Short tandem repeat marker based evaluation of mutation drift equilibrium in Krishna Valley cattle

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ABSTRACT

The present study was undertaken to evaluate the Krishna Valley cattle for the occurrence of genetic bottleneck based on microsatellite genotype data at 18 loci. Three different tests viz. sign test, standardized differences test and Wilcoxon sign rank test were utilized to evaluate the Krishna Valley cattle population for detecting the transient heterozygosity excess under different models of microsatellite evolution. Under infinite alleles model (IAM), all the three tests revealed significant heterozygosity excess while the stepwise mutation model did not reveal any such significant heterozygosity excess. Under the two phase model sign test and standardized differences test with the exception of Wilcoxon sign rank test revealed significant heterozygosity excess indicating the possible deviation of Krishna Valley cattle population from mutation drift equilibrium. However, the allele frequency distribution at different microsatellite loci revealed normal L-shape without any mode shift. The absence of mode shift indicates that the rare alleles have not been lost much, despite the severe reduction in the population size of Krishna Valley cattle.

Key Words: Krishna valley cattle, Genetic bottleneck, microsatellite

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INTRODUCTION

Krishna Valley cattle is a heavy draft breed of South India with its breeding tract lying on the banks of river Krishna. They are suitable for agricultural operations in black cotton soil of the region with varying milk producing capacity. Colour of animals varies from grey- white in males to white in females. Originally, Krishna Valley cattle were found to be distributed in the areas adjacent to Krishna river such as Ghataprabha and Malaprabha in Karnataka and Maharashtra states of India (Anonymous, 1926). The native breed tract included the districts of Satara, Sangali and Solapur in Maharashtra and Belgaum, Bijapur and Raichur districts of Karnataka. However, a recent survey of Ramesha et al. (2001) indicated a shift in the breed tract from different districts of Maharashtra and Karnataka to northern Karnataka alone. Only a few hundred true to type animals are now found in and around the villages of the Jamkhandi, Mudhol and Athani taluks (divisions within a district) of northern Karnataka (Karthickeyan et al., 2006a). Mechanization of agricultural operations, intensive cross breeding and non-availability of true type breeding bulls in its native tract have been attributed as major factors for reduction in the population of these animals. Such a reduction in the population might have affected the genetic variability in the population. Loss of genetic diversity may reduce the potential of small populations to respond to selective pressures and increased inbreeding may reduce population viability (Westemeier et al., 1998).

Populations that have experienced a recent reduction in their effective population size exhibit a correlative reduction in the allele numbers and heterozygosity (He) at polymorphic loci. With the advent of DNA markers such as minisatellites and microsatellites, it has become more feasible to detect genetic bottleneck in domesticated populations (Spencer et al., 2000). Microsatellite based genotypic data have been successfully used to evaluate different livestock populations viz. cattle (Sharma et al., 2006a,b; Pandey et al., 2006), buffalo (Kathiravan et al., 2009, 2010 and Kataria et al., 2010) sheep (Bhatia and Arora, 2007), goat (Aggarwal et al., 2007) and poultry (Tantia et al., 2006) for mutation drift equilibrium and genetic bottleneck events. The genetic variability of Krishna Valley cattle using microsatellite markers has already been reported by Karthickeyan et al. (2006b). However, no information is available with respect to genetic bottleneck events in Krishna Valley cattle population. Hence, the present study was undertaken with the objective of evaluating Krishna Valley cattle for mutation drift equilibrium and occurrence of genetic bottleneck in the recent past using microsatellite genotypic data.

