Molecular evidences of mutation-drift equilibrium in Punganur breed of cattle

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Abstract

To evaluate Punganur cattle for mutation drift equilibrium, three tests were performed under three different mutation models, viz., infinite allele model (IAM), stepwise mutation model (SMM) and two-phase model (TPM). The observed gene diversity (He) and expected equilibrium gene diversity (Heq) were estimated under different models of microsatellite evolution. All the 20 loci were found to exhibit gene diversity excess under IAM and TPM, while 19 loci were having gene diversity excess under SMM. All the three statistical tests, *viz.,* sign test, standardized differences test, and Wilcoxon sign rank test, revealed significant ($P<0.01$) deviation of Punganur cattle population from mutation-drift equilibrium under all the three models of mutation. Furthermore, the qualitative test of allele frequency distribution in Punganur cattle population revealed a strong mode shift from the normal L shaped form suggesting that the population had experienced genetic bottleneck in the recent past. The occurrence of genetic bottleneck might have led to the loss of several rare alleles in the population, which point towards the need for efforts to conserve this important cattle germplasm.

Key words: Bottle neck; Microsatellite; Mutation; Punganur cattle.

Introduction

Punganur cattle is the shortest humped dwarf cattle breed originating from Punganur area in Chittoor district of Andhra Pradesh. Punganur cattle are distributed in Chittoor, West Godavari and Krishna districts. Punganur breed (Figure 1 & 2) has short, long body and short neck. Coat colour is White, Grey, Black and Brown. Popular colour is white. Eyes are bright and prominent black circles around eyes. Horns vary in length and shape, thick at the base and grow outwards and backwards. Ears are short and erect. Well-developed massive and erect hump. Long tail and black switch of the tail touches the ground. Udder moderately developed with symmetrical teats. Back slightly rising to croup (Vinod *et al*., 2019). Genetic characterization of populations, breeds and species allows the evaluation of genetic variability, a fundamental element in working out breeding strategies and genetic conservation plans. Information about genetic diversity and differentiation of Punganur breed is essential to formulate appropriate conservation, breeding and sustainable management programmes in order to prevent extinction and genetic erosion of this breed. Microsatellite markers have been widely used for population genetic analyses of livestock species, as they are informative and can successfully elucidate the relationships between individuals and populations, assess within-breed genetic diversity and inbreeding, introgression from other species, genetic differentiation and admixture among breeds (Ghazy *et al*., 2013). Indigenous cows plays significant role in household income of marginal farmers in rural areas (Rahman & Gupta, 2016). Hence, the present study was undertaken to assess the mutation drift equilibrium of Punganur cattle using multilocus genotypic data at different microsatellite loci and to detect the occurrence of recent genetic bottleneck event, if any, in this population**.**

Bottleneck occurs when populations experience severe, temporary reduction in size. It influences the distribution of genetic variation within and among populations. Cornuet and Luikart, 1996 introduced heterozygosity excess as a method for detection of bottlenecks. This method is based on the premise that populations experiencing recent reduction in size develop an excess of heterozygosity at selectively neutral loci relative to the heterozygosity expected at mutation-drift equilibrium.

Materials and methods

A total of 50 blood samples were collected at random from Punganur cattle maintained at Livestock Research Station, Palamaner (35 number) and Farmers herds (15 number). The experiment was conducted at The Department of Animal Genetics and Breeding, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati. Tirupati, part of Rayalaseema region of AndhraPradesh state, located between the North Latitudes of 130 21’ 54’’ and 140-8’32’’ and between the Eastern Longitudes 790 5’ 42’’ and 800 4’ 10’’. The district covers an area of 9174 Square kilometres.

Microsatellite marker typing

A total of twenty microsatellites (BM1818, BM1824, BM2113, CSRM60, ETH10, ETH152, ETH225, HAUT27, HEL 1, ILSTS005, ILSTS006, INRA005, INRA035, INRA037, INRA063, MM12, SPS115, TGLA53 and TGLA227) were selected based on degree of polymorphism and genomic coverage (FAO, 2004).

