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Microsatellite based genetic diversity and mitochondrial DNA D-Loop variation in economically important goat breeds of Pakistan

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ABSTRACT

The present study was undertaken to analyze the genetic diversity of five economically important goat breeds of Pakistan, Beetal, Kaghani, Teddy, Nachi and Pahari. Fifteen microsatellite loci recommended by ISAG/FAO guidelines were investigated for measures of genetic variability, differentiation and population structure. The genetic variability in terms of allelic diversity and heterozygosity were moderate. The estimated inbreeding coefficient was low in all the investigated goat breeds and not significant. Overall, the populations were less diverse than Eurasian goat breeds, but did not exhibit signs of loss of diversity. Analysis of molecular variance (AMOVA) showed breed differences accounted for 5.42% of total genetic variation indicating low to moderate genetic differentiation among the investigated goat breeds. The genetic structure analysis revealed Teddy, Pahari, and Nachi as distinct breeds, while Beetal and Kaghani form a single genetic group distinct from the other three goats. The mitochondrial DNA control region sequences showed a total of 60 distinct haplotypes belonging to two major maternal lineages A and B1 with a frequency of 76.9% and 23.1% respectively. Comparison of mtDNA sequences from Pakistani, Indian and Iranian goats indicated distinct evolutionary history for Teddy, Beetal, Nachi and Pahari goats different from that of Indian and Iranian goats.
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1. Introduction

Pakistan is one of the largest goat rearing countries in the world following China and India with a population of 64.9 million, 182.9 million and 162 million respectively (FAOSTAT, 2014). During the last two decades, the population of goats in Pakistan has increased by more than 75% from 36.97 million in 1991–64.9 million in 2013 with an annual growth rate of 3.4% (Afzal and Naqvi, 2004; Khan et al., 2008; FAOSTAT, 2014). The goat production system in Pakistan is mostly extensive, low external input type and is dependent on grazing alone or with provision of some available fodder during certain seasons. Most of the goats in Pakistan are owned by smallholders with the flock size ranging from 6 to 15. About 70% of goats are distributed in flocks with size less than 50 while remaining goats are distributed in flocks of bigger size i.e. about 50–200 animals or more (Khan et al., 2008). One of the most important characteristics of Pakistani goat breeds, as compared with those from other geographic locations, is their adaptation to harsh production environments such as dry climate, poor quality forage, water shortage, high altitude and temperature extremes. Thirty-six goat breeds have been described in Pakistan (Afzal and Naqvi, 2004; Khan et al., 2008), and the most important ones in terms of population size, economic value and production indices are Beetal, Dera Din Panah, Teddy, Barbari, Kamori, Kaghani, Nachi and Pahari (Naqvi, 2006). Although goat is an important animal resource in the rural setup of Pakistan, some of the breeds are threatened with a relatively large risk of extinction in near future. Among all available goats, the population trend is available for only ten breeds, of which pop-

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ulation status of five breeds were found to be declining (Khan et al., 2008). Specific studies targeting the assessment of genetic diversity in Pakistani goat populations are very limited despite their importance in the livelihood of local livestock keepers (Sultana et al., 2003; Hussain et al., 2013). Assessment of genetic diversity and relationships among different goat breeds is essential to make decisions on conservation priorities (Ajmone-Marsan et al., 2014). The present study was undertaken to assess the genetic diversity, population structure and phylogeography of five economically important indigenous Pakistani goat breeds using microsatellite and mitochondrial DNA markers. The resulting information can be used in formulating national plans and strategies for sustainable improvement and conservation of these breeds in Pakistan.

2. Materials and methods

2.1. Animals and samples

To know the present diversity status of five Pakistani goat breeds (Beetal, Kaghani, Teddy, Nachi and Pahari), an intensive field survey was carried out in the most representative agro-ecological zones of Pakistan. Basically, Pakistan is divided into ten agro-ecological zones based on physiography, climate, land use and water availability namely, Indus Delta, Southern Irrigated Plain, Sandy Desert, Northern irrigated Plain, Barani (rainfall), Wet Mountains, Northern dry mountains, Western Dry Mountains, Dry western Plateau and Sulaiman Piedmont (FAO, 2004; Pakistan Agricultural Research Council (PARC), http://www.parc.gov.pk/index.php/en/component/content/article/43-maps/19-agrimaps). The field survey for the present study was conducted in Barani Lands (Teddy), Northern Irrigated Plains (Beetal, Teddy) Wet Mountains (Kaghani), Sandy Desert (Nachi), Western Dry Mountains (Pahari) and Sulaiman Piedmont zones (Pahari), the regions where the investigated breeds are mostly distributed. Although all the five breeds are distributed in the Punjab province of Pakistan, Beetal, Teddy and Pahari are also available in Khyber Pukhtunkhwa, Azad Jammu and Kashmir and Sindh provinces respectively. Beetal is a large sized breed distributed more commonly in Khyber Pukhtunkhwa and Northern Punjab, mainly in the districts of Multan, Sahiwal, Lahore, Faisalabad, Sargodha, Jhang, Okara, Jhelum, Gujranwala, Gujrat and Sialkot (Supplementary Figure SF1). Kaghani is concentrated in the Kaghan Valley in the districts of Abbotabad, Mansehra, Kohistan and Swat of Punjab. Pahari is distributed commonly in Loralai in Balochistan and D.G. Khan in Punjab. Nachi breed is found in Bahawalpur, Multan, and Sahiwal districts of Punjab while Teddy, although, widespread in the Punjab province, is concentrated in the districts of Gujrat, Jhelum, Sargodha, Rawalpindi in the Punjab and Kotli and Maripur in Azad Kashmir (Isani and Baloch, 1996; Afzal and Naqvi, 2004). A map indicating the sampling locations is provided in supplementary Figure SF2 and a brief description of the five investigated goat breeds is presented in table ST1. For each breed, blood samples were collected from unrelated goats belonging to multiple flocks across different villages. The farmers were interviewed in detail to ascertain the unrelatedness of collected samples. Blood was collected from the jugular vein into EDTA containing tubes. DNA purification was performed using the salting out protocol (Miller et al., 1988) and samples were stored at −20 °C until further processing. In addition to collection of blood samples for DNA preparation, data on the morphology of individuals and breed characteristics were recorded. Further, information regarding the existing goat breeds, distribution patterns and production purposes were retrieved from local livestock officers and farmers (Supplementary Table ST1). The number of samples collected from each of the five goat breeds include: Beetal (43), Kaghani (34), Teddy (41), Nachi (37) and Pahari (40).

