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MOLECULAR CHARACTERISATION OF KACHCHHI CAMEL (*Camelus dromedarius*) USING MICROSATELLITE MARKERS

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ABSTRACT

Molecular characterisation of Kachchhi camel was carried out using 16 microsatellite markers. A total of 74 blood samples were collected randomly from non-related animals and DNA was extracted. Sixteen microsatellite loci were amplified in a 5 multiplex PCR. Out of 16 microsatellite loci 14 were found to be polymorphic whereas, 2 were found monomorphic. The estimated mean allelic diversity was 3.1 (± 1.68), with a total of 51 alleles. The genetic variability within this breed was observed in terms of mean observed 3.18 ± 1.68 and effective number of alleles (2.0 ± 0.98), observed heterozygosity (0.36 ± 0.21), expected Nei's heterozygosity (0.421 ± 0.22). The PIC values ranged from 0.206 to 0.711. The Shanon's index ranged from 0.3960 to 1.5087 with mean 0.733 ± 0.450. These data were further used to verify if this population had undergone genetic bottleneck in the recent past. The result indicated existence of enough genetic variation and no bottleneck in this population.

Key words: Kachchhi camel, microsatellite markers, molecular characterisation

It is speculated that Kachchhi camel also declined steeply in last 10 years. As per the Livestock Census, 2007; there were 29,920 Kachchhi camels in the Gujarat state. However, some recent survey by local agency reports only 13,483 Kachchhi camels in the Kachchh district. The rapid decline in the Kachchhi camel population is attributed to several socio-economic reasons (Das *et al*, 2011) and is a cause of concern for the policy makers and the scientists. 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 is first step in the conservation programme. The microsatellite markers are considered as the most powerful genetic markers for characterisation of plant and animal genetic resources (Goldstein and Pollock, 1997). Several studies on establishing genetic relationships and differentiation based on microsatellite markers have been reported in livestock breeds, including camels (Arranz *et al*, 2001; Bjornstad and Roed, 2001; Fan *et al*, 2002; Ivankovic *et al*, 2002; Mburu *et al*, 2003; Vijh *et al*, 2007). The present study was planned to investigate the genetic variation in the Kachchhi breed of camel using sixteen microsatellite markers earlier used by Jianlin *et al* (2000).

Materials and Methods

Blood samples

A total of 74 blood samples were collected randomly from non-related animals belonging different areas of Kachchh district of Gujarat state aseptically into vaccutainers coated with EDTA (0.5 mM, pH 8.0).

Microsatellite loci

A total of 16 microsatellite markers - VOPL03, YWLL40, LCA66, LCA63, YWLL44, VOPL08, VOPL32, YWLL59, YWLL38, VOLP67, LCA59, LCA56, YWLL29, YWLL08, YWLL36 and VOPL10 were used to assess the extent of genetic variation in the Kachchhi camel.

DNA isolation and PCR based profiling

Genomic DNA was isolated from blood samples using standard phenol: chloroform extraction method (John *et al*, 1990). The purified DNA was quantified, aliquoted in small lots and stored at – 20°C for this study. PCR amplification was carried out in a final reaction volume of 25 µl using PCR Mastermix (MBI Fermentas) containing 0.05 U/µl Taq DNA polymerase (recombinant) in reaction buffer, $MgCl_2$ (4 mM) and dNTPS (0.4 mM of each) and 90-100ng DNA. Initially, all the 16 microsatellite markers

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were amplified individually in singleplex reaction for specific amplification. Later on, these markers were amplified in 5 multiplex PCR panels considering the annealing temperature of individual primers, dye label, amplification size and primer compatibility. The panels of microsatellite markers used are shown in table 1. The PCR amplification programme consisted of an initial denaturation temperature of 95°C for 5 min, followed by 35 cycles at 94°C for 45 sec, 55°C /53°C for 45 sec and 72°C for 45 sec. Final extension was carried out at 72°C for 10 min. The amplified products were sized by fragment analysis on ABI automated DNA sequence using GSLiz500 as size standard.

Computation and statistical analysis

The typing of individual animal for 16 microsatellite markers was carried out by Gene Mapper Software Version 4.1 (Applied Biosystem) and the Heterozygosity (Nei, 1978) and other genetic diversity variables were calculated using POPGENE computer package (Yeh *et al,* 1999). Polymorphism information content (PIC) values were calculated by using microsatellite tool kit. The probability of random mating in the population was estimated by Chi-square (χ^2) and likelihood ratio (G2) tests to examine Hardy-Weinberg equilibrium (HWE) at each locus. Bottleneck events in the population were tested

Table 1. List of microsatellite markers used in multiplex PCR.