The selected microsatellite markers complied with the recommendations of the FAO and the International Society for Animal Genetics (ISAG) (FAO 2004). Amplification of loci was performed in a final reaction volume 15 µl containing 100 ng of genomic DNA, 10 pM of each primer, 25 mM MgCl2, 200 Mm, dNTPs, 5 U/µl of Taq polymerase and 10X Taq buffer (Table 2). Optimization of PCR reaction was carried out for PCR assay buffer, MgCl2 and annealing temperature. PCR was carried out in Kyratec ® Thermal cycler (M/s JH BIO, Bangalore). A PCR programme with an initial denaturation at 94^{o} c for 5 minutes followed by 35 cycles of denaturation for 94^{o} c for 45 seconds with annealing temperature ranging from 52^{o} c to 66^{o} c (depending upon the primer used) for 45 seconds and an extension duration of 45 seconds at 72^{o} c was used. Final extension was done at 72^{o} C for 5 minutes followed by refrigeration at 4o c (Table 3). Amplified PCR products were checked on agarose gel electrophoresis with 50bp DNA ladder. Genotyping of animals was done based on allele size. The genotypes were scored based on the presence of a single band (homozygotes) or double bands (heterozygotes) on the agarose gel slab.

Statistical analysis for microsatellite data

Observed allele number (NA), expected Heterozygosity (HE) and Heterozygosity in equilibrium (HEQ) analysed under both Infinity Allele Model (IAM), TPM (Two-phase model) and Stepwise Mutation Model (SMM) performed by BOTTLENECK v1.2.02 software (Piry *et al.,* 2006). Statistical analyses SIGN TEST, Standardized Differences Test and WILCOXON TEST were performed to determine the loci number with excess Heterozygosity, which was based on the theory of a locus assuming mutation-drift equilibrium and that allele status changes was under IAM, TPM and SMM. The proportions of different allele classes were determined by using quantitative graphic method by MODE SHIFT TEST (Luikart *et al.,* 1998) that detected whether the distributions of allele frequencies follow L- shaped or not. All tests were evaluated by BOTTLENECK v1.2.02 software (Cornuet & Luikart 1996).

Fig 1: Punganur bull

Fig 2: Punganur Cow

Results and discussion

Bottleneck occurs when populations experience severe, temporary reduction in size. It influences the distribution of genetic variation within and among populations. Cornuet and Luikart, 1996 introduced heterozygosity excess as a method for detection of bottlenecks. This method is based on the premise that populations experiencing recent reduction in size develop an excess of heterozygosity at selectively neutral loci relative to the heterozygosity expected at mutation-drift equilibrium. Three tests were used to look for bottleneck in the Punganur cattle viz Sign test, Standardized difference test and Wilcoxon rank test. Sign test is a nonparametric test. The standardized difference test and Wilcoxon rank tests are parametric and more useful if the number of loci is more than twenty. In our study we utilized twenty loci and three models - Infinite allele model (IAM), Two phase model (TPM) and Stepwise mutation model (SMM). Under IAM, a mutation can involve any number of tandem repeats and always results in a new allele state not previously existing in population. SMM assumes that a mutation results in a change in one repeat unit either by insertion or deletion. It allows mutation to existing states and implies that two alleles differing by one repeat are more closely related than alleles that differ by many repeats. The SMM and IAM represent two extreme models of mutation (Chakraborty & Jin , 1992), and it is not appropriate to assume that all the loci evolved under either IAM or SMM. The true model of mutation for most loci is probably intermediate between IAM and SMM (Di Rienzo et al., 1994) and is called the TPM. The variance of two-phase model assumed in the present study was 70 % of one-step mutations and 30 % of multistep/infinite changes. The distribution of expected gene diversity (Heq) within Punganur cattle under the assumption of constant size population was derived from the frequency of observed alleles for the given sample size. The expected gene diversities calculated under the assumption of three possible mutation models, viz., IAM, SMM and TPM, are presented in Table 1. The mean expected equilibrium gene diversity across 20 microsatellite loci was lowest under IAM (0.7115± 0.016), while it was highest under SMM (0.804±0.013). Under TPM, the calculated mean equilibrium gene diversity was intermediate between IAM and SMM with a value of 0.763±0.015. This is expected, as SMM is a conservative

model with the estimated expected gene diversity always higher than the estimation when assumed IAM (Kataria et al., 2010).

