



Short communication

Genetic features of Patanwadi, Marwari and Dumba sheep breeds (India) inferred by microsatellite markers[☆]Basanti Jyotsana^{a,*}, Subhash Jakhesara^a, Ved Prakash^b, D.N. Rank^a, P.H. Vataliya^c^a Department of Animal Genetics and Breeding, College of Veterinary Science & A.H., Anand Agricultural University, Anand 388001, Gujarat, India^b Division of Animal Genetics & Breeding, Central Sheep and Wool Research Institute, Avikanagar, Rajasthan, India^c Cattle Breeding Farm, Junagadh Agricultural University, Junagadh, Gujarat, India

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ABSTRACT

India is rich in sheep biodiversity. Patanwadi, Marwari and Dumba are three important breeds of Gujarat. Dumba is a pastoralist breed with a majestic look, which is extensively used for crossbreeding with Patanwadi and Marwari ewes. Microsatellites were used to ascertain genetic variability, bottleneck and population structure using 20 microsatellite markers. The total number of alleles observed was 165, 181 and 160 in Patanwadi, Marwari and Dumba, respectively, depicting a wide range of variability. The overall heterozygosity, polymorphic information content (PIC) and Shannon index values were 0.62, 0.65 and 1.63, indicating high genetic diversity. The F_{IS} estimate reflected a high rate of inbreeding. Non-significant heterozygote excess on the basis of two-phase model (TPM), along with a normal 'L'-shaped distribution of mode-shift analysis test, indicated an absence of bottleneck. Population structure analysis revealed five different clusters and intermixing of individuals from three breeds in each cluster. The information generated from the present study will be valuable for setting conservation priority of the less-described breeds such as Dumba and will also add to the knowledge of previously characterised breeds.

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About 40 distinct breeds distributed in various agro-climatic zones (Acharya, 1982) show the high diversity of Indian sheep breeds. Besides rich variability, indigenous breeds possess many favourable traits such as disease resistance, better heat tolerance and feed conversion efficiency. Patanwadi, Marwari and Dumba are three important sheep breeds of Gujarat. Patanwadi sheep are medium- to large-

sized animals, with white coat and brown face and legs, mainly distributed in Patan, Mehsana, Surendranagar, Rajkot and Jamnagar districts of Gujarat. The majority of them are stationary. They are a superior carpet-wool-producing breed with the capacity to survive well under harsh environmental and management conditions. Marwari sheep are also medium-sized unicoloured animals with white coat and black face mainly found in the south-western districts of Rajasthan and Kachchh, Banaskantha, Mehsana and Surendranagar districts of Gujarat. Marwari sheep are a migratory breed, which migrates to central and south Gujarat during winter and comes back with the onset of monsoon. Dumba sheep are off-white in colour with a good body build, long legs and fat tail, mainly distributed in Surendranagar, Rajkot and Jamnagar districts of Gujarat. They have physical characters suited to a migratory habit. This breed has wool characteristics similar to Marwari sheep and milk-producing ability similar to Patanwadi sheep. These three breeds share overlapping geographical

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existence and Dumba rams are extensively being used for crossbreeding with Patanwadi and Marwari ewes. Hence, there is an urgent need to study the genetic variability and population structure of these breeds. Although they are not apparently facing an immediate risk of extinction, their small number requires demographic and genetic monitoring coupled with the survey of the environmental conditions. Keeping this in view, bottleneck analysis has been done. Dumba breed is still very less described. Hence, there is need to characterise this breed. The information generated from present microsatellite study will also be valuable for setting conservation priority, planning breeding strategy and providing an in-depth understanding of the less-described breed such as Dumba.

1. Materials and methods

A total of 150 blood samples were randomly collected from respective home tracts (50 Patanwadi, 50 Marwari and 50 Dumba). DNA was isolated following the Sambrook et al. (1989) method. Microsatellite loci were amplified in three different multiplexes (panel-I, panel-II and panel-III) comprising nine, five and six microsatellite markers, respectively. Polymerase chain reaction (PCR) was carried out in 15 µl reaction volume containing (30–60 ng) of DNA, 10× PCR buffer (Fermentas, Canada), 2 pmol of each primer (forward primer labelled at 5'-end), 50 mM MgCl₂, 10 mM dNTPs, 0.1 U of Taq DNA polymerase (Applied Biosystems, Foster City, CA - 94404, United States). PCR amplification was performed following an initial denaturation step of 95 °C for 5 min, followed by 35 cycles of 45 s at 95 °C, 45 s at 56 °C Ta (panel-I), 58 °C Ta (panel-II) and 45 s at 58 °C Ta (panel-III) and 45 s at 72 °C followed by a final extension of 10 min at 72 °C. One microlitre of multiplex PCR products was mixed with 0.25 µl of Genescan-500 LIZ Size Standard (Applied Biosystems) and 8.75 µl of Hi-Diformamide (Applied Biosystems) and subjected to electrophoresis. Fragments were resolved on ABI prism® 310 Genetic Analyser (POP-4; run temperature 60 °C) and collected data were analysed by GeneMapper® software.

