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Bottleneck analysis and genetic diversity in Mahabubnagar goats through microsatellite markers

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Abstract
In the present study a total of fifteen microsatellite loci were used for the assessment of genetic diversity and bottleneck analysis in Mahabubnagar goats obtained from the breeding tract of Mahabubnagar district, Telangana state. A total of 123 alleles with a mean of 10 ± 0.458 was observed. The effective number of alleles varied from 3.609 (MAF209) to 9.440 (INRABERN172) with a mean of 7.685 ± 0.36.The overall mean inbreeding coefficient (F_{IS}) was 0.101 ± 0.035 and the PIC values for all the loci were found to be more than 0.50. No mode shift was detected in the frequency distribution of alleles and a normal L-distribution was obtained indicating the absence of bottleneck in the population.

Keywords: Bottleneck analysis, genetic diversity, microsatellite markers and Mahabubnagar, goats

Introduction
Mahabubnagar goats are distributed in Mahabubnagar and in adjoining areas of Nalgonda and Rangareddy districts of Telangana state are well known for their production potential. The bodyweights of Mahabubnagar goats ranged from 2.16 ± 0.03kg to 18.81 ± 0.17kg at birth and 12 months age in males, while in females the values ranged from 2.11 ± 0.03kg to 16.97 ± 0.15kg at birth and 12 months age respectively (Ekambaram *et al*. 2010) [1]. Phenotypic and genetic characterization would provide comprehensive information on this group of goats and would pave way for its characterization and recognition as a separate breed. The analysis of genetic diversity by using microsatellite markers provides information about the presence or absence of bottleneck in the population.

Materials and methods
Blood samples were obtained from 40 mahabubnagar goats and genomic DNA was isolated by standard phenol- chloroform method (Sambrook and Russel, 2001) [2]. A total of 15 microsatellite markers were utilized for genotyping. The markers were chosen from the list recommended by FAO were amplified using a thermalcycler with a PCR reaction mixture (12 .5µ1) containing 25 mM Mgcl2, 10mM dNTPs, 10x buffer, 60pM of each primer, 50ng of template DNA and 1 unit of Taq DNA polymerase. The The PCR products were genotyped using 8% denaturing polyacrylamaide gel and then visualized after silver staining. The allele sizes were determined with the help of 50 bp DNA marker. The microsatellite genotype data were analysed by GenALEx 6.502 software. BOTTLENECK version 1.2.02 was used to know the reduction of effective population size in the investigated Mahabubnagar goat population (Piry *et al*. 1999) [3].

Results and discussion
Mean number of alleles (n_a), Effective number of alleles (n_e), Shannon's Information Index (I), Observed heterozygosity (H_o), Expected heterozygosity (H_e), Within population inbreeding estimates (F_{IS}), Polymorphism Information content (PIC) and Out crossing rates for 15 number of microsatellite loci are presented in Table 1. A total of 123 alleles were observed across all the loci were observed with a mean of 10 ± 0.458 alleles per locus and were on par with values reported by Mishra *et al.* (2012) [4] in Konkan Kanyal goats; Hassen *et al.* (2012) [5] Ethiopian indigenous goat populations. Genetic diversity measures the genetic variations among the population in terms of estimation of heterozygosity. The overall mean observed and expected heterozygosity was found to be 0.778± 0.034 and 0.863±0.011 reflecting the existence of ample variation in the population at this loci and this may be related to the breeding history and environment of population.

High heterozygosity values obtained in the present study may be due to intermixing of different populations probably during grazing period. The overall mean within population inbreeding coefficient (F_{IS}) was 0.101 revealing wide genetic variability and the population was out bred in nature as indicated by negative F_{IS} value by some loci. The overall mean PIC value (0.848) of all the loci genotyped in the Mahabubnagar goats was above 0.5.

Bottleneck Analysis:

When a population experiences reduction of its effective population size, the reduction in the allele number is at a faster rate than reduction in heterozygosity, *i.e.*, when the locus is at mutation-drift equilibrium, the observed heterozygosity is larger than the expected heterozygosity from the observed number of alleles. Three tests (sign test, standard difference test and wilcoxon test) under three mutation models namely infinite allele model (IAM), two phase model (TPM), step-wise mutation model (SMM) used to find the heterozygosity excess using the bottleneck programme and results were shown in Table 2. The first test suffers from low statistical power. The second test is not very useful since it requires at least 20 polymorphic loci. The Wilcoxon test provides relatively high power and it can be used with as few as four polymorphic loci and any number of individuals (15-40 individuals and 10-15 polymorphic loci is recommend to achieve high power (Mahmoudi *et al*., 2013). Results revealed that under sign rank test, observed heterozygosity excess (Hoe) was significantly high than the expected heterozygosity excess (Hee) under IAM ($P<0.05$), TPM and SMM ($P<0.01$) models of microsatellite evolution and hence population is in mutation drift equilibrium. Positive T2 values under Standardised difference test provided significant gene diversity excess ($P<0.01$) for all the three models. Highly significant P-value under Wilcoxon test revealed the heterozygosity excess under IAM, TPM and SMM models, agreed the acceptance of mutation-drift equilibrium (Deepika *et al*., 2012) [7]. In addition qualitative graphical method (Mode-shift indicator test) of Luikart *et al*. (1998) [8] was also used to visualize the allele frequency spectra. The microsatellite alleles were classified into 10 frequency classes and found normal L-shaped distribution where alleles with low frequencies (0.01-0.1) are the most abundant (Fig 1.) which reflects that the population has not undergone any recent reduction in size.

