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Application of cattle microsatellite markers to assess genetic diversity of Indian yaks

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Yak (*Poephagus grunniens*) is a unique bovine species of economical and cultural importance to the tribal population living in the difficult terrains in the foothills of Himalayas. In India, there are around 71 000 yaks found in Kargil and Leh districts in Ladakh division and Doda districts of Jammu and Kashmir (47 000), West Kameng and Tawang districts of Arunachal Pradesh (13 000), North and East districts of Sikkim (7 000) and Chamba, Lahaul-Spiti and Kinnaur districts of Himachal Pradesh (4 000) and also in few numbers in Kumaon and Garhwal region of Uttarakhand (Fig.1). In India, yaks are mainly distributed in the Himalayan states of Himachal Pradesh, Jammu and Kashmir, Sikkim and Arunachal Pradesh in an area of approximately 14 000, 23 000, 2 000 and 1 500 sq km, respectively (Ramesha *et al.* 2008).

Microsatellite markers have been widely used for assessing the genetic diversity, mapping of QTLs, population structure and relationship in and amongst various populations in several livestock species. However, very few genetic studies on yaks were successfully conducted using bovine microsatellites (Dorji *et al*. 2002, Minqiang *et al*. 2003) and no report is available on Indian yaks. In India, distribution of yaks is restricted to isolated pockets where avoiding close breeding is difficult as dominant bulls mate most of the females in a herd. Hence, genetic characterization of Indian yaks was performed using 30 microsatellite markers with the principal aim of diversity analysis.

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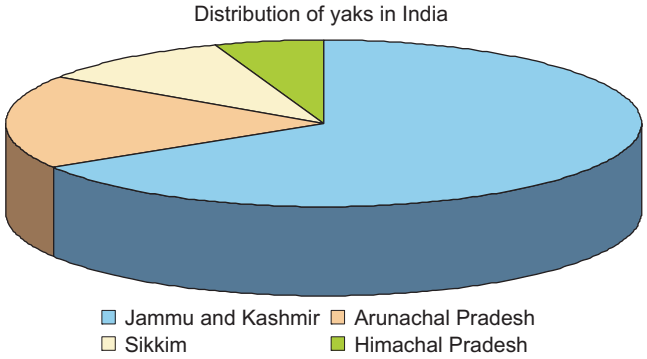

Fig. 1. Yak population in the Indian states.

Blood samples (148) from unrelated yaks were collected from all the yak rearing states of India and DNA was isolated by high salt method as described by Montgomery and Sise (1990). DNA was diluted to a final concentration of 50ng/ml and stored at 4°C. A total of 30 bovine microsatellite markers distributed across the bovine genome were selected and procured to screen the yak populations. All the selected markers were originally identified in cattle and evaluated for genetic diversity analysis in Swiss yaks (Nguyen *et al.* 2005). The total reaction mix of 50 µl contained 2 µl (100ng) of template DNA, 1µl (100 pmol) each primer, 2 µl (150 µM) each of dNTP, 2 unit of *Taq* DNA polymerase and 1X PCR buffer and distilled water. Amplifications were carried out in a thermal cycler for 30 cycles. The temperature profile of PCR was initial denaturation at 95°C for 5 min followed by 30 cycles with denaturation at 94°C for 30 s, primer annealing at the optimal temperature for each primer pair (range 46° C to 70° C) for 45 s and 72°C for 45 s with final extension at 72°C for 8 min. Products were resolved through 8% polyacrylamide gel electrophoresis (PAGE) and sized using silver staining. Various factors such as amount of PCR product, denaturing solution, voltage, running time, acrylamide: bisacrylamide ratio, acrylamide concentration and temperature were optimized and used for further studies. The gels were analyzed using gene tool software.