Fig. 1 Expected and observed number of loci with gene diversity excess as estimated by sign test under different mutation models

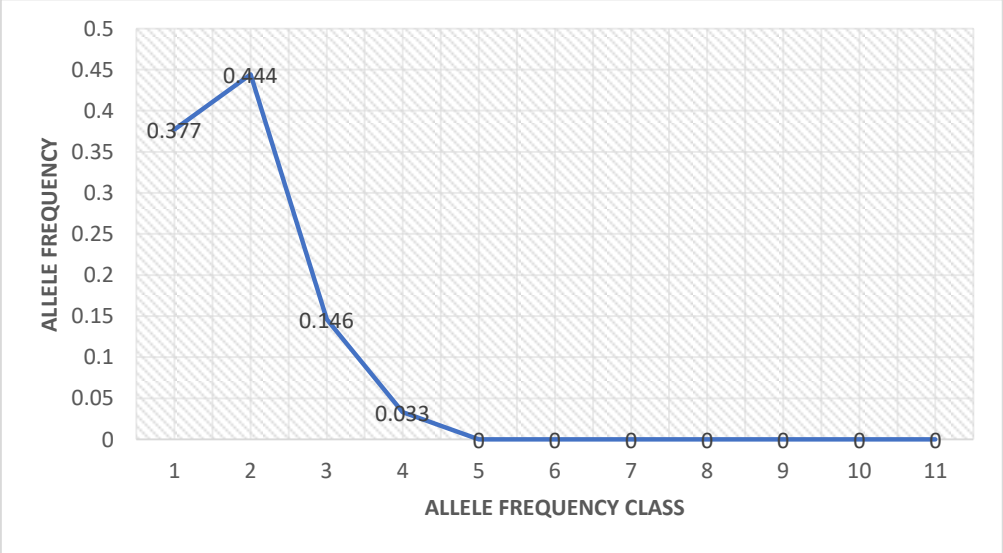

Fig. 2 Mode shift in the allele frequency spectra of Punganur cattle indicating the occurrence of recent genetic bottleneck

The expected gene diversities (Heq) estimated under three models of microsatellite evolution were compared to the observed gene diversity (He) to establish the presence of gene diversity excess or deficit at each locus within Punganur cattle population. Three statistical tests, viz., Sign test, Standardized differences test, and Wilcoxon sign rank test, were performed for each mutation model (Table 2). The results of sign test showed all the 20 investigated loci exhibiting heterozygosity excess under IAM and TPM, while 19 loci showed heterozygosity excess under SMM. The expected numbers of loci with heterozygosity excess under these three mutation models were 11.37, 11.34, and 11.17, respectively (Fig. 3). Thus, sign test revealed significant heterozygosity excess ($P<0.01$), indicating the deviation of Punganur cattle population from mutation drift equilibrium under all the three models of microsatellite evolution. Standardized differences test was performed by comparing the calculated T2 statistic (the difference between observed and expected gene diversities divided by standard deviation of the corresponding distributions of gene diversities) to an N (0, 1) distribution. The T2 values were found to be significantly positive ($P<0.01$), under all the three models of mutation with the values of 6.087 (IAM), 5.494 (TPM), and 4.315 (SMM). Similarly, the one-tail Wilcoxon sign rank test for gene diversity excess revealed significant deviation ($P<0.01$), of Punganur cattle from mutation drift equilibrium.

The standardized differences test is a parametric test, while Wilcoxon sign rank test is nonparametric similar to sign test. The overall statistical power of standardized difference test and Wilcoxon sign rank test are higher than that of sign test. These tests for heterozygosity excess can detect recent historical bottleneck events

Table 1: Estimated observed (He) and expected (Heq) gene diversities under different mutation models of microsatellite evolution

