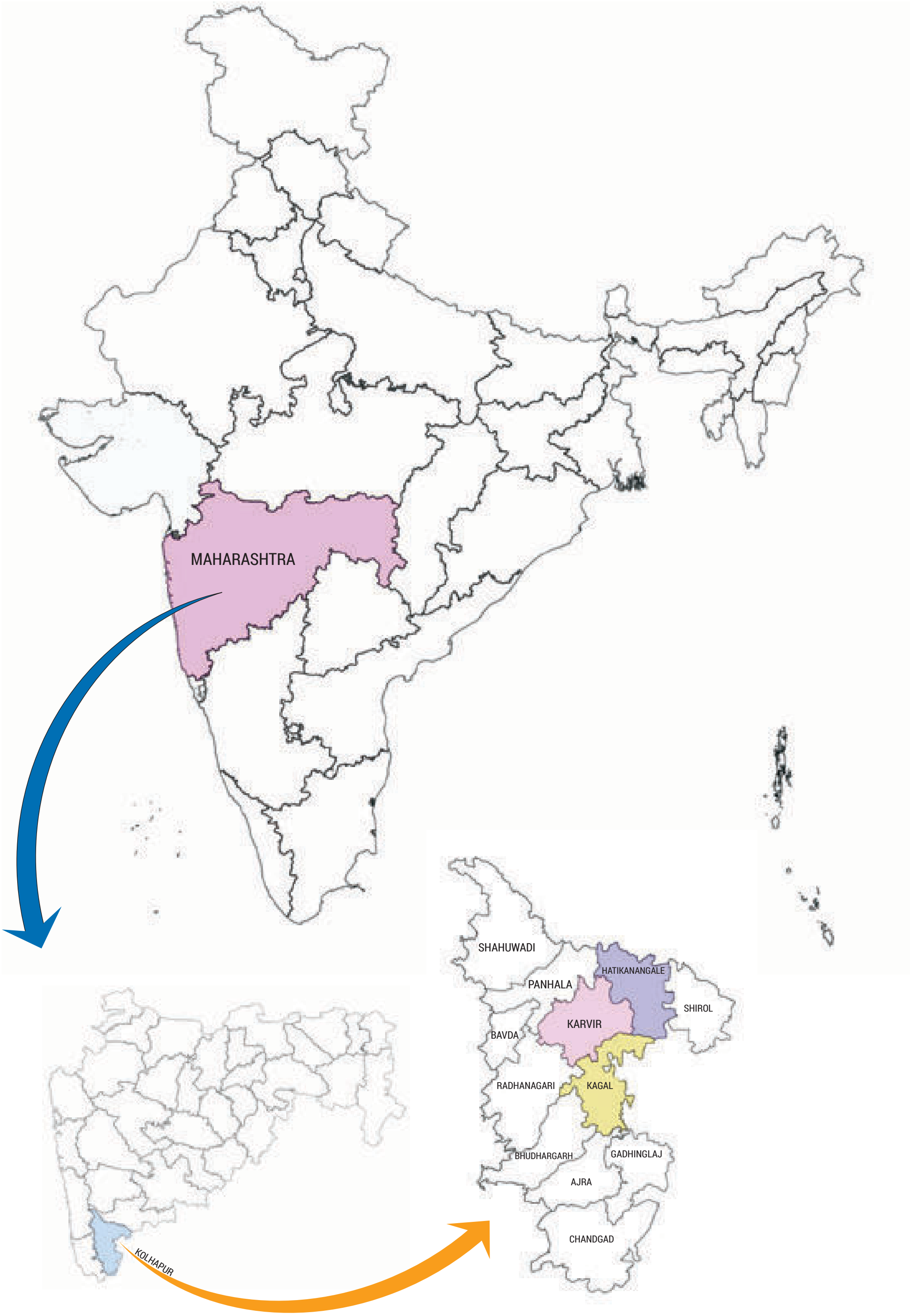


Fig 1: Distribution Area of Kolhapuri Sheep

Agro-climatic Conditions

Kolhapur, one of the industrially and agriculturally developed district, lies in the south-west of Maharashtra between 15° 43' to 17° 17' North latitude and 73° 40' to 74° 42' East longitude and spreads across the Deccan Plateau in the rain shadow region of the Sahyadri mountain ranges on the southernmost tip of the state. The average height above MSL varies between 390 to 900 metres. Kolhapur city is situated on the bank of river Panchaganga, also known as 'Dakshin Kashi'. It lies at an altitude of 605 m MSL. Kolhapur is famous in the country for production of jaggery, Kolhapuri chappals, silver jewellery, and Indian wrestling. It is one of the leading and shining examples in the co-operative movement of India.

The climate of Kolhapur is moderate and pleasant. The temperature has a relatively narrow range between 10°C to 35°C. Summer is comparatively cooler but more humid than neighbouring places. Maximum temperature rarely exceeds 38°C. Three distinct seasons viz. hot (March-May), rainy (June-October) and cold (November-February) are experienced. The district receives rain from the South-West as well as North-East monsoons. The rainfall is unevenly distributed in the district. It varies from a mean annual rainfall of 480 mm in the eastern Shirol Taluka to around 6000 mm along the Western Ghats in Gaganbawada Taluka. The average rainfall in Kolhapur district is around 1900 mm.