MATERIALS AND METHODS

Blood sample collection and microsatellite genotyping: A total of 50 blood samples collected from Krishna Valley cattle distributed in Bangalore and Jamkhandi, Mudhol, Bilgi taluks of Bagalkot district of Karnataka were utilized for the present study. Adequate care was taken by interviewing the farmers in detail to ensure unrelatedness of sampled animals. The DNA for the study was isolated from peripheral blood by a rapid, non-enzymatic method as described by Lahiri and Nurnberger (1991). A total of 18 different microsatellite loci recommended by FAO for diversity analysis in cattle were analyzed. The amplification of DNA was carried out using thermal cycler (MJ Research) with the PCR reaction mixture of 20ml. The mixture was prepared by adding 50-100ng of template DNA; 1.5mM MgCl2; 5 picomoles each of forward and reverse primers; 0.75 units of Taq DNA Polymerase and 100mM dNTPs. Amplification was carried out with annealing temperatures ranging from 51°C to 60°C for different primers for

30 cycles. PCR products were resolved by running on 6% denaturing polyacrylamide gel electrophoresis and visualized by silver staining. Allele sizes were estimated by running a 10 bp DNA ladder (Invitrogen, USA) along with the PCR products and genotypes scored manually.

Statistical analysis: Different measures of genetic variation like observed and effective number of alleles, observed and expected heterozygosities, polymorphism information content, test for Hardy-Weinberg equilibrium (HWE) and FIS were computed using different softwares as reported earlier (Kathiravan et al., 2009). The variation between allelic diversity and heterozygosity was exploited as the basis for statistical tests to evaluate the mutation drift equilibrium of Krishna Valley cattle populations. To determine the significant number of loci with heterozygosity excess, three tests viz. sign test, standardized differences test and Wilcoxon sign rank test were performed under three different mutation models viz. infinite alleles model (IAM), stepwise mutation model (SMM) and two phase model (TPM). Further, a qualitative test of mode shift was performed to evaluate the frequency distribution of alleles at different microsatellite loci using BOTTLENECK program (Piry et al., 1999).

RESULTS AND DISCUSSION

In total, 93 alleles were observed at 18 microsatellite loci with a mean of 5.13 per locus. The number of observed alleles ranged from 3 (HAUT 027) to 8 (ILSTS 033). Allele size varied from 94 to 290 for different loci and their frequency ranged between 0.0104 and 0.7614 (Table 1). Polymorphic Information Content (PIC) was found to be varying 0.3793 (ILSTS 006) to 0.7787 (CSRM 060) with a mean of 0.628. Except ILSTS 006 and ETH 152, all other loci showed high PIC values suggestive of high polymorphic nature. Observed heterozygosity varied from 0.3636 (ILSTS 006) to 1.000 (ILSTS 011, HEL005, ETH 003) while the expected heterozygosity (gene diversity) varied from 0.4021 (ILSTS 006) to 0.8065 (CSRM 060) with the means of 0.840 and 0.673 respectively. Thirteen out of 18 microsatellite loci revealed significant deviation from Hardy Weinberg equilibrium. The mean observed heterozygosity was found to be higher than the mean expected heterozygosity in Krishna Valley cattle population.

Table 1. Observed number of alleles and their frequency at different microsatellite loci in Krishna Valley cattle

Locus	No. of alleles	A	B	C	D	E	F	G	H
ILSTS005	6	0.2727	0.0795	0.1818	0.0341	0.1818	0.25		
ILSTS006	5	0.0455	0.7614	0.1023	0.0795	0.0114			
ILSTS011	4	0.3776	0.0918	0.3776	0.1531				
ILSTS030	4	0.2188	0.1771	0.4062	0.1979				
ILSTS033	8	0.0625	0.1771	0.0417	0.1458	0.5104	0.0417	0.0104	0.0104
ETH003	5	0.0761	0.2826	0.4891	0.0652	0.087			
ETH010	4	0.0778	0.4444	0.1889	0.2889				
ETH152	5	0.7604	0.0833	0.0208	0.0625	0.0729			
ETH225	6	0.0581	0.0465	0.186	0.0814	0.2791	0.3488		
INRA005	5	0.1889	0.3111	0.2444	0.2111	0.0444			
HEL001	5	0.1279	0.2791	0.3953	0.093	0.1047			
HEL005	6	0.2206	0.0441	0.1029	0.3382	0.0588	0.2353		
HEL009	5	0.1364	0.0455	0.3977	0.3864	0.0341			
BM1818	6	0.1042	0.0938	0.4896	0.1458	0.1146	0.0521		
MM008	4	0.0444	0.4778	0.4556	0.0222				
HAUT027	3	0.2128	0.4149	0.3723					
CSSM066	6	0.1333	0.0444	0.5	0.0556	0.1556	0.1111		
CSRM060	6	0.1702	0.2872	0.1277	0.1702	0.0638	0.1809		