2.2. Microsatellite genotyping

The laboratory work flow consisted of (1) DNA purification, (2) DNA quality and quantity estimation by agarose gel electrophoresis and spectrophotometry, (3) PCR amplification using microsatellite primers, (4) PCR product visualization in agarose gel electrophoresis, (5) preparation of PCR product for multiplex genotyping using a capillary sequencer (ABI 3730 DNA Analyzer – Applied Biosystems), (6) electropherogram analysis using GeneMapper software (Applied Biosystems) for allele size estimation and (7) statistical analysis using different software packages. Fifteen ISAG/FAO recommended microsatellite markers for diversity analysis in goats were used: ILSTS029, ILSTS11, ILSTS005, BMS1494, MAF035, MAF70, SRCRSP3, BM1818, SPS113, INRA0132, CSRD247, OARAE54, ETH10, OARFCB20 and MCM527 (FAO, 2011).

2.3. Sequencing mitochondrial DNA control region

Mitochondrial DNA control region was amplified and sequenced in 77 goats belonging to the five investigated breeds (Beetal (32), Kaghani (9), Nachi (11), Pahari (10) and Teddy (15)). Additionally, 14 Pak-Angora goats were also sequenced. Thus, a total of 91 sequences from six Pakistani goat breeds were generated in the present study. Primers were designed to amplify 1607 bp mitochondrial control region using online tool Primer 3 version 4.0 (http://bioinfo.ut.ee/primer3-0.4.0/). The primer sequences used were GTMT-F 5′ **cagcagctagcaccattgaa 3**′ and GTMT-R 5′ AAGCGAGGC GTTGTAAGCTA 3′. Polymerase chain reaction was performed in a total reaction volume of 20 μl with the following cycling conditions: initial denaturation at 95° C for 15 min followed by 30 cycles of 95° C for 1 min; 60° C for 1 min; 72° C for 1 min with a final extension at 72° C for 10 min. Purified PCR products were sequenced using Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, USA) on an automated Genetic Analyzer ABI 3100 (Applied Biosystems, USA). In addition to 91 Pakistani goats, 69 sequences from five Iranian goat breeds (Abadeh (14), Khalkhali (15), Naini (15), Taleshi (12) and Turki-Ghashghaei (13)) generated from another study were included for comparative analysis. A 636 bp control region sequence common to both Pakistani and Iranian sequence datasets that correspond to positions 15469–16104 of the complete mitochondrial genome (NCBI GenBank Accession No. GU229278) was utilized for estimating diversity and relationship among different breeds.

2.4. Statistical analysis

Measures of genetic variability including information on number of alleles, identification of private alleles, allele frequencies, allelic richness and observed and expected heterozygosities were obtained using GENEPOP software (Raymond and Rousset, 1995) available at http://genepop.curtin.edu.au/. Polymorphism information content (PIC) estimates were obtained using a customized Perl script, following methodology described by Botstein et al. (1980). GENEPOP was also used for the calculations of within-population inbreeding (F_{IS}) (Wright, 1951) and exact tests of heterozygote excess and deficit for each marker and each breed. Pairwise estimations of F_{ST} and R_{ST} were obtained using the SPAGeDi 1.3 software (Hardy and Vekemans, 2002). The effective number of migrants between pairs of breeds per generation (N_m) was calculated based on private alleles and corrected for sample size using GENEPOP. Inter-individual allele sharing distances were obtained using MICROSATELLITE ANALYZER (MSA) version 3.15 (Dieringer and Schlötterer, 2003). The neutrality of the microsatellites used in this study was evaluated by comparing the markers against neutral expectations in a distribution of F_{ST} vs. heterozygosities under an island model of migration using LOSITAN version 1 for Linux

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