There are six main rivers of Kolhapur, the Warana, the Panchanganga, the Dudhganga, the Vedganga, the Hiranyakeshi and the Ghatprabha. These rivers rise in the Sahyadri hills and flow south-east, east or north-east across the Kolhapur plateau towards the Krishna. The Warana has the south-eastern trend and it serves as the boundary between Kolhapur and Sangli district. However, all these rivers are seasonal. The Panchanganga has blessed the people of the district and has boosted its agricultural economy significantly.

Soil is very important resource because it acts as medium for the cultivation of crops. Kolhapur district is endowed with brownish, reddish and black soil types. Western hilly region soil is red to brownish red having high iron content, which is suitable for paddy crops and fruits cultivation. The central region has brown fertile soil while the eastern region has black soil. Due to presence of phosphorous in the soil, the Kolhapur district is very suitable for plantation of sugarcane and tobacco. The district exhibits a mixed cropping pattern. Broadly food grain and cash crops are grown. Rice, sorghum, ragi, pulses, groundnut, soybean, oil seeds and sugarcane are the Kharif crops. Wheat, sorghum, chickpea and maize are major Rabi season crops. Kolhapur is leading district in sugarcane cultivation and sugar industry. The feed resources in the district are maize, jowar, babool, karoo, peepal, chinch and ber. (Source: Agricultural Statistical Information, Maharashtra; Maharashtra State Gazetteer; Internet)

Morphometric Characteristics

Kolhapuri sheep are large sized, mutton sheep of Maharashtra. These are mottled (black colour mingled with varying shades of brown and off white) having a straight backline. Belly is medium deep. Ears are medium, flat, drooping, and alert. Both sexes are polled. Some males have thick and corrugated horns, and some ewes also have thin and short horns. Animals are very active, have strong herding tendency and suitable for walking long distances. Tail is thin and medium in size. Hindquarter is heavier. Wool is coarse, hairy and dense. Wool colour fades with advancement of age. Head, face, belly and legs are devoid of wool. Nose line is straight. Udder is round with cylindrical teats and pointed tip.




Body Biometry

Average body weight and body biometry of adult Kolhapuri sheep are presented in Table 2 while average body weight of lambs are shown in Table 3. The average body weights of ewes and rams were 31.8±0.26 and 43.3±0.92 kg respectively. In adult females; the body length, height at wither, chest girth, paunch girth, ear length and tail length were 71.0±0.17, 68.2±0.16, 75.4±0.22, 73.5±0.22, 16.9±0.17, 15.0±0.09 cm. respectively; and the corresponding values in adult males were 78.6±0.47, 75.8±0.46, 81.4±0.58, 79.2±0.59, 16.9±0.38, 16.6±0.24 cm. respectively. Substantial sexual dimorphism was observed in all seven morphometric traits (Table 2). Males were 36 % heavier than females. Coefficient of variation of the morphometric traits varied from 3.7 to 16.5%. Coefficient of variation was slightly higher in males as compared to females. The difference may be ascribed to the smaller sample size in males. Up to 3 months of age, body weight of male and female lambs ranged between 2-21 and kg 2-17 respectively. 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. Average weight of two-tooth animals was 27.6 kg in females and 36.8 kg in males. The body biometry reflects that Kolhapuri sheep are large in size with medium tail.

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

Morphometric Trait	Female (253)			Male (60)			Sexual dimorphism (m/f)
	Mean ± SE	Coefficient of variation	Range	Mean ± SE	Coefficient of variation	Range	
BW	31.8±0.26	13.2	21-44	43.3±0.92	16.5	28-58	1.36
BL	71.0±0.17	3.8	64-79	78.6±0.47	4.7	71-86	1.11
HW	68.2±0.16	3.7	63-76	75.8±0.46	4.7	68-83	1.11
CG	75.4±0.22	4.6	66-87	81.4±0.58	5.5	71-88	1.08
PG	73.5±0.22	4.8	65-88	79.2±0.59	5.8	69-89	1.08
EL	16.9±0.17	16.2	5-21	16.9±0.38	17.7	6-21	1.00
TL	15.0±0.09	10.5	11-20	16.6±0.24	11.3	13-20	1.11

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 Kolhapuri lambs

Age (month)	Female		Male	
	Average ± SE	Range	Average ± SE	Range
0-3	10±0.49 (63)	2-17	10.8±0.75 (50)	2-21
3-6	16.4±0.29 (38)	13-21	21.6±1.74 (8)	17-30
6-12	22.5±0.46 (68)	15-31	28.4±1.53 (18)	18-38

Socio-economic status of the shepherds/sheep farmers

Kolhapuri sheep are mostly reared by shepherds / farmers belonging to Dhangar and Ramoshi communities. 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 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.

Seventy sheep farmers were interviewed from Kagal, Karvir and Hatkanangale talukas of Kolhapur district. The average family size was 5.9 with 2.2 males, 1.9 females and 1.8 children. The overall literacy was 46.4 %. The literacy among male, female and children was 33.1%, 46.3% and 62.9% respectively. Besides rearing sheep, 27%, 86%, 41%, and 29% also maintained cattle, goat, buffalo and chicken respectively, whereas 13% maintained both cattle and buffalo.

Flock Size and Breed Purity

In 70 flocks, the average flock size was 79.5 with 61.6 ewes, 2.3 rams and 15.6 lambs. Most of the farmers kept two or three rams. The percentage of Kolhapuri sheep in the surveyed flocks was 70.7. Madgyal rams were observed in some of the flocks. Better growth and comparatively higher price have tempted the farmers to breed the ewes with Madgyal rams. State government is also providing Madgyal rams. But aversion to crossing with Madgyal rams was observed amongst some of the farmers due to poor adaptations and higher cost of maintenance.