In a non-bottlenecked equilibrium population, approximately 50% of the loci sampled are expected to have a slight excess of gene diversity and 50% will have a slight deficiency of gene diversity, resulting from genetic drift and sampling error (Luikart and Cornuet, 1998). Populations that have experienced a recent reduction of effective population size exhibit a correlative reduction of allele numbers (k) and gene diversity. But the allele number is reduced faster than the gene diversity. Thus in a recently bottlenecked population, the observed gene diversity is higher than the expected equilibrium (mutation drift) gene diversity. The distribution of expected gene diversity within Krishna Valley cattle (Table 2) 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. infinite alleles model (IAM), stepwise mutation model (SMM) and two phase model (TPM) were compared to the Hardy-Weinberg gene diversity to establish the presence of gene diversity excess or deficit at each locus within Krishna Valley cattle population. Three statistical methods viz. Sign test, Standardized differences test and Wilcoxon sign rank test were performed for each mutation model.

Table 2. Observed and expected equilibrium heterozygosity at various loci in Krishna Valley cattle when assumed under different models of microsatellite evolution

Locus	No. of alleles	Observed Heterozygosity (He)	Expected equilibrium heterozygosity (Heq)			
			IAM	TPM (30 -70)	TPM (50 -50)	SMM
ILSTS005	6	0.807	0.637	0.702	0.679	0.751
ILSTS006	5	0.41	0.565	0.638	0.627	0.688
ILSTS011	4	0.698	0.491	0.549	0.535	0.611
ILSTS030	4	0.732	0.49	0.541	0.525	0.613
ILSTS033	8	0.694	0.731	0.78	0.774	0.819
ETH003	5	0.678	0.567	0.637	0.619	0.695
ETH010	4	0.692	0.482	0.554	0.529	0.614
ETH152	5	0.414	0.577	0.631	0.622	0.693
ETH225	6	0.77	0.641	0.697	0.679	0.751
INRA005	5	0.778	0.572	0.639	0.617	0.688
HEL001	5	0.746	0.573	0.629	0.622	0.689
HEL005	6	0.782	0.636	0.697	0.675	0.748
HEL009	5	0.685	0.575	0.634	0.613	0.694
BM1818	6	0.719	0.635	0.697	0.682	0.75
MM008	4	0.574	0.487	0.55	0.524	0.607
HAUT027	3	0.658	0.374	0.426	0.406	0.48
CSSM066	6	0.706	0.638	0.698	0.681	0.747
CSRM060	6	0.824	0.635	0.699	0.683	0.745

Among the 18 microsatellite loci analyzed in the present investigation, Krishna Valley cattle population was found to have heterozygosity excess at 15 and 10 loci when assumed under IAM and SMM respectively (Figure 1). Sign test revealed significant heterozygosity excess under IAM while the observed heterozygosity excess was not found to be statistically significant under SMM. Standardized differences test revealed significant positive T2 value of 3.374 under IAM while the observed T2 value was found to be negative under SMM. Wilcoxon sign rank test also revealed similar results with respect to IAM and SMM. Thus all the three tests revealed significant heterozygosity excess under infinite alleles model, while the stepwise mutation model did not reveal any such significant heterozygosity excess (Table 3). Similar type of contradictory nature of populations under different mutation models has been reported in Indian poultry (Tantia et al., 2006) and Marathwada buffaloes of Central India (Kathiravan et al., 2009). It is important to note that the calculation of expected equilibrium heterozygosity depends on the model of mutation used to analyze the loci being studied (Cornuet and Luikart, 1996).