LOCUS	N_A	H_E	H_{EQ}					
			IAM (Infinite alleles model)		TPM (Two-phase model)		SMM (Stepwise mutation model)	
			T2	P Value	T2	P Value	T2	P Value
BM1818	8	0.873	0.726	0.001*	0.778	0.005*	0.821	0.026*
BM1824	8	0.843	0.727	0.036*	0.779	0.110 NS	0.818	0.299 NS
BM2113	6	0.745	0.627	0.177NS	0.692	0.328 NS	0.744	0.407 NS
CSRM60	11	0.897	0.815	0.007*	0.850	0.062 NS	0.875	0.180 NS
ETH10	9	0.878	0.758	0.004*	0.805	0.014*	0.842	0.105 NS
ETH152	7	0.858	0.682	0.000*	0.741	0.003*	0.788	0.019*
ETH185	6	0.821	0.634	0.0040*	0.694	0.011*	0.749	0.052 NS
ETH225	6	0.813	0.630	0.014*	0.692	0.026*	0.746	0.075 NS
HAUT27	8	0.874	0.731	0.0010*	0.777	0.003*	0.820	0.017*
HEL 1	10	0.901	0.786	0.002*	0.830	0.004*	0.861	0.023*
ILSTS005	6	0.821	0.633	0.004*	0.692	0.013*	0.745	0.040*
ILSTS006	8	0.868	0.726	0.002*	0.776	0.010*	0.819	0.054 NS
INRA005	5	0.752	0.566	0.045*	0.633	0.093 NS	0.686	0.2080
INRA035	7	0.872	0.763	0.009	0.826	0.048	0.858	0.168
INRA037	6	0.824	0.634	0.004*	0.694	0.009*	0.747	0.0310*
INRA063	8	0.879	0.730	0.000*	0.776	0.000*	0.819	0.005*
MM12	10	0.886	0.785	0.009*	0.834	0.046*	0.862	0.172 NS
SPS115	8	0.829	0.723	0.063*	0.777	0.201 NS	0.816	0.469 NS
TGLA53	7	0.858	0.688	0.000*	0.745	0.001*	0.788	0.009*
TGLA227	14	0.905	0.866	0.155	0.891	0.383 NS	0.909	0.366 NS
Mean± S.E	158	0.849±0.0104	0.7115±0.0167		0.763±0.0155		0.804±0.0131	

Table 2: Different tests for mutation drift equilibrium in Punganur cattle

Test		IAM	TPM	SMM
Sign Test	No. of Loci with H_e Excess	20	20	19
	No. of Loci with He Deficiency	0	0	1
	P- Value	0.00006*	0.00006*	0.00059*
S D Test	T_2 Value	6.087	5.494	4.315
	P- Value	0.0000*	0.0000*	0.0000*
Wilcoxon Sign Rank Test	P- Value (One Tail Test for H_e Excess)	0.0000*	0.0000*	0.0000*

up to 0.25–2.5 Ne generations after the initiation of population decline. In the present study, all the three tests revealed significant deviation of Punganur cattle from mutation drift equilibrium, indicating the occurrence of recent genetic bottleneck. Furthermore, the population was found to have significant deviations when assumed under all the three models of microsatellite evolution. For any given data set, IAM predicts lower equilibrium gene diversity than SMM, and hence, it is more likely to indicate significant heterozygosity excess. Punganur cattle were found to deviate from equilibrium gene diversity even under the statistically more conservative stepwise mutation model, thus providing unequivocal genetic basis for demographic bottleneck. Furthermore, the test for mode shift in frequency distribution of different alleles was performed in Punganur cattle. This is a qualitative method for detection of genetic bottleneck. In non-bottlenecked populations, a large proportion of rare alleles are expected. However, bottleneck events are expected to cause alleles with low frequency, i.e., rare alleles to become less abundant in the population than alleles with intermediate frequencies. In such cases, the plotting of proportion of different alleles against allele frequency classes will cause mode shift from the normal L-shaped distribution. In the present study, Punganur cattle revealed a mode shift suggesting the loss of low frequency alleles from the population (Fig. 4).

Very few studies have reported on possible occurrence of genetic bottleneck based on microsatellite data, viz., Dexter cattle population (Bray et al., 2009), certain African cattle breeds (Luikart et al., 1998), Valais Blackneck (Glowatzki-Mullis et al., 2008). Earlier studies on different Indian cattle breeds did not reveal any indication of recent occurrence of genetic bottleneck (Sodhi et al., 2006; Pandey et al., 2006; Sharma et al., 2008; Sharma et al., 2009).

Conclusion

Population size is important for the maintenance of genetic variation, and it should be large enough to avoid strong genetic drift and inbreeding. The occurrence of genetic bottleneck in Punganur cattle is important, as increased demographic stochastics will reduce the adaptive potential and probability of persistence of the breed. There is a strong and urgent need for conservation and genetic improvement of these animals to avoid further loss of alleles and genetic variation. Farmers must be made aware of the importance of scientific breeding principles such as keeping at least one bull per 20–30 cows, the practice of selection among the breeding bulls, and castration of scrub bulls. To begin with, a breed society and bull mother farm needs to be established in the native tract. Purebred bulls can be distributed to the farmers under open nucleus mode with bidirectional exchange of germplasm between farm and field. The occurrence of genetic bottleneck might have led to the loss of several rare alleles in the population, which point towards the need for efforts to conserve this important cattle germplasm.

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