Management Practices

Kolhapuri 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. Water is provided 2-3 times a day depending upon availability of water source and weather conditions. In pastoral areas, sheep normally graze within a radius of about 3 km of a watering point. The sheep flocks are migratory. Rainfall is good in Kolhapur district. Sheep remain on migration from June to October/November (till Dussehra). Migration is mostly restricted within

the state in adjoining districts viz. Sangli, Solapur and Pandharpur. Some farmers also migrate to Bijapur (Karnataka). Majority of farmers provide housing especially during night. Generally it is open, thatched, fenced and adjacent to owner's house. The boundaries of the enclosures are made of tree branches/bushes/rope or wire netting. While on migration the flocks are housed in open fields with temporary fencing of either ropes or iron wires. Sheep prefer to graze on hillsides and steep slopes and provide a means for improving forage utilization and soil fertility on areas not accessible to farm equipment. 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. 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 or in the open fields. In addition to suckling mother's milk, most of the farmers feed the lambs on goat and buffalo milk. Approximately 400 ml milk per day is fed till the lamb attains the age of 45-60 days, thereafter, maize and groundnut cake are fed. Neem, Babool and Ber leaves (a source of protein) are also fed to lambs. Rams are also given groundnut, jowar and maize.

Disease and health problems of sheep are closely associated with management and nutrition. Preventing disease reduces economic losses and improves animal welfare. 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. 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 5-12 %. Still births and abortions are also reported.

Breeding and Reproduction

In sheep husbandry, lamb production is the primary objective, which is affected by the reproductive rate. Optimal reproductive rates are essential to profitable sheep production. Management affects the reproductive rates. Shepherds keep breeding

rams with ewes. August-October is the main lambing season and March-April, the minor. Age and weight at puberty in females are around 13-18 months and 27 kg. Age at first lambing is 18-24 months. In farmers' flocks, lambing percentage on the basis of ewes available is 75-85%. Lambing interval is 6-8 months. Litter size is single. Age at first breeding in males is 15-18 month and weight at two teeth age is around 37 kg. Breeding life of a ram is 6-7 years. Some of the farmers exchange the rams in order to avoid inbreeding. Rams are selected on the basis of body size and conformation. Lambs for raising future breeding rams are selected at the age of 2-3 months based on height, body length, face and weight (12-15 kg). Body weight, height, fine Roman nose, large scrotum, soft and long wool are the preferred traits for selection of rams. Lambs of ewes which give more milk are selected for future stock. Daily milk yield is 200-250 ml and lactation length is 60-75 days. Madgyal crosses are less preferred by shepherds in Kolhapuri breeding tract due to poor adaptations and high cost of maintenance. The conservative estimates given by farmers indicated that maintenance cost of Madgyal crosses increase by 10% whereas returns are to the tune of 5% only as compared to pure Kolhapuri sheep. They pointed that Madgyal crosses fall sick in winter season. Their wool quantity and staple length reduces. The wool is coarser than that of Kolhapuri sheep.

Wool Production

Kolhapuri sheep are mainly reared for mutton but wool is also utilized for making local products viz. Ghoghri (blanket) and Jena (carpet). The earnings from the coarse and short wool are little. Sale price of wool was reported as ₹ 20 per kg. Sheep are shorn twice a year (March/April and October/November). Wool yield per year is around 500-600 grams.

Lamb Marketing

Lamb prices have a large influence on profitability and viability of sheep husbandry as it derives majority of the income from sale of lambs. Lamb prices vary by year, season, body condition, sex and marketing method. In India demand for mutton is increasing due to increasing human population, urbanisation and income. Due to gap between demand and supply of mutton, the sale prices of lambs are also increasing every year. Kolhapuri lambs are marketed between the age of 3 and 4 months. Butchers and traders purchase the lambs on visual appraisal, generally, in groups. Male lambs are priced higher than female lambs. 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 ₹3000-3500 whereas its female counterparts bring ₹ 2500-3000 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 healthy ram is higher priced (₹ 10000-11000) than a corresponding ewe (₹ 7000-8000). Old aged ram (6-7 years) procures a price of ₹ 7000-8000 depending upon the body condition. According to farmers' conservative estimates, a flock of 40 generates an income of ₹ 50000 from lambs whereas total expenditure incurred is around ₹ 20000 per year.

Socio-economic and Cultural Values

Kolhapuri sheep act a mobile bank and meet out majority 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 the honour of Biroba (a deity of shepherd) 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. Providing 5-10 sheep to daughter in her marriage was a common practice in the past which has been abandoned now. On the occasion of Deepawali festival, the sheep flock is worshiped. One ewe and ram are garlanded and turmeric tilak is put on their forehead. Sweets are prepared and marriage like ceremony of the ram and ewe is performed. Ram fighting is also arranged. Weak ram will run away. Running competition for ewes is also held. Owner runs and his/her ewes run after him/her. Winner is declared based on minimum time taken to reach the goal. Most of the shepherds follow the practice of sacrificing a lamb every three years with a belief that family and flock health will be maintained. The lamb for sacrifice is earmarked at its birth which remains in the flock for a period of three years.

