

Genetic characterisation of Jaisalmeri camel using microsatellite markers

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Six New World Camelidae microsatellite primer pairs were used to investigate the genetic polymorphism in Jaisalmeri camel. Polymerase chain reactions were carried out for 30 unrelated camels of Jaisalmeri breed. The amplification products were resolved in 6% (denaturing) urea PAGE and stained with silver nitrate. All the 6 microsatellite primer pairs were found polymorphic in Jaisalmeri camel. The number of alleles ranged from 2 to 5. The expected heterozygosity ranged from 0.32 to 0.651 and the polymorphic information content ranged from 0.268 to 0.588. The results indicated the utility of these microsatellite loci for studying genetic polymorphism in dromedary breeds.

Keywords: breeds, genetic characterisation, Jaisalmeri camel, microsatellite

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India has camel genetic resources consisting mainly of single humped Arabian camel (*Camelus dromedarius*) and a few double humped camel (*C. bactrianus*). The western part of India has three main breeds of which Jaisalmeri is well-known for riding and long distance travel in the hot arid desert. Conservation of genetic diversity is important for long-term genetic improvement to meet the requirement of growing population and unforeseen challenges arising through changing production systems and agro-climatic conditions. Characterisation of breeds/strains is the first step in the conservation programme.

There exists significant effect of breed on the production of hair, milk, draught potential, speed and stride¹⁻³. However, no significant differences could be detected by cytogenetic and biochemical studies^{4,7}. Earlier study using PCR-RAPD revealed high genetic similarity among the indigenous camel breeds⁸. The microsatellite markers are now considered as the most powerful genetic markers for characterisation of plant and animal genetic resources⁹.

Therefore, this study was planned to investigate the genetic variation in the Jaisalmeri breed of camel using 6 New World Camelidae microsatellite loci.

The blood samples were collected from unrelated camels of Jaisalmeri breed maintained at the National Research Center on Camel, Bikaner and from the breeding tract of the breed.

DNA was isolated using phenol-chloroform method¹⁰ with minor modifications and 6 New World Camelidae microsatellite primer pairs¹¹⁻¹³ were selected for the present study (Table 1). PCR amplifications were carried out in 25 µl reactions containing 50 ng DNA, 5 pmol each primer (Geneset, Singapore and Genei, India), 1.5 U Taq DNA polymerase, 2.5 mM each dNTP, 2.5 µl 10X Taq DNA polymerase buffer containing 10 mM Tris-HCl (pH 9.0), 1.5 mM MgCl₂, 50 mM KCl and 0.01% gelatin. The PCR amplification programme, performed on Eppendorf Mastercycler Gradient, consisted of an initial denaturation temperature of 95°C for 5 min, then 30 cycles at 94°C for 45 sec, 50-60°C for 1 min, depending on the primer pair used (Table 1), and 72°C for 1 min. Final extension was carried out at 72°C for 15 min. The microsatellite bands were resolved by 6% Urea Polyacryl Amide Gel Electrophoresis (Bio-Rad: Sequi-Gen® GT), stained with silver nitrate¹⁴ and sized using Gene Tools (Syngene) software. The allele frequency, expected heterozygosity and polymorphic information content were calculated¹⁵⁻¹⁶.

All the 6 microsatellite primer pairs used in the present investigation were found to produce polymorphic alleles in Jaisalmeri camel. The number of alleles, allele size range, annealing temperature, observed and expected heterozygosity along with the polymorphic information content are presented in Table 1. The number of alleles ranged from 2 to 5 at different microsatellite loci. Most polymorphic loci were VOLP-10 and YWLL-44 with 5 alleles at each locus. The expected heterozygosity ranged from 0.32 (YWLL-09) to 0.651 (VOLP-10) and the polymorphic information content ranged from 0.268 (YWLL-09) to 0.588 (VOLP-10).

