



Evaluation of within-breed genetic diversity in Krishna Valley cattle: an endangered breed of south India

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Krishna Valley breed of cattle is an endangered draught breed, restricted to few villages in the Jamkandi *taluk* of Karnataka whose population size with the true-to-type animals is less than 100 in number. It has the capacity to undertake heavy work in black cotton soil in the valleys of river Krishna (Ramesha *et al.* 2001). This breed is claimed to be an admixture of at least 3 distinct types; Gir from Kathiawar, Ongole from erstwhile Madras Presidency and local beasts of the Mysore basic type. The Krishna Valley cattle is quite distinguishable from other breeds of cattle in south India with their typical horn pattern; characteristically black scrotum and muzzle; and black patches over the knee and fetlock joints.

One of the main reasons for reduction in the population is lack of availability of breeding Krishna Valley bulls resulting from the preference of farmers for Khillari cattle and mechanization of agricultural operations. Not much effort had been made to preserve the Krishna Valley breed except a few *Goshalas*, and no genetic improvement measures are being undertaken (Karthickeyan *et al.* 2006). Studying the genetic variation within this breed provides us the base genetic architecture of the breed helping in better conservation management. Hence, this study was carried out with bovine-specific micro-satellites through genotyping with automated DNA sequencer.

Blood samples were collected from Krishna valley breed of cattle, unrelated by ancestry, inhabiting villages in Mudurakhandi, Kallolli and Savalagi villages in Jamkhandi a *taluks* of northern Karnataka. The genomic DNA was isolated by phenol chloroform method (Sambrook *et al.* 1989). A total of 25 micro-satellite markers recommended by FAO (<http://www.fao.org/dad-is>) were amplified with specific primer sets. Each 15 µl PCR reaction mixture contained 50–100 ng of genomic DNA, 10 X PCR assay buffer, 1.5 mM MgCl₂ and dNTPs (each 25mM), 5'-end fluorescent-labelled (FAM, HEX, TET-CE and TAMRA) primers (10 p mol each) and IU TaqDNA polymerase. The annealing temperatures used for different primers varied

from 55° to 63°C. The PCR products were confirmed electrophoretically on a 2% agarose gel and the amplicons were then resolved through automated DNA sequencer and analysed by genotyping software (Gene Mapper). Micro-satellite allele frequencies, effective number of alleles, tests for Hardy-Weinberg equilibrium (HWE), observed and expected heterozygosities and F-statistics were calculated (Yeh *et al.* 1999). Polymorphism information content (PIC) was calculated using PIC calculator (Bolstein *et al.* 1980) and bottleneck analysis was done to check any recent reduction in the population (Cornuet and Luicart 1996).

Out of 25 micro-satellite markers, 2 markers, Hel1 and ETH3, were monomorphic and the rest 23 exhibited polymorphism and hence considered to analyse the genetic diversity within the breed. The micro-satellite parameters estimated in Krishna Valley cattle are presented in Table 1. In this analysis, 169 alleles with the number of observed alleles ranging from 3 (TGLA227) to 11 (HEL9 and ILSTS034) with a mean of 7.35±0.50 was recorded; while the effective number of alleles ranged from 1.18 to 6.87 with an overall mean of 3.91±0.29 across all loci. The results were similar to that of Kherigarh cattle (Pandey *et al.* 2006) where 4 to 10 alleles were observed across each locus; but Nimari (Sharma *et al.* 2010) and Malvi (Thakur *et al.* 2010) cattle breeds had higher number of alleles across each locus than Krishna valley breed of cattle ranging from 3 to 15 and 4 to 18 respectively. In this study, 88 % (22 out of 25 loci) of the markers had more than 4 alleles indicating that the markers selected are effective in evaluating the genetic diversity of the breed.

The sizes of the alleles in Krishna Valley breed of cattle ranged from 84 (TGLA227) to 304 (ILSTS006) bp and the frequencies were from 0.0104 (196, 200, 208 bp alleles in CSSM66; 213 and 219 bp alleles in ETH10; 137, 141 and 143 bp alleles in ETH225; 150 bp allele in HEL9; and 178 and 180 bp alleles in TGLA53) to 0.9167 (84 bp allele in TGLA227). From the most frequent alleles observed in Krishna Valley cattle at each locus and the individual allele frequencies, it could be inferred that the population (despite small population size) has high allelic diversity ($N_a = 7.35$), by retaining most of the alleles; but with small frequencies.

The mean polymorphism information content (PIC)

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Table 1. Microsatellite variability in Krishna Valley cattle