Genetic Diversity

Genotypic data from 25 microsatellites (Table 4) was used to assess the genetic diversity of Kolhapuri sheep. The various variability measures estimated across 25 loci in Kolhapuri sheep are shown in Table 5. All the microsatellites amplified well and were polymorphic in nature with more than 7 alleles. A total of 313 distinct alleles were detected across the analysed microsatellite loci. The investigated 25 ovine microsatellites represented 19 autosomal chromosomes in sheep. These microsatellite loci also exhibited high level of genetic variability as revealed by a wide range of alleles (N_a) which varied from 7 (BM757) to 19 (CSRD247). The effective number of alleles (N_e) per locus ranged from 1.89 (BM6506) to 11.10 (CSRD247). The allele frequency data revealed considerable variation in the distribution of allele frequencies across loci (0.005 to 0.703, Fig 2). The low frequency of the most common alleles (< 95%) at each investigated locus further supported the polymorphic nature of the used microsatellites and their utility in the measurement of diversity indices based on genetic polymorphism studies. The observed and expected heterozygosity values of the microsatellite loci ranged from 0.283 (CSRD247) to 0.919 (OarCP20) and 0.471 (BM6506) to 0.910 (CSRD247) respectively. The use of microsatellites with a wide range of heterozygosity reduces the risk of overestimating genetic variability, which might occur with microsatellites exhibiting only high heterozygosity.

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

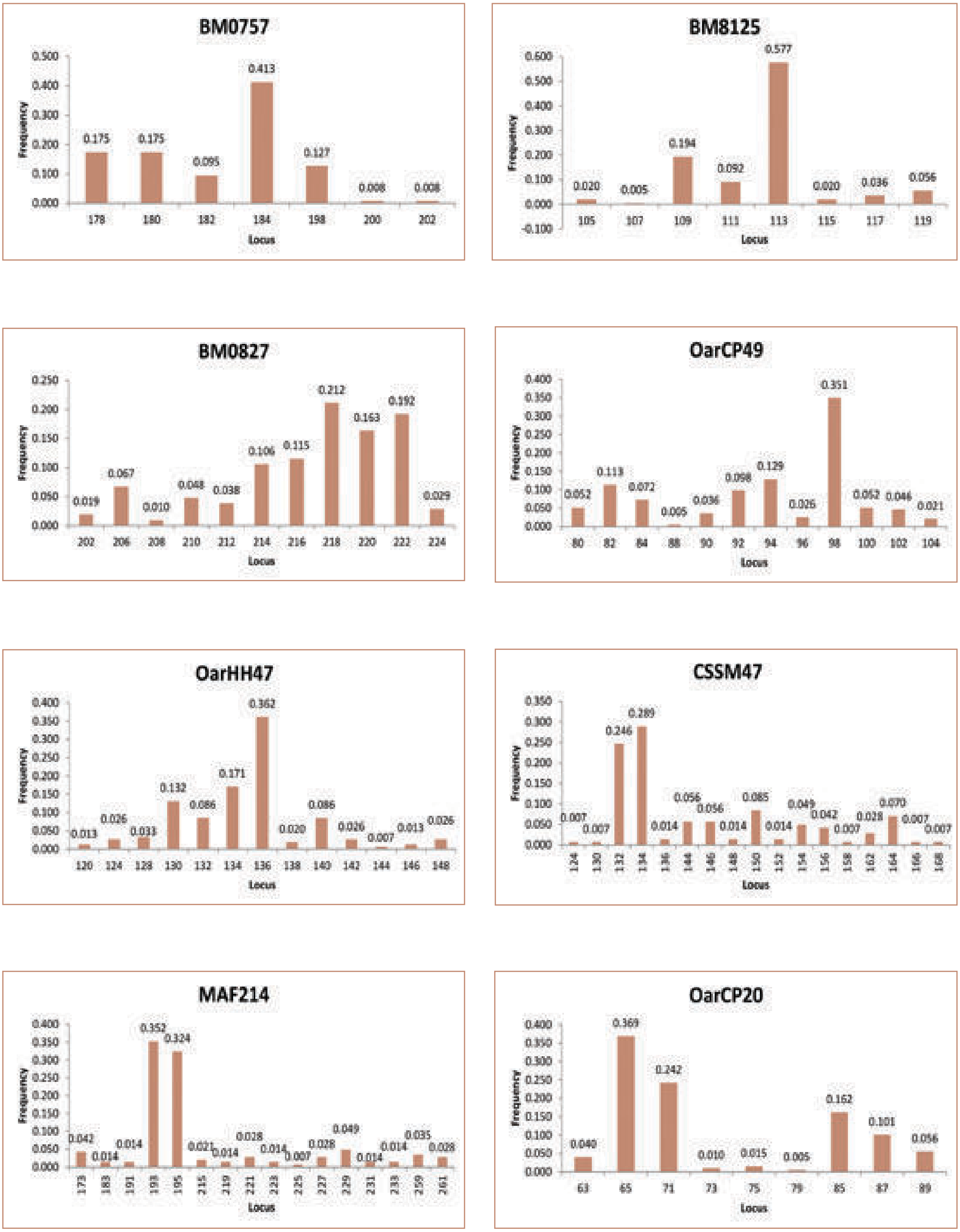
Locus	Primer sequence	Type of repeat	Size range (bp)	Chr. no.	GenBank Acc. no.
BM757	tgg aaa caa tgt aaa cct ggg ttg agc cac caa gga acc	$(GT)_{17}$	178-198	9	G18473
BM827	ggg ctg gtc gta tgc tga g gtt gga ctt gct gaa gtg acc	-	214-224	3	U06763
BM1314	ttc ctc ctc ttc tct cca aac atc tca aac gcc agt gtg 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 $(CA)_7$TA $(CA)_{25}$	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	$(AC)_{17}$TTGCGTGT $(CA)_4$	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	$(GT)_6$GC $(GT)_{15}$	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

