Journal of Entomology and Zoology Studies 2020; 8(6): 1249-1252

Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

JEZS
Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(6): 1249-1252
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Received: 02-09-2020
Accepted: 15-10-2020

Bottleneck effect in Poonchi sheep

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Abstract

The present study was performed to reveal genetic bottleneck in Poonchi sheep breed (*Ovis aries*) raised in Poonch district of Jammu & Kashmir in India using genotypes for microsatellite markers. In the present study, six microsatellite markers, namely, MAF70, SPS113, OarFCB128, OarFCB48, BM1329 and MAF209 were used to study the genetic bottleneck in the Poonchi sheep. The number of alleles examined by all the microsatellite markers in the present study ranges from 4 to 9. Moreover, the highest and lowest expected heterozygosity (H_e) were observed to be as 0.879 and 0.725 for MAF70 and BM1329, respectively. The expected numbers of loci with heterozygosity excess in Poonchi sheep were 3.51 ($P<.05$), 3.60 ($P<0.05$) and 3.62 ($P>0.05$) for Infinite Allele Model (IAM), Two Phase Model of Mutation (TPM) model and Stepwise Mutation Model (SMM), respectively in Sign test. The IAM, TPM and SMM values for one tail for heterozygosity excess in Wilcoxon rank test were significant ($P<0.05$) indicated all the loci deviates from mutation-drift equilibrium. None normal 'L' shaped distribution of mode-shift test suggested that there was recent bottleneck in the existing population, providing important information for formulation of sheep breeding strategies and conservation of sheep breed.

Keywords: Genetic bottleneck, Poonchi sheep, microsatellite, heterozygote, India

Introduction

There are 44 different sheep breeds have been reported in India [1]. Poonchi sheep is a well known sheep population of Jammu & Kashmir. The name of Poonchi is derived from its native breeding tract Poonch district of Jammu & Kashmir state, India. The population is spread over Poonch district and area of Reasi and Rajouri districts, adjacent to Poonch district. This sheep population is reared mainly by the Bhakarwal, Gujjars, Pahari and other nomadic communities [2].

Livestock diversity needs to be conserved because intermixing and/or the resulting genetic drift in the population overtime leads to reduced adaptation capability and disease resistance [3]. In most of the developing countries, valuable sheep breeds, possessing unique genetic makeup, are at the risk of extinction mainly due to inadvertent crossbreeding between neighboring breeds. These breeds need to be characterized for their morphological and genetic features to define selection and conservation strategies [4].

Bottleneck effect is a phenomenon where the genetic variation in the population is reduced due to the genetic drift. Microsatellite data subjected to statistical analysis to test whether the populations have undergone recent genetic bottleneck. Because historical population sizes and levels of genetic variation are seldom known, methods for detecting bottlenecks in the absence of historical data would be useful. The quantitative methods suitable for analysis of microsatellite data for detection of recent bottlenecks in (100-200) generations was described [5]. No information regarding bottleneck effect of the Poonchi sheep population is available till date. Therefore, the present study was aim to determine the genetic bottleneck in Poonchi sheep by using molecular markers.

Materials and Methods

Animal material and DNA extraction

A total of 30 unrelated blood samples were collected from Poonchi sheep of Poonch and Rajouri districts of Jammu & Kashmir. Blood samples (4-5 ml) were obtained from jugular vein using vacuitaners treated with 15% EDTA as anticoagulant and stored at -20 °C until DNA extraction. Genomic DNA was extracted from blood samples of Poonchi sheep by GeneJET whole blood genomic DNA purification Kit (Thermo Scientific).

Polymerase chain reaction and fragment analysis

PCR amplifications were performed with some modifications in annealing temperatures (Table 1). A total of six microsatellite markers namely MAF70, SPS113, OarFCB128, OarFCB48, BM1329 and MAF209, were used for characterization study. The amplicons were checked in 3.0% agarose gel. PCR products were dissolved in denaturing polyacrylamide gels 8% Urea-PAGE. Genotyping were done by visualizing the bands and different alleles were identified as different bands.

Table 1: Primer sequence of different microsatellites used in the study

S. No.	Marker	Primer sequence (5՝>3’)	No. of bp	T_A
1.	MAF70	f-cacggagtcacaaagagtcagacc r-gcaggactctacggggcctttgc	24 23	63°C
2.	SPS113	f-cctccacacaggcttctctgactt r-cctaacttgcttgagttattgccc	24 24	55°C
3.	OarFCB128	f-attaaagcatcttctctttatttcctcgc r- cagctgagcaactaagacatacatgcg	29 27	56°C
4.	OarFCB48	f-gagttagtacaaggatgacaagaggcac r-gactctagaggatcgcaaagaaccag	28 26	64.5°C
5.	BM1329	f-ttgtttaggcaagtccaaagtc r-aacaccgcagcttcatcc	22 18	51.5°C
6.	MAF209	f-gatcacaaaaagttggatacaaccgtg r-tcatgcacttaagtatgtaggatgctg	27 27	56.9°C