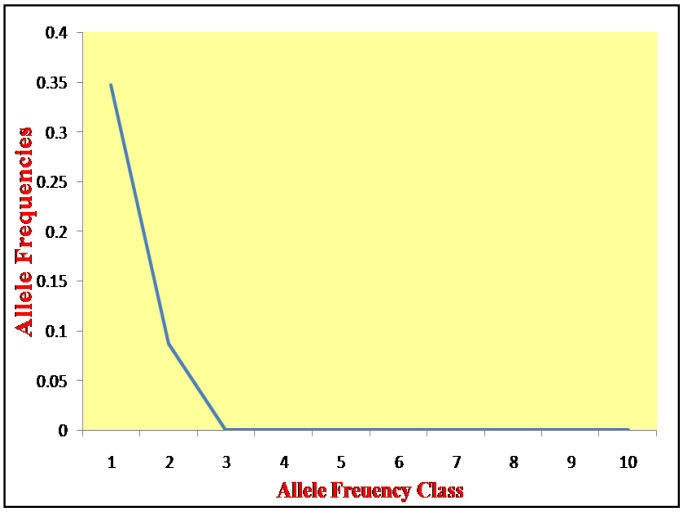

Fig 1: L-shaped mode-shift graph showing lack of bottleneck in Mahabubnagar goats.

Table 1: Mean number of alleles (n_a), Effective number of alleles (n_e), Shannon's Information Index (I), Observed heterozygosity (H_o), Expected heterozygosity (H_e), Expected heterozygosity (H_e), Fixation Indices (F_{IS}), Polymorphism Information content (PIC) and Out crossing rates at various microsatellite loci studied

Locus	Na	ne	I	H_o	H_e	F_{IS}	PIC	out cross
INRA023	9.000	7.512	2.092	0.900	0.867	-0.038	0.852	1.079
OarAE54	9.000	6.404	2.002	0.795	0.844	0.058	0.825	0.890
SRCRSP8	10.000	7.511	2.161	0.744	0.867	0.142	0.853	0.750
SPS113	10.000	8.593	2.215	0.846	0.884	0.042	0.872	0.918
INRABERN172	12.000	9.440	2.347	0.950	0.894	-0.063	0.884	1.133
OarFCB20	10.000	7.960	2.177	0.700	0.874	0.199	0.861	0.667
MCM527	10.000	8.519	2.214	0.842	0.883	0.046	0.870	0.912
ETH10	11.000	8.399	2.244	1.000	0.881	-0.135	0.869	1.312
MAF209	5.000	3.609	1.440	0.538	0.723	0.255	0.684	0.593
INRABERN185	11.000	7.191	2.164	0.775	0.861	0.100	0.846	0.818
P19(BYA)	10.000	7.643	2.170	0.821	0.869	0.056	0.856	0.893
TCRVB6	11.000	7.901	2.192	0.775	0.873	0.113	0.860	0.797
SRCRSP7	9.000	7.064	2.061	0.700	0.858	0.185	0.842	0.688
BM6444	13.000	9.357	2.397	0.775	0.893	0.132	0.884	0.766
DRBP1	10.000	8.177	2.191	0.513	0.878	0.416	0.865	0.412
Mean	10.000	7.685	2.138	0.778	0.863	0.101	0.848	0.842

Table 2: Mutation–drift equilibrium, heterozygosity excess/deficiency under three mutation models in Mahabubnagar goat population

Model		Infinite alleles model (IAM)	Two-phase model (TPM)	Step wise mutation model (SMM)
Sign test (No. of loci with heterozygosity excess)	Expected	9.11	8.91	8.83
	Observed	15	15	14
	Probability	0.00056	0.00040	0.00403
Standardized differences test	T2 values	4.963	4.314	3.161
	Probability	0.00000	0.00001	0.00079
Wilcoxon sign rank test	Probability of heterozygosity excess	0.00002	0.00002	0.00003

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