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Analysis of Gene Diversity of Domestic Goats for Conservation of Genetic Resource

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Abstract

The study was conducted to analysis of gene diversity of domestic goats for conservation of genetic resource for maintains the future breeding options. The study was conducted in well maintained goat farm at the Central Institute of Research on Goats, Makhdoom, Farah, Mathura. The experimented goats evaluated by bottleneck analysis were carried out in microsatellite in six Indian goat breeds i. e. Barbari, Jakhrana, Sirohi, Marwari, Black Bengal and Pashmina. The sample size ranged for each breeds from 35 to 49 in all population. The most diverse goat breeds were the Marwari and Jakhrana which had the highest total no. of alleles (TNA) of 181 and 167 and highest mean number of alleles (MNA) 10.6 and 9.8 respectively. The least diverse breeds was the Pashmina, which had lowest TNA of 130 and lowest MNA of 7.6 respectively. The total gene diversity varies from 0.77 to 0.79 over the breed. The highest gene diversity between individuals was 0.3009 in Black Bengal and lowest was 0.2511 in Marwari goat. This analysis showed that gene diversity was quite sustained in all the analyzed population.

Key words : Diversity, Goats, Conservation, Resource, Genetic, Microsatellite and Bottleneck

Introduction

An assessment of genetic variability in domestic goats is a first step towards conservation of genetic resources for maintaining breeding options in order to satisfy the demand of marketable changes. Genetic diversity is one of the three forms of biodiversity recognized by the world conservation union (IUCN) as deserving conservation. The need to conserve genetic diversity within population is based on two arguments. The necessity of genetic diversity for evaluation to occur, and expected relationship between hetrozygosity and population fitness. Because loss of genetic diversity is related to inbreeding and inbreeding maintence of genetic diversity the changing phase of agricultural practices, a few breeds have been used on a large scale for immediate economic gain. It is therefore necessary to conserve genetic variation as well as to estimate the genetic uniqueness of the individual breeds. As a lot of genetic diversity exists in Indian breeds with respect to production potential, adaptability and disease resistance parameters, it is necessary to study the Indian goat breeds at genomic level. From an agricultural standpoint, the world's 700 million goats provide reliable access to meat, milk, skins, and fiber for small farmers particularly in developing countries like India. In spite of its unique characteristics, there is a lack of concern for the conservation and improvement of Indian breeds under field conditions. However, there is worldwide

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recognition of the need for the conservation of livestock diversity (FAO, 1995 [5]), and for characterization of breeds and populations including their genetic differentiation and relationships. These unique characteristics are the result of evolutionary forces and their interactions over longer periods of time. However, these adaptation and unique characteristics might have been diluted due to intermixing, sub-structuring and consequent genetic drift in the population over time. Therefore, an investigation of genetic variation within the breed, and its structure may help to analysis these factors, and provide genetic information to be used in the conservation and improvement of this breed of goats.

Materials and Methods

The study was conducted in well maintained goat farm at the Central Institute of research on Goats, Makhdoom, farah, Mathura. In this study mainly six breeds were considered i.e. Barbari, Jakhrana, Sirohi, Marwari, Black Bengal and Pashmina. The blood samples were collected from natural habitat of the goat breeds and at least two samples were collected from each village. An effort was made to collect samples from unrelated individuals based on information provided by farmers. Blood samples were collected from each animal using EDTA vacutainer and stored at –20°C till further use. PCR amplification was carried out in MJ research Thermal cycler. Each 10ml reaction mixture consisted 10hg of template DNA, 1X buffer, 200mM dNTPs, 2.5mM MgCl2, 1U of AmpliTaq Gold (Perkin Elmer) and 10pM of each primer. Samples (PCR products) were prepared by mixing with the loading dye (formamide: bluedextrin; 5:1) and GS-ROX500 (0.5ml/sample) and denatured (94°C for 2mins) and electrophoresed in 5% Long Ranger (FMC) gel using ABI 377 automated DNA sequence (PerkinElmer). Raw data was analysed using GeneScan and Genotyping software's (Perkin-Elmer). Microsatellite analysis was carried out to test for signatures of recent population bottlenecks in all goat breeds.