2. Statistical analysis

Allele frequencies, observed number of alleles and effective number of alleles were analysed using Microsatellite Analyser Software (MSA) version 4.0. The polymorphic information content (PIC) (Botstein et al., 1980) was calculated with PopGene version 1.31 using marker allelic frequencies as follows:

$$PIC = 1 - \sum_{i=1}^j p_i^2 - 2 \sum_{i=j+1}^j \sum_{j=1}^{i-1} p_i^2 p_j^2$$

where p_i and p_j are the frequencies of the i th and j th alleles at a locus with alleles in a population, respectively. Observed heterozygosity, expected heterozygosity and within breed heterozygosity deficit were estimated using FSTAT 2.9.3.2 software. The BOTTLENECK program (Piry et al., 1999) was applied to determine the existence of the bottleneck effect in the investigated sheep population, if any. Under the TPM (Di Rienzo et al., 1994), all the three tests that is, Sign test, standardised differences test and Wilcoxon rank test were applied to detect recent bottleneck, if present, and also to determine whether a population exhibits a significant number of loci with gene diversity excess. The genetic structure of the populations was investigated using the software STRUCTURE version

2.2 (Pritchard et al., 2000). The program STRUCTURE infers the number of populations into which the analysed genotypes can be divided. The program estimates the natural logarithm of the probability $\ln P(D)$ of given genotype, being part of a given population K . To choose the appropriate number of inferred clusters (K), 12 different runs fitting K from 2 to 13 were performed. All runs used a burn-in period of 200 000 iterations and a period of data collection of 200 000 iterations.

3. Results and discussion

A total of 207 alleles were detected across the pooled population with 10.35 mean number of alleles indicating the highly polymorphic nature of these markers. The mean numbers of alleles observed were 8.25, 9.05 and 8.00 in Patanwadi, Marwari and Dumba breeds, respectively. A total of 165 alleles were found in Patanwadi, 181 in Marwari and 160 in Dumba sheep (Table 1). The allele size range obtained at each locus was comparable in all the breeds and in agreement with the data published for European and Indian sheep breeds (Stahlberger-Saitbekova et al., 2001; Arora and Bhatia, 2004; Mukesh et al., 2006; Sharma et al., 2006). The Shannon index and PIC showed the informativeness of the loci under study with an overall mean of 0.65 and 1.63. Since microsatellite markers exhibiting average PIC values higher than 0.5 are considered highly informative (Botstein et al., 1980), thus, with the exception of CSSM 47 and BM6506 (PIC < 0.5), all other loci were very informative. The observed heterozygosities were high, approaching unity, indicating high genetic variability. The highest average observed heterozygosity (H_o) was seen in Marwari (H_o = 0.6380) followed by Dumba (H_o = 0.6360) and Patanwadi (H_o = 0.6058). Average heterozygosity found was comparable with other Indian sheep breeds such as Garole sheep (Sodhi et al., 2003), Muzafarnagri breed (Arora and Bhatia, 2004), Hassan sheep (Sharma et al., 2006) and Nilgiri sheep (Haris et al., 2007). A test for Hardy-Weinberg Equilibrium (HWE) was carried out to evaluate the significant inbreeding occurring at each locus in each population and overall loci in the three populations. Mean estimates of F -statistics obtained were found to be 0.1133 (F_{IS}), 0.1311 (F_{IT}) and 0.0201 (F_{ST}). The high F_{IS} and F_{IT} values indicated high level of inbreeding within and among the populations and also pointed towards low genetic differentiation between the populations. Multilocus F_{ST} values indicated that only 2.0% of the total genetic variation was explained by a breed difference, the remaining 98% corresponding to differences among individuals. The F -estimates obtained in this study were in contrast with most studies in the livestock populations where these estimates were not significantly different from zero even for rare breeds. Diez-Tascon et al. (2000) reported F_{IS} values between 0.023 and 0.096 in related Merinos populations and 0.052–0.113 by Santos-Silva et al. (2008) in Portuguese sheep breeds. The shortage of heterozygotes and excess of homozygotes ($F_{IS} > 0$) exhibited by three populations might be attributed to a number of factors, namely assortative mating, linkage with loci under selection and population heterogeneity. Similar heterozygote deficit was reported in Hassan sheep by Sharma

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