Locus	No. of alleles	Alleles size (bp)	PIC	Heterozygosity		Heterozygote deficit (F_{IS})	χ^2 value
				Observed	Expected		
BM1824	4	179–193	0.518	0.4468	0.5951	0.2411	14.20*
CSRM60	6	92–110	0.6107	0.3111	0.6452	0.5124	71.32**
CSSM008	6	183–201	0.6406	0.4000	0.7086	0.4240	81.75*
CSSM033	9	158–186	0.7992	0.7907	0.8306	0.0369	57.39*
CSSM66	10	178–208	0.777	0.7917	0.8129	0.0159	31.96 ^{NS}
ETH10	6	207–219	0.6441	0.7292	0.7083	–0.0402	24.67 ^{NS}
ETH225	9	137–157	0.7242	0.7500	0.7673	0.0123	54.69*
HAUT024	7	106–124	0.7141	0.5957	0.7586	0.2063	49.92**
HEL5	9	147–167	0.743	0.4255	0.7806	0.4490	139.80**
HEL9	11	146–168	0.8041	0.8125	0.8351	0.0168	56.84 ^{NS}
ILSTS005	6	180–192	0.7556	0.8542	0.7974	–0.0825	12.54 ^{NS}
ILSTS006	8	276–304	0.6093	0.4545	0.6651	0.3087	152.90**
ILSTS011	4	260–268	0.3801	0.3696	0.4233	0.1174	7.31 ^{NS}
ILSTS033	5	139–149	0.7012	0.3333	0.7571	0.5522	51.45**
ILSTS034	11	142–200	0.7515	0.8140	0.7814	–0.0539	40.88 ^{NS}
INRA005	7	135–149	0.7339	0.7292	0.7772	0.0519	116.18**
INRA035	9	100–124	0.8202	0.8333	0.8493	0.0085	143.75**
INRA063	7	176–188	0.626	0.6383	0.6831	0.0556	16.02 ^{NS}
MM12	6	106–122	0.5906	0.1379	0.6425	0.7815	94.74**
MM8	5	119–139	0.6293	0.7021	0.6889	–0.0302	6.02 ^{NS}
TGLA122	11	136–164	0.8377	0.6304	0.8638	0.2622	184.17**
TGLA227	3	84–88	0.1472	0.1000	0.1576	0.3548	8.84*
TGLA53	10	152–180	0.7139	0.7500	0.7430	–0.0201	34.50 ^{NS}
Mean / range	7.35±0.50	84 - 304	0.6640±0.03	0.5826±0.05	0.7075±0.03	0.1818±0.05	

*Significant ($P \leq 0.05$); **significant ($P \leq 0.01$); ^{NS} not significant.

value for all the 25 loci was 0.6640 ± 0.03 and among them 21 markers had PIC values of more than 0.5 indicating that these markers can be effectively used for genetic diversity analysis. Reports were similar to those reported by Pandey *et al.* (2006) as 0.669 ± 0.097 in Kherigarh cattle and Sharma *et al.* (2010) as 0.64 in Nimari cattle. The expected heterozygosity (H_e) ranged from 0.1576 (TGLA227) to 0.8638 (TGLA122) with a mean of 0.7075 across all loci. This value is comparable to that of Kherigarh cattle (0.717; Pandey *et al.* 2006). The heterozygosity values observed in Krishna valley cattle reflects the existence of genetic variation in the population, even though the population size is dwindling. Since the breed has high mean heterozygosity value, it has an ability to recover from the set back in population size and would brave harsh and extreme cold climatic conditions prevailing in the breeding tract.

Totally, 18 loci showed positive F_{IS} values indicating the deficit in heterozygotes. The overall F_{IS} value (18.18 %) indicated that the population has suffered a moderate amount of inbreeding, which is due to reduction in the size of the population and also the practice of non-random mating within population. Similar heterozygote deficit (0.188) was noticed in Kherigarh population (Pandey *et al.*

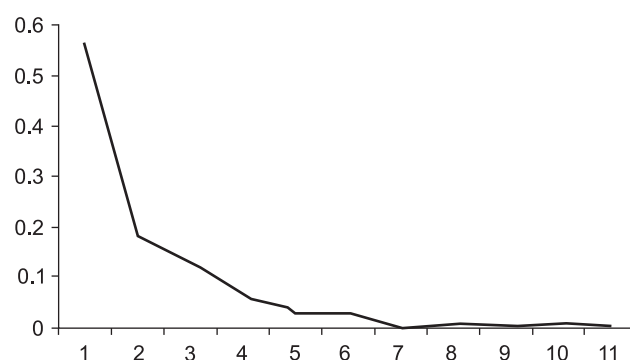


Fig. 1. Graphical representation of allele frequency in Krishna Valley cattle showing L-shaped curve.

2006) and on the other hand, heterozygote excess was found in Punganur cattle (-0.001 ; Chenna Kesvulu *et al.* 2009). While, in another 4 Indian aboriginal populations (Binjharipuri, Ghumsuri, Motu and Kumauni cattle of Odisha), it was 9.4% (Sharma *et al.* 2012). Though positive F_{IS} values were observed in 18 loci, the excess of heterozygotes found in the other loci reflects the wide genetic variability as a result of admixture of various breeds

in the formation of Krishna Valley.

The χ^2 tests revealed that 14 loci deviating from the equilibrium proportions may be due to consequences of small population size. Further, bottleneck analysis showed that the breed was apparently non-bottlenecked where the mode-shift for the frequency distribution of alleles had a L-shaped curve (Fig. 1). But yet, the step-wise mutation model (SMM) showed high significance ($P < 0.01$) in the probability when subjected to Sign, Wilcoxon rank and Standardized differences tests, and that would be resulting in slight irregularity in L-shaped curve which hints the visible reduction in its population observed in the breeding tract. The curve still being L-shaped is due the presence of rare alleles, despite severe reduction in the population size.

SUMMARY

Krishna Valley breed, an endangered draught breed of cattle, evaluated for within breed genetic diversity. The study revealed 169 alleles with the observed number of alleles ranging from 3 to 11. The mean PIC value was high (0.6640 ± 0.03) and 21 loci possessed the PIC values of more than 0.5. Despite small size of the population, Krishna Valley breed of cattle possesses moderately high within-breed genetic diversity (0.7075 ± 0.03) resulting from retention of some rare alleles in small frequencies. Overall F_{IS} value indicated that the population has suffered high amount of inbreeding which is due to very small population size and also the practice of non-random mating due to unavailability of bulls. But departure of the majority of the loci from Hardy-Weinberg equilibrium and high F_{IS} value (18.18 %) urge the breeders and policy makers to take immediate conservation measures of the breed.

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