All the six New World Camelidae microsatellite primer pairs successfully amplified the polymorphic DNA in Jaisalmeri camel (*C. dromedarius*). The present results suggest that a single panel of microsa-

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Table 1—Amplification of microsatellite loci in Jaisalmeri breed of camel (*C. dromedarius*) using New World Camelidae primers

Locus	Primer 5' → 3'	Alleles(n)	Size (bp)	Temp (°C)	H _o	H _e	PIC
VOLP-08	CCATTACCCCATCTCTC TCGCCAGTGACCTTATTTAGA	3	143-146	50	0.300	0.605	0.526
VOLP-10	CTTTCTCCTTTCTCCCTACT CGTCCACTTCCTTCATTTTC	5	250-265	55	0.630	0.651	0.588
YWLL-09	AAGTCTAGGAACCGGAATGC AGTCAATCTACACTCCTTGC	2	160-162	53	0.388	0.32	0.268
YWLL-44	CTCAACAATGCTAGACCTTGG GAGAACACAGGCTGGTGAATA	5	96-108	55	0.630	0.585	0.533
YWLL-58	GGCATCTCTTCTCATCAAT GACATCTCCAACCTTGAATC	3	173-176	51	1.00	0.597	0.516
YWLL-59	TGTGCAGGAGTTAGGTGTA CCATGTCTCTGAAGCTCTGGA	2	115-117	53	0.660	0.444	0.345

H_o, observed heterozygosity; H_e, expected heterozygosity

tellite primer pairs can be used for studying genetic diversity in closely related breeds and species of family Camelidae.

The number of alleles at different marker loci and their frequencies are simple indicators of the genetic variability. The number of alleles and allele size range observed in the present investigation is in close agreement with that of Jianlin *et al*¹³, who reported 3 (146-150 bp), 7 (250-268 bp), 3 (158-109 bp), 3 (105-109 bp) and 2 (109-111 bp) alleles (size range) respectively for VOLP-08, VOLP-10, YWLL-09, YWLL-44 and YWLL-59 locus in dromedary. Obreque *et al*¹² also reported 3 alleles each at VOLP-08(148-152 bp) and VOLP-10 (231-235 bp) locus in Alpacas. However, Lang *et al*¹¹ reported 9, 11, 6 and 10 alleles in Alpacas and Llamas at YWLL-09 (154-180 bp), YWLL-44 (86-120 bp), YWLL-58 (175-194 bp) and YWLL-59 (96-136 bp) locus, respectively. These results suggest the existence of good amount of genetic variation in Alpacas, Llamas and dromedary breeds.

In the present investigation, the expected heterozygosity was lowest (0.32) at the locus YWLL-09 followed by YWLL-59 with the expected heterozygosity of 0.44. In rest of the four microsatellite loci viz., VOLP-08, VOLP-10, YWLL-44 and YWLL-58, the expected heterozygosity was more than 58%. The observed and expected heterozygosity reported by Jianlin *et al*¹³ and Obreque *et al*¹² are similar to the present findings. The results indicate that the above 6 loci can very well be utilized for further genetic studies, which may include characterisation, conservation and production enhancement.

The Polymorphism Information Content (PIC) is another important measure of DNA polymorphism. Besides being a measure of genetic variation, it is also used in the context of gene mapping. The values of PIC are lower than heterozygosity for the corresponding marker because in PIC, a quantity is subtracted from heterozygosity that corresponds to the probability of offspring being uninformative. The PIC values ranged from 0.268 to 0.588 with more than 0.5 PIC values at four loci. The PIC values reported by Lang *et al*¹¹ at the YWLL-09, YWLL-44, YWLL-58 and YWLL-59 loci in Llamas and Alpacas are relatively higher due to more number of alleles at these loci. The present and the earlier results suggest the probable existence of relatively less genetic variation in dromedary as compared to Alpacas and Llamas at these loci. Nevertheless, the above markers, due to good number of alleles and enough heterozygosity, can be used to study the genetic diversity of the family Camelidae.

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