This analysis was carried out on 49 DNA samples with 17 microsatellite markers as reported by Rout et al. [12]. For these 17 loci, genetic variation was quantified using measures of the total number of alleles, number of polymorphic loci, observed and expected heterozygosity per locus, allelic richness using, population gene diversity within population and between population using by BOTTELENECK, AGArst and Metapop. Genetic bottleneck was detected using microsatellite data by three approaches, heterozygote excess, mode-shift, and M ratio test. We first used the M ratio (the mean ratio of the number of alleles to total range in allele size) [3] as implemented in AGA_{rst} [7], because of its consistent performance in identifying populations with known bottlenecks. M ratio calculates the changes that occur after a bottleneck in the distribution of allele sizes relative to the number of alleles in a population. It has been established that an M ratio less than 0.71 signifies a bottleneck [3].

The BOTTLENECK programme [10] was used as an alternative measure of genetic bottlenecks to test for excess gene diversity relative to that expected under mutation-drift equilibrium. The heterozygosity excess method exploits the fact that allele diversity is reduced faster than heterozygosity during a bottleneck, because rare alleles are lost rapidly and have little effect on heterozygosity, thus producing a transient excess in heterozygosity relative to that expected in a population of constant size with the same number of alleles [3, 10]. To determine the population "genetic reduction signatures" characteristic of recent reductions in effective population size (Ne), the Wilcoxon's heterozygosity excess test [3] and the allele frequency distribution mode shift analysis [9] were performed using BOTTLENECK [3]. The heterozygosity excess method was used to analyse the population, and the data for the heterozygosity excess test were examined under the two-phased model (TPM; 95% stepwise mutation model with 5% multistep mutations and a variance among multiple steps of 12), which is considered best for microsatellite data [3,11]. We also analysed the allele frequency distribution for gaps. A qualitative descriptor of allele frequency distribution

(the mode-shift indicator), which is reported to discriminate between bottlenecked and stable population [3], was obtained using the programme BOTTLENECK.

Table1. Describe The H excess and H deficiency in IAM and TPM model by bottleneck in all breeds.

Model	**Parameter**	**Breed**						
Iam model	H excess	Jamunapari	Jakhrana	Pashmina	Barbari	Sirohi	Marwari	Black Bengal
		16	15	17	16	15	15	14
	H deficiency	1	2	0	1	2	2	3
	Probability	0.00192	0.00073	0.00011	0.0014	0.01181	0.01270	0.04735
Tpm model	H excess	6	14	17	15	9	13	8
	H deficiency	11	3	0	2	8	4	9
	Probability	0.04152	0.04706	0.00012	0.00912	0.37455	0.12955	0.21898

.Parameters for T.P.M.: Variance = 5.00 Proportion of SMM = 95.00%, Estimation based on 1500 replications; Hee: Heterozygosity excess expected; Hd: Heterozygosity deficiency; P: Probability; IAM: Infinite Allele Model, TPM: Two Phase Model; SMM: Stepwise Mutation Model

Metapop

A software for the management and analysis of subdivided populations in conservation programs. The analysis metapop can perform on the data into two main methods.

1) Population analysis
2) Population management

On the basis of Metapop. We were trying to find out the population analysis for conservation priorities. Population analysis divided in several sections:

1. Alleles per locus, 2. Average frequencies of subpopulation, 3. Average frequencies of whole population and the result of population analysis the co ancestry, inbreeding and genetic distances, the contribution of each subpopulation to the global gene diversity, the gain/loss of diversity because of the removal of each subpopulation, and the relative contribution of each subpopulation to a pool of maximal diversity if the option has been required. Bootstrap confidence intervals for the main parameters are given when the option is used.

Results and Discussion

Results of the study are presented in two parts: (a) Bottleneck detection in Indian goats and (b) Gene diversity of goats.

(a) Bottleneck Detection in Indian Goats

Bottleneck detection in Barbari, Jakhrana, Pashmina, Sirohi, Black Bengal and Marwari goats was presented in Table 2. The comparison between both estimates was different in some breeds due to variation in sample size. The sample size ranged for each breeds from 35 to 49 in all population. The most diverse goat breeds were the Marwari and Jakhrana which had the highest total no. of alleles (TNA) of 181 and 167 and highest mean number of alleles (MNA) 10.6 and 9.8 respectively. The least diverse breeds was the Pashmina, which had lowest TNA of 130 and lowest MNA of 7.6 respectively. The M-ratio ranged for each breed from 0.858 (similar in both breeds Pashmina and Sirohi) to 0.795 to 0.735 in Jakhrana and Barbari. This was significantly higher than critical value.