Genetic variation was quantified by calculating observed

Table 1. Bovine microsatellite markers tested for polymorphism in Indian yaks

Micro-satellite markers	Expected hetero-zygosity	Observed hetero-zygosity	PIC	No. of alleles	Heterozygote deficiency (F_{is})	Other studies (number of alleles)*			
						Ritz (1977)	Hanotte (2000)	Minqiang *et al.* (2003)	Nguyen *et al.* (2005)
ETH185	0.619	0.585	0.587	5	0.204	-	-	7	3
ETH225	0.659	0.689	0.619	6	–0.033	5	4	6	7
ETH152	0.628	0.677	0.621	6	–0.085	-	-	8	6
INRA005	0.599	0.562	0.581	5	0.207	-	-	7	5
TGLA126	0.601	0.528	0.579	5	0.082	5	-	4	5
TGLA227	0.457	0.516	0.479	4	–0.088	5	-	4	4
HEL9	0.689	0.527	0.617	5	0.146	-	-	8	5
ETH10	0.732	0.819	0.713	7	–0.124	5	3	-	9
BM2113	0.703	0.779	0.681	7	0.0218	-	4	8	7
BM1824	0.413	0.369	0.351	3	0.0515	5	4	6	4
HEL13	0.514	0.559	0.498	6	–0.075	-	-	4	6

*-, Not analyzed.

and effective number of alleles, observed heterozygosity, expected heterozygosity and within group heterozygotes deficiency (F_{IS}) were computed using POPGENE program (Yeh *et al*. 1999). PIC was calculated using the following formula suggested by Botstein *et al*. (1980). An exact test of POPGENE (Yeh *et al*. 1999) was done to determine the deviations from Hardy-Weinberg Equilibrium (HWE).

Out of 30 microsatellite markers tested on a panel of 9 animals, 27 (90%) successfully amplified yak genomic DNA, of which 23 (85%) were polymorphic with 2–7 allele numbers. Eleven microsatellites showing three and more distinct alleles per locus were further analyzed. The amplification results of 11 microsatellite loci tested on a panel of 50 animals are presented in Table 1. The marker BM1824 gave least alleles (3) while ETH10 and BM2113 showed 7 alleles. Hanotte (2000) observed only 3 alleles for ETH 10 while in the present study we observed 7 alleles. Across the 11 microsatellites studied, a total of 69 distinct alleles were detected. The PIC values were high (> 0.5) in most of the primers studied which proved the utility of genetic diversity analysis. Nguyen *et al*. (2005) also observed high heterozygosity with ETH10 in Swiss yaks. The results of the χ^2 test of Goodness of fit proved that majority of the loci showed significant chi square values suggesting departure from HWE. The observed heterozygosity (Ho) ranged from 0.369 to 0.819 while expected heterozygosity ranged from 0.413 to 0.732 (Table 1).

F_{is}, values were negative for five loci indicating an absence of inbreeding. Other loci gave F_{is} values ranging from 0.0218 to 0.207 indicating presence of varying degrees of inbreeding. The mean F_{is} value observed was 0.2959±0.108. The deviation from Hardy-Weinberg Equilibrium in majority of the loci could be due to reduction in population size. The investigation showed high heterozygosity and mild inbreeding in Indian yaks. The study validated the usefulness of the bovine microsatellite markers for genetic diversity analysis in yaks confirming that cattle microsatellite loci represent an important source of polymorphic markers for yak which is similar to the earlier observations of Ritz *et al*. (2000), Dorji *et al*. (2002) and Minqiang *et al*. (2003) on yaks from different geographical origin. Indian yaks had high allelic diversity indicating genetic variation. High PIC values justified the microsatellite markers selected for the study.

SUMMARY

Bovine microsatellite markers were used to assess their applicability for population genetic studies in Indian yaks. Out of 30 microsatellite markers tested 27 (90%) successfully amplified yak genomic DNA, of which 23 (85%) were polymorphic with allele number ranging from two to seven. A total of 69 alleles were detected. The Polymorphic Information Content (PIC) values were high (> 0.5) in most of the primers studied. The observed heterozygosity (Ho) in Indian yaks ranged from 0.369 to 0.819, while expected heterozygosity ranged from 0.413 to 0.732. The study validated the usefulness of the cattle microsatellite markers for genetic diversity analysis in yaks.

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