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CONTENTS

Study on bottleneck analysis in Udaipuri goat of Uttarkhand

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ABSTRACT: This study was undertaken for genetic diversity and bottleneck analysis in Udaipuri goat found in Pauri Garhwal district of Uttarakhand using a set of twenty microsatellite markers. Blood samples were collected from 30 unrelated animals from the breeding tract. All the twenty microsatellite loci were amplified successfully and a total of 96 alleles were observed across all the loci. When bottleneck analysis was performed, sign test revealed the observed heterozygosity excess was higher than the expected utilizing IPM, TPM and SMM. The expected heterozygosity (Hee) values were 11.31, 11.66 and 11.85 while the observed heterozygosity (He) were 20, 18 and 14 for IAM, TPM and SMM, respectively. Under standardized test, T_2 values were positive (4.958, 3.766 and 2.032 utilizing the IAM, TPM and SMM, respectively) indicating a gene diversity excess that is caused by reduction in effective population size.

Key words: Bottleneck analysis, genetic diversity, microsatellite markers, Udaipuri goat

Udaipuri is another breed existing in Uttarakhand which is not yet characterized and also not recognized. This is a small, meat type goat surviving in the Western Himalayan region of Uttarakhand. The native tract of this goat is Ajmer and Udaipur patties (from Dugadda to Yamkeshwar) in Pauri district of Garhwal division of Uttarakhand. The size of the goat is small and the body is compact and of tan colour, covered with short hairs. The head is smaller in length with small to medium forehead. The contour of head is convex and the ears are medium and pendulous. They have a tapering muzzle with a roman nose. Their horns are curved and turned backward. The top line is almost straight and the flank is moderate in size. Their legs are small, lean and straight. The tail is short and thin (Sarma*, et al.,* 2018). The genetic diversity analysis of Chaugarkha goat of Uttarakhand was done by the Ganie *et al.* (2017).

In the absence of information about the genetic aspects of each breed accessible for a breeding programme, improvement of local breeds is often ignored which favours the introduction of germplasm from exotic breeds, for which more information is available. Therefore, there is a need to characterize indigenous goat populations genetically to further utilize the genetic resource effectively. The present study was undertaken to examine the pattern of microsatellite variation within Udaipuri goat population and has also tried to highlight the detailed information to access the knowledge on the genetic diversity and differentiation of the breed. This study has been a made in such a way to provide a comprehensive database of genetic variation among goat population, to identify group representing homogenous population and also to identify the genetically distinct groups. This study may help in extracting inferences regarding planning of sustainable improvement and conservation programme for Udaipuri goat at local or national level.

MATERIALS AND METHODS

The present study aimed at assessing the genetic diversity within the Udaipuri breed of goat which is found in the Pauri Garhwal district of Uttarakhand. The experiment materials used for the investigation considered 30 unrelated goats from Udda, Jiyadhamraag, Bithyani, Thanger, Kandi, Kosli, Manjhera villages of Pauri Garhwal of Uttarakhand. Under sterile conditions, 10 ml of venous blood was collected aseptically from jugular

vein of each animal in a 15 ml polypropylene centrifugation tube containing 2 ml ACD as anticoagulant. The tubes were gently inverted about four to five times to facilitate the proper mixing of blood and the anticoagulant. The blood samples were transported from field to the laboratory in an icebox containing icepacks. Utmost care was taken to avoid the exposure to direct sunlight during transportation. The samples were stored in the refrigerator at -20^{0}C till DNA isolation. The isolation of genomic DNA from blood samples was done using the protocol described by Sambrook and Russel (2001). In this study, 20 microsatellite primers were used including ILSTS- 058, ILSTS-059, ILSTS-34, ILSTS-005, ILSTS-019, ILSTS-049, ILSTS-008, ILSTS-087, ILSTS-033, ILSTS-044, ILSTS-030, ILSTS-002, ILSTS-065, ILSTS-029, ILSTS-082, RM-088, OarFCB-304, OarJMP-29, RM-4 and ETH-225 which are belonging to different chromosomes. Typical polymerase chain reaction (PCR) testing was carried out under these conditions: 100ng/ µl of target DNA was used in 20-µl PCR reaction containing 10x PCR buffer (with $MgCl_2$ 20mM), dNTPs (200 mM each), forward primer (20 pmol/ µl), reverse primer(20 pmol/µl), Taq DNA polymerase(5U/µl) and DNase free water. The PCR protocol used included initial denaturation 95^{0}C for 5 min, denaturation 95^{0}C for 1 min, Primer annealing at 52-60^{0}C for 30 seconds extension at 72^{0}C for 1 min, 30 cycles of denaturation to extension step, then a final extension at 72^{0}C for 10 min. Until the PCR amplicons were taken out from the thermocycler, and was hold at 4^{0}C. The conformation of PCR amplification was done by 1.5% agarose gel electrophoresis. Eight per cent Urea Polyacrylamide Gel Electrophoresis (PAGE) was employed to resolve the Microsatellite alleles. Polyacrylamide gels are impregnated with soluble silver ion (Ag^+) and developed by treatment with a reductant. Following the gel documentation on a gel-doc system and capturing of the images, scoring of the alleles was done manually accomplished by the joint observation of two independent observers. This was done to avoid any false calling of alleles. Using different colour markers, marking of the bands over the gel, which was wrapped in transparent sheet, was done in bright light. The electronic Vernier Calliper was used to get the size of the bands in millimeter over the gels. Size of the alleles was determined by using the INCHWORM (version 1.02) software. A careful amalgam of manual as well as machine logic was applied by the independent observers so as to get the scoring very accurately. After scoring, the genotyping table was prepared in M.S Excel format which was processed further for feeding into the population analysis software (POPGENE software version 1.31).