The M-ratio was less than 0.71, diagnostic value of genetic bottlenecks, the average M- ratio was large and above the critical significant value (MC) in the population suggesting that is a not suffered severe or long lasting genetic bottleneck (Arranz et al. 1998 [10], Gaza and Williamsons 2001 [9] and Ganai and Yadav 2001 [15]). The Marwari M ratio ranged 0.426 which was significantly the critical value.

Table 2. Bottleneck Detection in Indian goat breeds

Breed	Sample size	No. of Total Alleles (TNA)	Alleles /Locus (MNA)	M ratio	Variance M	Ne*
Barbari	35	137	8.1	0.735	0.054	8284
Jakhrana	49	167	9.8	0.795	0.096	12415
Pashmina	34	130	7.6	0.858	0.054	9934
Black Bengal	48	151	8.9	0.809	0.015	11554
Marwari	35	181	10.6	0.426	0.080	14180
Sirohi	49	150	9.0	0.858	0.014	11839

The variance M ranged for 0.054 similar in both breeds Barbari and Pashmina. Similarly the Black Bengal and Sirohi had the lowest M variance of 0.015 to 0.014 respectively, However the Jakhrana and Marwari had highest M ratio of 0.096 to 0.080 respectively. Heterozygosity excess test and the mode shift indicators demonstrated that there was no sign of recent reduction in effective population size (Ne) in the all breeds.

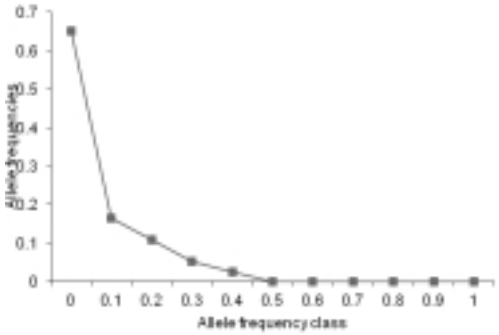

Figure 1: L-shaped mode shift graph showing the absence of bottleneck in all goats' population breeds.

(b) Gene Diversity of Goats

The majority of analyses of genetic variation in studies for conservation purposes are focused on gene diversity. The contribution of each population to gene diversity of goats is present in Table 3. The total gene diversity varies from 0.77 to 0.79 over the breeds. The highest contribution of gene diversity was from Black Bengal. The contribution to the between individuals was highest in Black Bengal goat (0.3009). The lowest contribution to between individual vary diversity was 0.2511 by Marwari goat. This analysis showed that gene diversity was quite sustained in all the analysed population. Our finding were in close an accordance with the most of finding of Saitbekova et al. 1999 [16], Kim et al. 2002 [13], Wang et al. 2004 [18] and Agha et al. 2008 [2].

Conclusion

Population structure and bottleneck analysis were carried out in microsatellite data in six Indian goat breeds, (Barbari, Pashmina, Black Bengal, Sirohi, Marwari and Jakhrana. The data was analysed by bottleneck software, AGARst and Metapop software population analysis showed average allele no. 8.10 high expected heterozygosity was high indecently that all population. Mutation -drift- equilibrium, heterozygosity excess/ deficiency under different mutation model generated by the BOTTLENECK showed that there were significant deficiencies of heterozygosity. The mode shift test did not detect any distortion of allele frequency and showed a normal 'L' shaped distribution in all population. The analysis indicated that the population has not any bottleneck recently and was a constant size population. The total

Table 3. Total Gene diversity within and between individuals and Gene diversity within in the breeds Sub population

Subpopulation	Gene diversity within individuals (GDT)	Gene diversity Between individuals (GDWI)	Gene diversity Within subpopulation (GDBI)	Total gene diversity (GDWS)
Pashmina	0.4957	0.2529	0.7786	0.7786
Marwari	0.4907	0.2511	0.7718	0.7718
Jakhrana	0.4971	0.2911	0.7881	0.7881
Barbari	0.4911	0.2819	0.7729	0.7729
Black Bengal	0.4988	0.3009	0.7997	0.7997
Sirohi	0.4977	0.2873	0.7850	0.7850

gene diversity varies from 0.77 to 0.79 over the breed. This analysis showed that gene diversity was quite sustained in all the analyzed population. Conserved will be much more difficult when the population becomes genetically impoverished and is effective and easy to implement when the populations are genetically stable. Therefore, it is necessary to initiate necessary steps to conserve the breed for future use.

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