Table 3. Different tests for mutation drift equilibrium in Krishna Valley cattle

Tests		IAM	TPM	SMM
Sign test	No. of loci with He excess	15	15	10
	No. of loci with He deficiency	3	3	8
	P-value	0.024	0.029	0.450
Standardized differences test	T2-value	3.374	1.790	-0.722
	P-value	0.000	0.037	0.235
Wilcoxon sign rank test	P-value (one tail test for He excess)	0.001	0.054	0.248

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. Hence, for any given data set, the IAM predicts lower equilibrium heterozygosity than the SMM and hence it is more likely to indicate that significant heterozygosity excess exists. The true model of mutation for most loci is probably intermediate between the IAM and SMM (Di Rienzo et al., 1994). Consequently the actual expected equilibrium (Heq) for a given locus probably lies between the Heq values calculated under IAM and SMM. In the present study variance for TPM was kept as 30, while the percent proportion of SMM in TPM was kept as 70%. Under the two phase model (70% SMM-30%IAM) sign test and standardized differences test with the exception of Wilcoxon sign rank test revealed significant heterozygosity excess indicating the possible deviation of Krishna Valley cattle population from mutation drift equilibrium. Reduction in the Krishna Valley cattle population to only few hundreds in its native tract corroborates the findings of the present study.

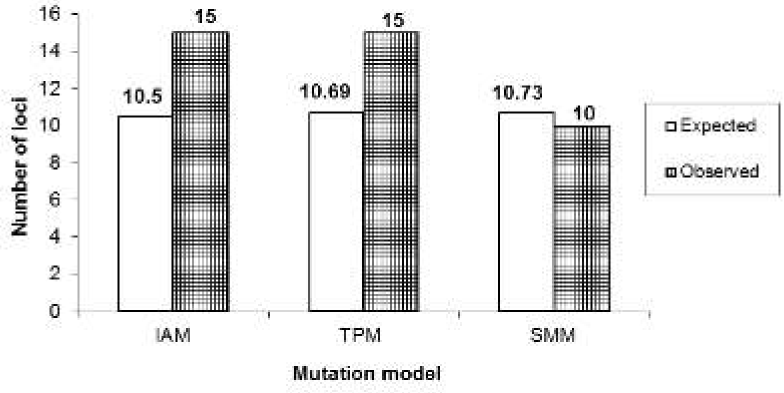

Figure 1. Expected and observed number of loci with heterozygosity excess as estimated by sign test under different mutation models

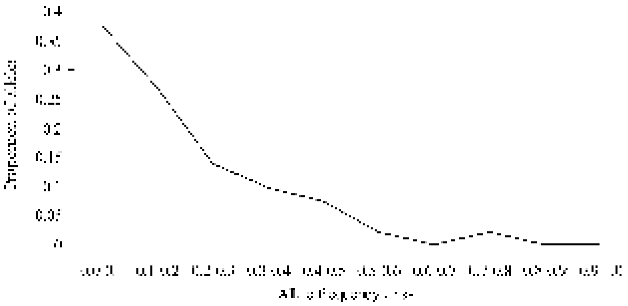

Figure 2. Allele frequency distribution in Krishna Valley cattle showing L-shaped normal curve without any mode shift

Further, Krishna Valley cattle population was evaluated by the qualitative test for mode shift, which utilizes the frequency distribution of different allele classes. The bottlenecks are expected to cause alleles at low frequency to become less abundant than alleles present in intermediate frequency leading to a mode shift. Allele frequencies in Krishna Valley cattle population revealed normal L-shape (Figure 2) without any mode shift. In conclusion, the present study revealed significant deviation of Krishna Valley cattle population from mutation drift equilibrium. Although, it was found that rare alleles have not been lost much, severe reduction in the population size of Krishna Valley cattle is a cause of great concern. The potential loss of valuable genes is imminent unless corrective steps are taken to increase the population in its native breed tract.

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