Statistical Analysis

The population variability parameters taken out from molecular study was derived using software POPGENE32 developed by Francis C. Ye and Rong-cai Yang, University of Alberta and Tim Boyle, Centre for International forestry Research, in August 1999 (downloaded from https://www.ualberta.ca/~fyeh/popgene_download.html).

Bottleneck Analysis

The Bottleneck (Version 1.2.02) was used to test for departure from mutation drift equilibrium based on heterozygosity excess or deficit. This analysis was performed to know whether the population has suffered any bottleneck (reduction in number) in the past (Piry *et al.*, 1999)

RESULTS AND DISCUSSION

In the present study, considering 20 microsatellite loci and three test, the Sign Test, Standardized Test and Wilcoxon Test under which comes the three mutation models- Infinite Allele Model (IAM), Two Phase Model (TPM) and Stepwise Mutation Model (SMM) were studied (Table 1).

Under the Sign test the expected heterozygosity excess (Hee) were 11.31, 11.66 and 11.85, while the observed heterozygosity was 20, 18 and 14 for IAM, TPM and SMM models respectively. If a population shows higher observed heterozygosity than expected for majority of loci, than it is considered to experience a recent bottleneck or reduction (Sharma *et al.*, 2008[b]). The results under these models showed that the observed heterozygosity was higher than the expected. The excess heterozygosity in Udaipuri

goat could be attributed to recent population reduction. Under the standardized test (T_2 test), the T_2 values utilizing the IAM, TPM and SMM are 4.958, 3.766 and 2.032, respectively. The positive values of Standardized test under bottleneck analysis suggests the gene diversity excess that is caused by reduction in effective population size, while negative values reveal an expansion in the population (Mahmoudi *et al.*,2013). Under the Wilcoxon test the probability values for heterozygosity excess are 0.00, 0.00 and 0.01812 for IAM, TPM and SMM, respectively. These values were found to be less than 0.05 ($P < 0.05$) for all the three models. Wilcoxon Test revealed that the population has undergone recent bottleneck assuming all the three mutation models.

Similar observation for observed heterozygosity excess were reported by Aggarwal *et al.* (2007)in Mehsana goat under IAM and TPM whereas the Hee values were 15.01 and 14.88, while He were 17 and 15 respectively. Whereas under model SMM, there was heterozygosity deficiency with Hee value of 14.75 and He value 4.00. Under Wilcoxon test considering the IAM model showed value 0.03335 ($P < 0.05$) and they stated the Mehsana goat population has undergone bottleneck utilizing IAM. While the Standardized test utilizing SMM revealed significant heterozygote deficiency (T_2 values = -8.748, $P < 0.01$). However, all the test utilizing SMM and mutation drift equilibrium utilizing TPM showed significant heterozygosity deficiency and they safely concluded that Mehsana goat population has not suffered any bottleneck.

Sharma *et al.* (2008[a]) reported in Barbari goat the Sign test, Standardized test and Wilcoxon test showed significant departure from mutation drift equilibrium and concluded that the population has not undergone any bottleneck in the past.