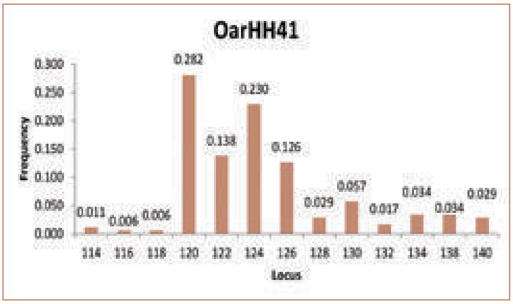
Table 5. Genetic diversity indices across 25 microsatellite markers in Kolhapuri sheep

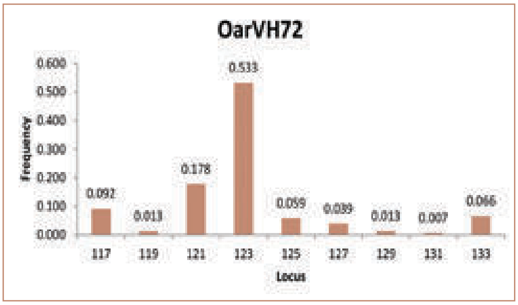
Locus	N_a	N_e	H_o	H_e
BM0757	7	3.897	0.587	0.743
BM0827	11	7.014	0.654	0.857
BM1314	14	5.475	0.313	0.817
BM6506	8	1.890	0.341	0.471
BM6526	11	4.654	0.517	0.785
BM8125	8	2.606	0.561	0.616
CSRD247	19	11.103	0.283	0.910
CSSM31	15	10.595	0.557	0.906
CSSM47	17	5.938	0.493	0.832
HSC	17	9.779	0.500	0.898
INRA63	14	6.958	0.625	0.856
MAF214	16	4.194	0.535	0.762
OarAE129	12	4.981	0.402	0.799
OarCP20	9	4.236	0.919	0.764
OarCP34	13	6.863	0.462	0.854
OarCP49	12	5.649	0.649	0.823
OarFCB128	15	8.266	0.580	0.879
OarFCB48	12	7.413	0.640	0.865
OarHH35	14	6.610	0.607	0.849
OarHH41	13	5.715	0.598	0.825
OarHH47	13	5.100	0.671	0.804
OarHH64	10	4.320	0.632	0.769
OarJMP08	11	5.528	0.577	0.819
OarJMP29	13	4.374	0.721	0.771
OarVH72	9	2.996	0.592	0.666
Mean	**12.52**	**5.846**	**0.561**	**0.798**

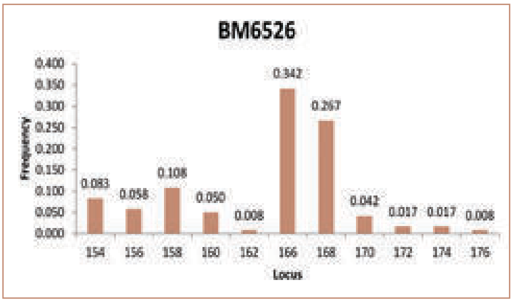
N_a: Observed number of alleles; N_e: Effective number of alleles; I: Shannon information index; H_o: Observed heterozygosity; H_e: Expected heterozygosity

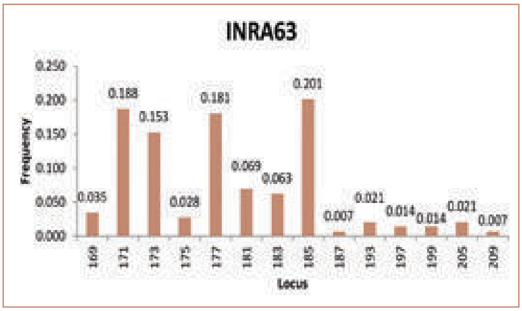
Fig 2: Allele Frequency Distribution at 25 Microsatellite Loci in Kolhapuri Sheep