Bottleneck analysis

In order to detect the recent effective sample size reductions, the program bottleneck uses the allelic frequencies and it computes for each sample of size and therefore, for each locus, the distribution of the heterozygosity from the observed number of alleles (k_o), given the sample size (n) under the assumption of mutation-drift equilibrium is expected to occur. Bottleneck effects were tested with Sign, Standardized differences and Wilcoxon sign–rank tests under the different mutation models such as Infinite Allele Model (IAM), Two Phase Model of Mutation (TPM) model and Stepwise Mutation Model (SMM) in Bottleneck software version 1.2.02 (1 000 simulation) [6]. The BOTTLENECK program was applied to determine if there had been past bottlenecks in population [5]. It tests for the departure from mutation drift equilibrium based on heterozygosity deficient or excess. The bottleneck compares heterozygosity expected at Hardy-Weinberg equilibrium to the heterozygosity expected at mutation drift equilibrium in same sample that has the same size and same number of alleles. The qualitative graphical method was employed to visualize the allele frequency spectra [5]. The allele frequency distribution was established in order to see whether it is approximately L-shaped (as expected under mutation-drift equilibrium) or not (recent bottlenecks provoke a mode shift).

Results and Discussion

Genetic bottleneck analysis was performed to investigate whether there was a bottleneck in Poonchi sheep population. The data set obtained was tested according to three different mutation models known as Infinite Allele Model (IAM), Two Phase Mutation Model (TPM) and Stepwise Mutation Model (SMM) reported under the assumption that all loci fit mutation-drift equilibrium [5, 7, 6].

The number of alleles examined by all the microsatellite markers in the present study ranged from 4 (MAF90) to 9 (BM1329) (Table 2). Moreover, the highest and lowest expected heterozygosity (H_e) were observed to be as 0.879 and 0.725 for MAF70 and BM1329, respectively with an average value of 0.809. Lower estimates of H_e were reported in Muzzafarnagri & Marwari sheep population [8]; in Tibetan sheep [9] and Michni population, Hashtnagri population & in Balkhi populations of Pakistan [10]. Higher mean H_e in Chios sheep population and lower H_e in Gökçeada and Çine Çaparı sheep populations were reported [11].

Table 2: Bottleneck analysis under three microsatellite evolution models in Poonchi sheep

Microsatellite locus	Observed alleles (k_o)	He	I.A.M.				T.P.M.				S.M.M.			
			Heq	S.D.	DH/sd	P	Heq	S.D.	DH/sd	P	Heq	S.D.	DH/sd	P
MAF70	4	0.725	0.609	0.119	0.978	0.185	0.655	0.093	0.754	0.270	0.679	0.082	0.557	0.377
SPS113	6	0.823	0.724	0.088	1.111	0.087	0.759	0.065	0.976	0.146	0.787	0.05	0.708	0.274
OarFCB128	7	0.808	0.766	0.071	0.597	0.323	0.796	0.057	0.215	0.522	0.822	0.042	-0.342	0.297
OarFCB48	5	0.824	0.730	0.078	1.211	0.080	0.753	0.069	1.022	0.149	0.773	0.057	0.893	0.214
BM1329	9	0.879	0.847	0.05	0.638	0.304	0.871	0.033	0.242	0.534	0.882	0.028	-0.124	0.360
MAF209	5	0.797	0.689	0.097	1.119	0.084	0.723	0.08	0.934	0.141	0.746	0.068	0.755	0.237

The significance of Sign test, Standardized differences test and Wilcoxon sign rank test were presented in Table 3. The expected numbers of loci with heterozygosity excess in Poonchi sheep were 3.51 ($P<.05$), 3.60 ($P<0.05$) and 3.62 ($P>0.05$) for Infinite Allele Model (IAM), Two Phase Model of Mutation (TPM) model and Stepwise Mutation Model (SMM), respectively in Sign test (Table 3).

These significant results in Sign test indicate that, due to mutation-drift equilibrium, the Poonchi population has undergone a recent genetic bottleneck. Similar findings were reported for IAM, SMM and TPM models in Michni and Hashtnagri populations of Pakistan [10] in Sign test.

On contrary to present findings, significant sign test values was obtained for SMM model in Balkhi sheep population of Pakistan [10].

Non-significant results in Sign test for all the models were reported in Tibetan sheep population [9].

Table 3: Test for null hypothesis under three microsatellite evolution models for bottleneck analysis in Poonchi sheep

Mutation Models	Sign test			Standardized differences test		Wilcoxon test		
						One tail for H deficiency	One tail for H excess	Two tails for H excess or deficiency
	Hee	He	P	T2	P	P	P	P
IAM	3.51	6	0.03978	2.308	0.01050	1.00000	0.00781	0.01563
TPM	3.60	6	0.04676	1.691	0.04540	1.00000	0.00781	0.01563
SMM	3.62	4	0.55054	0.999	0.15898	0.97656	0.03906	0.07813

Abbreviations and symbols: Hee: expected number of loci with heterozygosity excess; He: observed number of loci with heterozygosity excess; T2: test 2; P: probability value for heterozygosity excess; IAM: infinite allele model; TPM: two-phase model; SMM: stepwise mutation model;

*$P<0.05$ showing significant differences between the observed and expected values for heterozygosity excess.