Sharma *et al.*(2008[b]) examined the Beetal goat for bottleneck analysis. Under Sign test utilizing IAM and TPM He was reported significantly higher than Hee. Though for SMM Hewas significantly lower than Hee Under Standardized test utilizing IAM, TPM and SMM, T_2 values were -0.703, -1.576 and -5.418, respectively, negative thus accepted the null hypothesis of mutation drift equilibrium. Under Wilcoxon test P value (for one tail for heterozygosity excess) were 0.245, 0.524 and 0.952 utilizing IAM TPM and SMM respectively which was more than 0.05 ($P > 0.05$) and reported the absence of bottleneck in Beetal goat population.

Dixit *et al.* (2013) studied the Surti goat and reported under sign test significant heterozygosity excess utilizing IAM and TPM ($P < 0.001$) but non-significant heterozygosity excess utilizing SMM. Under standardized test positive T_2 values were

Table 1: Mutation drift equilibrium, heterozygosity excess/deficit under different models in Udaipuri goat

Models	Sign Test	Standardized	Wilcoxon Test
IAM	Hee = 11.31	T_2 = 4.958	P (One tail of H deficiency): 1.0000
	Hd = 0	P = 0.00	P (One tail of H excess): 0.0000
	He = 20		P (Two tail of H excess and deficiency): 0.0000
	P = 0.00001		
TPM	Hee = 11.66	T_2 = 3.766	P (One tail of H deficiency): 1.0000
	Hd = 2	P = 0.00008	P (One tail of H excess): 0.0000
	He =18		P (Two tail of H excess and deficiency): 0.0000
	P = 0.00228		
SMM	Hee = 11.85	T_2 = 2.032	P (One tail of H deficiency): 0.98362
	Hd = 6	P = 0.02109	P (One tail of H excess): 0.01812
	He =14		P (Two tail of H excess and deficiency): 0.03623
	P = 0.22849		

Parameters for T.P.M: Variance = 30.00 Probability = 70.00%; Estimation based on 1000 replication; IAM: Infinite Allele Model; TPM: Two Phase Model; SMM: Stepwise Mutation Model; Hee: Heterozygosity excess expected; Hd: heterozygosity deficiency; He: heterozygosity excess (observed); P: Probability.

observed. Utilizing IAM, TPM and SMM T_2 values were 5.220 ($P < 0.001$), 3.781 ($P < 0.001$) and 1.762 ($P < 0.001$). All the test under sign test except SMM showed heterozygosity excess in Surti goat population. They concluded that the heterozygosity excess could be attributed to recent population reduction and further reported that the population had suffered bottleneck.

Mahmoudi *et al.* (2013) analyzed the Najdi goat for bottleneck and found the Hee to be 7.52, 7.65 and 7.71 while He 13, 11 and 10 for IAM, TPM and SMM respectively. The results under these models showed that the observed heterozygosity (H_e) was higher than the expected (Hee). Under standardized test T_2 values were positive that indicated gene diversity excess which they reported to cause from reduction in effective population size. Under Wilcoxon Test probability values were significantly lower than 0.01 ($P < 0.01$) utilizing IAM and TPM. Though utilizing the SMM $P > 0.01$ and thereby stating the population to deviate from mutation drift equilibrium.

Kharkar *et al.* (2015) reported a heterozygosity excess under IAM and TPM models, while mutation drift equilibrium under SMM model in Berari goat. The standardized test revealed significant heterozygosity deficiency under SMM with $T_2 = -4.873$, $P < 0.01$ in Berari goat population. There results under Sign test indicated the absence of bottle neck for IAM and TPM.

The Udaipuri goat has been brought by the migrants from Udaipur, Rajasthan. These migrants while moving and settling in Uttarakhand, took few goats along with them. Years of evolutionary forces has led these goats to develop into a unique breed and now better called as Udaipuri. Bottleneck or reduction in the population size may have caused due to colonization event. As a new population was developed from a small subset of the ancestral population, consequently the gene frequencies have varied from that of the ancestral population as a whole. This might have been the cause for the reduction in the population size observed in Udaipuri goat.

CONCLUSION

Under Wilcoxon test, the probability (P) values for heterozygosity excess were 0.00, 0.00 and 0.01812 for IAM, TPM and SMM, respectively. These values were found to be less than 0.05 ($P < 0.05$) for all the three models. Wilcoxon Test showed that the population has undergone recent bottleneck assuming all the three mutation models. So, from these findings we can conclude that bottleneck analysis under sign test, standardized test and Wilcoxon test utilizing the three models viz., IAM, TPM and SMM, Udaipuri goat revealed to suffer a reduction in the population size and there is substantial genetic variability across all the studied loci in Udaipuri goat population in spite of heterozygote deficiency at Hardy-Weinberg equilibrium and genetic bottleneck analysis.

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