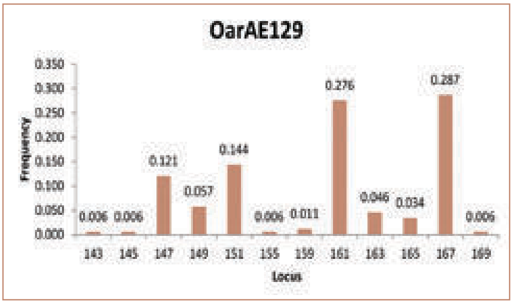


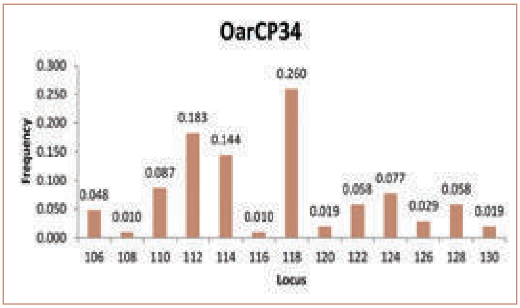

OarHH41
Frequency
Locus

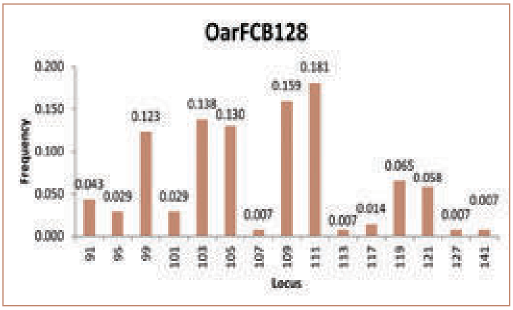

OarVH72
Frequency
Locus

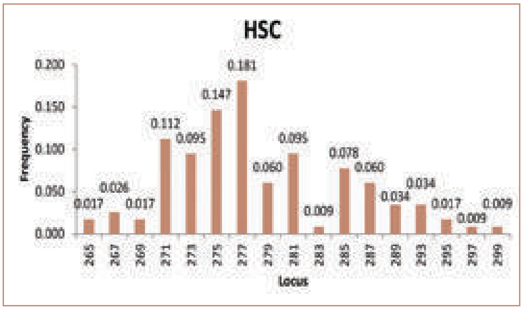

BM6526
Frequency
Locus

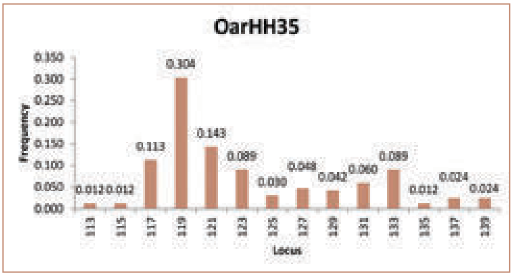

INRA63
Frequency
Locus

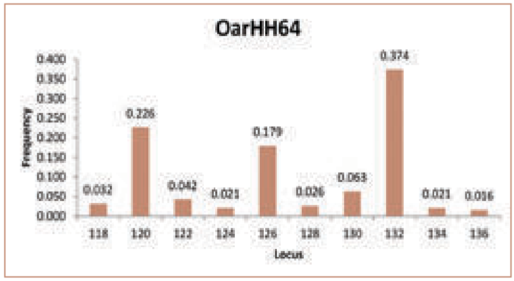

OarAE129
Frequency
Locus

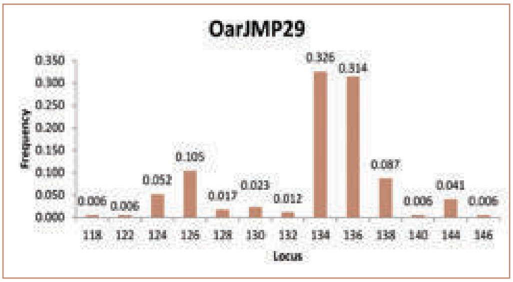

OarCP34
Frequency
Locus

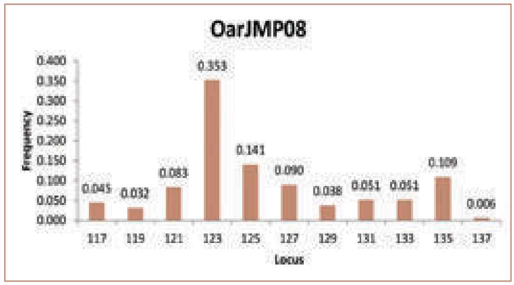

OarFCB128
Frequency
Locus

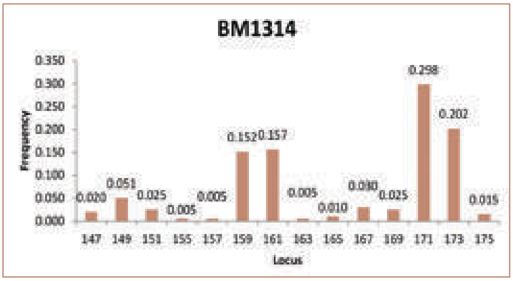

HSC
Frequency
Locus

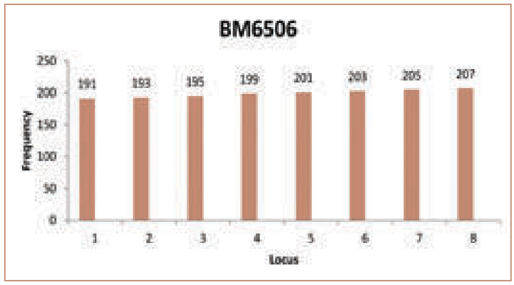

OarHH35
Frequency
0.304
0.143
0.113
0.089
0.089
0.060
0.048
0.042
0.030
0.024
0.024
0.012 0.012
0.012
113 115 117 119 121 123 125 127 129 131 133 135 137 139
Locus

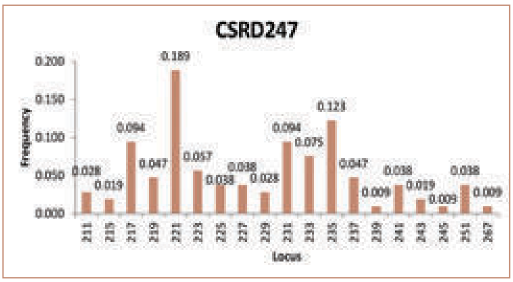

OarHH64
Frequency
0.374
0.226
0.179
0.063
0.042
0.032
0.026
0.021
0.021
0.016
118 120 122 124 126 128 130 132 134 136
Locus