In the present study Standardized differences test were significant ($P<0.05$) for IAM and TPM models whereas, for SMM model the test was non-significant (Table 3). Similar findings were reported for Michni sheep population whereas, in Hashtnagri and Balkhi sheep populations the test were significant for SMM models [10]. Significant IAM (P=0.01216) & SMM (P=0.00382) values and non-significant TPM (P=0.39403) values for Standardized differences test were reported in Tibetan sheep [9].

Wilcoxon rank test was significant for one tail heterozygosity excess for all the three evolution models. Similar findings were reported in Michni, Hashtnagri and Balkhi sheep population for IAM and TPM models [10]. On contrary to present findings the expected numbers of loci with heterozygosity excess in Gökçeada, Chios and Çine Çapari were reported to be 7.75 (P>0.05), 7.76 ($P<0.05$) and 7.61 ($P<0.05$) in TPM in the Wilcoxon rank test [11]. Non-significant Wilcoxon test for heterozygosit excess in all three mutational models were reported in Kilakarsal sheep population [12]. Wilcoxon rank tests (one tail for heterozygosity excess) were significant for IAM model and non-significant for TPM and SMM models in Tibetan sheep [9].

It is known that the infinite allele model (IAM) and the stepwise mutation models (SMM) cause inconsistent results in studies using microsatellites. Therefore, it is reported that the two-phase mutation model (TPM) is the most useful model to test the heterozygosity excess in the bottleneck tests performed with microsatellites [13, 14, 6]. On the other hand, it has been reported that the Wilcoxon test, which has high statistical confidence even in bottleneck analysis studies using a limited number of loci (<20), can be used with high confidence in bottleneck studies [6]. The population studied was found to be bottlenecked by the Wilcoxon test according to the two-phase mutation model (TPM). Therefore, it can be concluded that serious demographic bottlenecks have been experienced in the Poonchi sheep population studied given that considering the TPM model of Wilcoxon test results.

Mode-Shift test

A mode-shift graph (Fig. 1) was obtained using allele frequency classes of six microsatellite markers to identify potential bottlenecks in the studied populations as a second method. As it can be seen from mode-shift graph, normal L-shaped distribution was not found. None normal 'L' shaped distribution of mode-shift test suggested that there was recent bottleneck in the existing population. This finding suggested the population had gone detectably large, recent genetic bottleneck (last 40-80 generations). The results indicated that the unplanned and indiscriminate mating prevalent in the breeding tract leading to small effective population size/or mating between relatives and consequent genetic drift. Similarly, Hashtnagri and Balkhi populations showed deviation from the normal L-shape an indicator of genetic bottleneck in the populations [10].

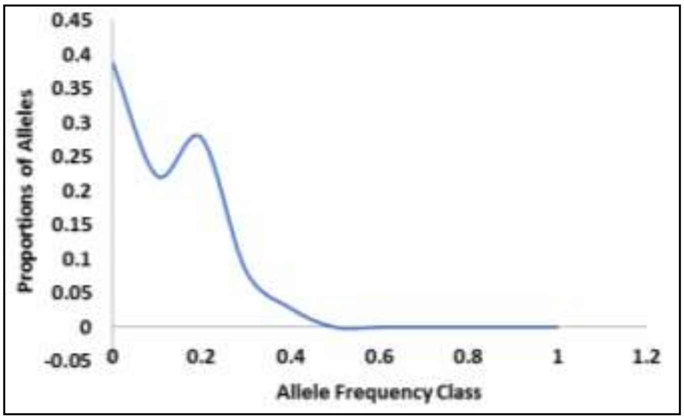

Fig 1: Mode Shift Test for Bottleneck in Poonchi Sheep

On the other hand, the typical L-like distribution of the allele frequencies obtained in the Mode shift test was obtained in Jalauni sheep of India [15], Muzzafarnagri Sheep [16], Kilakarshal sheep [12] and Tibetan sheep [9]. Michni population followed a normal L-shaped distribution of allele frequencies showing the population in mutation drift equilibrium [10]. Mode-shift graph for bottleneck in the Gökçeada (GA), Chios (CH) and Çine Çaparı (ÇÇ) of Turkey showed the normal L-shaped curve and there was no genetic bottleneck in the studied populations [11].

Conclusions

In conclusion, the present study has revealed an important knowledge about the genetic diversity in Poonchi sheep. It can be said that microsatellites used in the study have a high potency for the determination of bottleneck in the Poonchi sheep breed. The Poonchi population was found in the state of genetic bottleneck suggesting reduction in the population sizes in the recent past. Obtained results will be of benefit to the efforts for animal breeding and genetic conservation studies. The strong inference that the sheep breed studied has undergone major bottlenecks is also important for animal breeding program or conservation programs implemented. Further, integrating genetic improvement programmes for this breed with market oriented production strategies will raise the economy of its rearers and thereby ensure its sustainable conservation.

Acknowledgments

The authors would like to thank sheep breeders of Poonch district for allowing us to collect the blood samples for the present study.

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