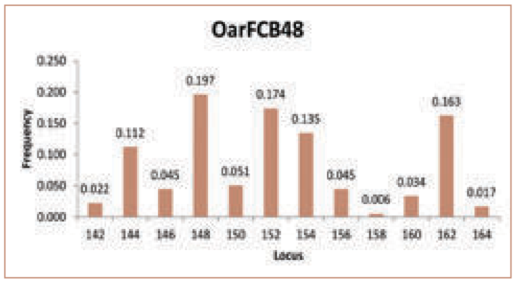

OarJMP29
Frequency
0.326
0.314
0.105
0.087
0.052
0.041
0.023
0.017
0.012
0.006 0.006
0.006
0.006
118 122 124 126 128 130 132 134 136 138 140 144 146
Locus

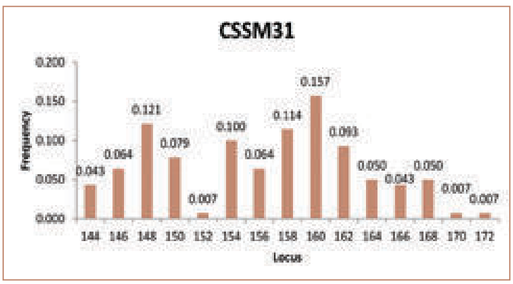

OarJMP08
Frequency
0.353
0.141
0.109
0.090
0.083
0.051 0.051
0.045
0.038
0.032
0.006
117 119 121 123 125 127 129 131 133 135 137
Locus


BM1314
Frequency
0.298
0.202
0.152 0.157
0.051
0.030
0.025
0.025
0.020
0.015
0.010
0.005
0.005
0.005
147 149 151 155 157 159 161 163 165 167 169 171 173 175
Locus


BM6506
Frequency
191 193 195 199 201 203 205 207
1 2 3 4 5 6 7 8
Locus


CSRD247
Frequency
0.189
0.123
0.094
0.094
0.075
0.057
0.047
0.047
0.038
0.038
0.038
0.038
0.028
0.028
0.019
0.019
0.009
0.009
0.009
211 215 217 219 221 223 225 227 229 231 233 235 237 239 241 243 245 251 267
Locus


OarFCB48
Frequency
0.197
0.174
0.163
0.135
0.112
0.051
0.045
0.045
0.034
0.022
0.017
0.006
142 144 146 148 150 152 154 156 158 160 162 164
Locus


CSSM31
Frequency
0.157
0.121
0.114
0.100
0.093
0.079
0.064
0.064
0.050
0.050
0.043
0.043
0.007
0.007
0.007
144 146 148 150 152 154 156 158 160 162 164 166 168 170 172
Locus

Polymorphic Information Content

The PIC values varied from 0.437 (BM6506) to 0.904 (CSRD247) with a mean of 0.776 (Fig 3). High PIC values (> 0.5) for majority of the markers employed (96%) further substantiated their utility in biodiversity evaluation of native Indian sheep breeds. High average estimates of PIC are suggestive of suitability of used set of markers to other applications such as parentage verification and linkage mapping studies.

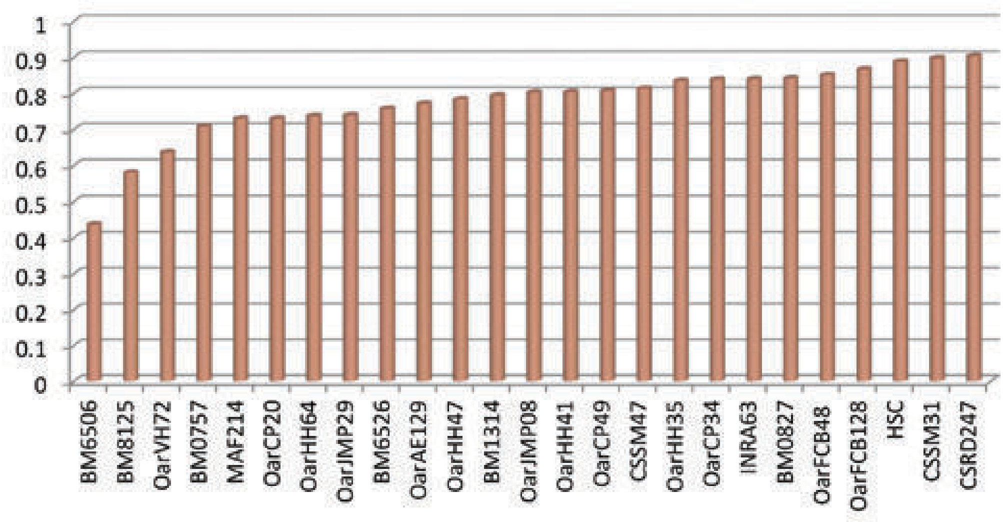


Fig 3: Polymorphism Information Content of 25 microsatellite markers in Kolhapuri sheep

Intra Breed Genetic Variation

The intra breed genetic variation is indicated by the overall allele diversity (mean number of observed alleles over a range of loci (Na), observed heterozygosity (H_o), gene diversity (expected heterozygosity, H_e) values, and within population inbreeding estimate (F_{IS}). The allele diversity, considered to be a reasonable indicator of genetic variation within the population, displayed high genetic variation (12.52) in Kolhapuri sheep. The observed heterozygosity (0.561) and gene diversity

(0.798) values of Kolhapuri were relatively similar to those of other domestic sheep breeds investigated earlier (Arora and Bhatia 2004; 2006; Arora et al 2010). The mean observed heterozygosity values, though lower than the expected values, did not exhibit significant differences when tested using ANOVA ($P>0.05$), which suggested random mating in Kolhapuri. The high value of gene diversity indicated that the population had retained the presence of several alleles although at low frequencies. This implied a substantial amount of genetic variability in Kolhapuri that might be used in planning breeding strategies particularly in populations of small sizes.

This population also showed a high heterozygote deficit value (F_{IS}=0.288). The high heterozygote deficiencies could be due to segregation of non-amplifying (null) alleles or Wahlund effect (population substructure). In the present study it was not possible to estimate the extent of null alleles as no pedigree records were available for analysis and blood samples were taken from unrelated animals. However, the heterozygote deficiency in Kolhapuri sheep may be due to possibility of Wahlund effect resulting from pooling samples (within breed) from different breeding flocks i.e. different villages and flocks in the same area.

The similar tendencies of the three variables viz., allele diversity, mean observed heterozygosity and gene diversity estimates observed in Kolhapuri sheep showed the investigated population to be under mutation drift equilibrium. These measurements, however, behave differently when a population bottleneck is followed by a rapid population expansion. Efforts made to study recent bottleneck effect (up to 40-80 generations) in the investigated sheep population by using the Mode shift test revealed a normal L-shaped curve (Fig 4). This finding clearly suggested the absence of a recent reduction in the effective population size or a genetic bottleneck and further supported Kolhapuri sheep as being a non-bottlenecked population under mutation drift equilibrium.

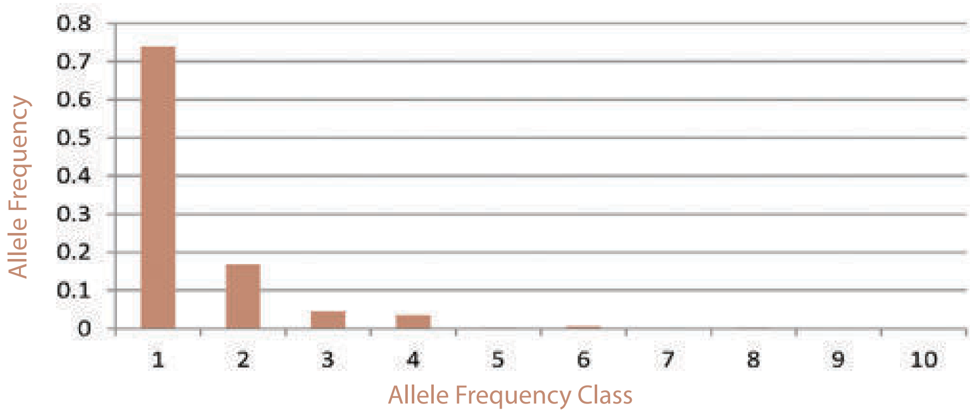


Fig 4: Allele frequency distribution in Kolhapuri sheep revealing absence of genetic bottleneck

Conclusion

Characterization based on morphological traits can provide to some extent a reasonable representation of the differences among the breeds, though not exhaustive, it serves as the foundation upon which molecular genetic analysis can be built. This study characterises Kolhapuri sheep at both levels. It defines its morphometric standard and also provides molecular genetic sketch of the ecotype. The study showed marked sexual dimorphism in the Kolhapuri sheep. Rams were 36% heavier than ewes. The averages for BW, BL, HW and CG in Kolhapuri adult males found in the study were similar to those reported by Yadav et al. (2013) in Bellary sheep. The results in adult females of the present study are also similar to those reported by Yadav et al. (2013) and Yadav and Arora (2014). Coefficients of variation for all traits ranged from 3.7 to 16.5% and were similar to those obtained by Yadav et al. (2013) and Yadav and Arora (2014) in Bellary, Munjal and Muzaffarnagri sheep. The holistic approach on phenotypic characterization as well as genetic diversity analysis will provide an insight for its conservation and improvement programmes. Accurate classification of Kolhapuri sheep vis-à-vis their neighbouring ecotypes will not only have impact on management of these animals but also reduce misidentification by sheep traders and help in the conservation of genetic resources. Kolhapuri sheep are well adapted to harsh climatic conditions of Deccan plateau and are valuable for mutton production in Maharashtra. With disease resistance, good lamb survivability, good mothering instincts and good flocking, Kolhapuri sheep have a lot to offer shepherds and mutton industry in Maharashtra. Concrete and concerted efforts would contribute to multiplication of its population. The following recommendations may be advantageous in proliferation of its population.

Recommendations

1. Madgyal crosses are less preferred by shepherds in Kolhapuri breeding tract due to poor adaptations and higher cost of maintenance. Increase in diseases incidence in winter, reduction in wool quantity and staple length and increase in wool diameter are some of the areas of concern observed by the farmers in Madgyal and Kolhapuri crosses. The conservative estimates given by farmers indicated that maintenance cost of Madgyal crosses increase by 10% whereas returns are 5% as compared to Kolhapuri sheep. However, these observations need to be validated by conducting appropriate studies by related institutes of the region/state. Till then, crossbreeding with Madgyal rams should be avoided.

2. To check the erosion in genetic purity, pure-breeding should be encouraged among the farmers. Purebred rams should be provided to the farmers. The essential action of establishing a nucleus flock and implementing it as open nucleus breeding system with exchange of rams between nucleus herd and farmers flocks may be taken.

3. The system of unorganized marketing of live animals (selling animals purely on the basis of visual appraisal of their age, form and weight, and sex) 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.

4. A breed society could provide crucial support for recognition of a breed at national level. 'Kolhapuri Sheep Breeder Society' may be formed and the population may be registered as a breed.

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Concluding a successful characterization and classification project on sheep ecotypes of Maharashtra is really a great pleasure. 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.

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