Panel	Marker	5' to 3' sequence	5′ Label	Annealing temp.
Panel-1	VOPL03	F: AGACGGTTGGGAAGGTGGTA	HEX	55°C
		R: CGACAGCAAGGCACAGGA		
	YWLL40	F: CACATGACCATGTCCCCTTAT	TAMRA	55°C
		R: CCAGTGACAGTGTGACTAAGA		
	LCA66	F: GTGCAGCGTCCAAATAGTCA	TAMRA	55°C
		R: CCAGCATCGTCCAGTATTCA		
	LCA63	F: TTACCCAGTCCTTCGTGGG	FAM	55°C
		R: GGAACCTCGTGGTTATGGAA		
	YWLL44	F: CTCAACAATGCTAGACCTTGG	TAMRA	55°C
		R: GAGAACACAGGCTGGTGAATA		
Panel-2	VOPL08	F: CCATTCACCCCATCTCTC	FAM	55°C
		R: TCGCCAGTCACCTTATTTAGA		
	VOPL32	F: GTGATCGGAATGGCTTGAAA	FAM	55°C
		R: CAGCGAGCACCTGAAAGAA		
	YWLL59	F: TGTGCAGGAGTTAGGTGTA	FAM	55°C
		R: CCATGTCTCTGAAGCTCTGGA		
Panel-3	YWLL38	F: GGCCTAAATCCTACTAGAC	HEX	55°C
		R: CCTCTCACTCTTGTTCTCCTC		
	VOLP67	F: TTAGAGGGTCTATCCAGTTTC	ROX	55°C
		R: TGGACCTAAAAGAGTGGAG		
	LCA59	F: TGTGCAGGAGTTAGGTGTA	ROX	55°C
		R:CCATGTCTCTGAAGCTCTGGA		
	LCA56	F: ATGGTGTTTACAGGGCGTTG	ROX	55°C
		R: GCATTACTGAAAAGCCCAGG		
	YWLL29	F: GAAGGCAGGAGAAAAGGTAG	ROX	55°C
		R: CAGAGGCTTAATAACTTGCAG		
Panel-4	YWLL08	F: CCATTCACCCCATCTCTC	TAMRA	55°C
		R: TCGCCAGTCACCTTATTTAGA		
Panel-5	YWLL36	F: AGTCTTGGTGTGGTGGTAGAA	HEX	53°C
		R: TGCCAGGATACTGACATTCAT		
	VOPL10	F: CTTTCTCCTTTCCTCCCTACT	HEX	53°C
		R: CGTCCACTTCCTTCATTTC		

by the Bottleneck program (Cornuet and Luikart, 1996).

Results and Discussion

A total of 16 microsatellite markers earlier used and recommended by Jianlin *et al* (2000) were analysed. Out of the 16 microsatellite markers, 14 loci were found to be polymorphic whereas 2 loci YWLL40 and YWLL08 were monomorphic with a size of 172bp and 155 bp, respectively. The number of alleles in the polymorphic markers ranged from 2 (VOPL32, YWLL59, LCA56 and YWLL29) to 7 (VOPL10). The numbers of alleles, heterozygosity, Shannon diversity, FIS value and polymorphic information content (PIC) values for various microsatellites markers as well as, the Chi-square and likelihood ratio test performed to examine HWE at each locus are shown in table 2.

In the present work, all the 16 New World Camelidae microsatellite primer pairs were successfully amplified in 5 multiplex panels in Kachchhi camel. A total of 51 alleles were observed at 16 microsatellite loci. The number of alleles at different marker loci, their frequencies and heterozygosity are simple indicators of the genetic variability. YWLL40 and YWLL36 microsatellite loci were found monomorphic which is in agreement with earlier observation on Kachchhi camel breed by Mehta *et al* (2007), who additionally also found YWLL29 microsatellite loci monomorphic. The observed and expected mean number of alleles (MNA) were 3.18 and 2.06, respectively. Comparable estimates are observed in other dromedary camel breeds e.g. 2-5 alleles in Jaisalmeri Indian camel (Gautam *et al,* 2004), 2-7 alleles in Bikaneri Indian camel (Mehta *et al,* 2007), 4-6 alleles in Baladi, Somali, Sudani, Maghrabi and Mowallad camels (Karima *et al,* 2011). However, Vijh *et al* (2007) observed higher genetic variability in 4 Indian breeds including Kachchhi (Kutchi) breed. They observed 8.04, 7.30, 6.39, and 7.43 MNA for Bikaneri, Jaisalmeri, Kutchi and Mewari Indian camel breeds. These microsatellites have exhibited higher number of alleles (2 to 19) in South American dromedary camelids including tulu (♂ bactrian camel × ♀ dromedary) (Sasse *et al,* 2000). This New World Camelidae series of microsatellites are considered as suitable microsatellites for Old World Camelidae (Jianlin *et al,* 2000; Mburu *et al,* 2001; Al-Swailem *et al,* 2009). However, they have invariably given less

Table 2. Observed (no) and effective (ne) number of alleles, allele size range, observed heterozygosity (Ho), expected heterozygosity (He), polymorphism information content (PIC), Shannon diversity Index (I), FIS value and Chi-square and G square probability across 16 microsatellite loci in Kachchhi camel.

Microsatellite Locus	Observed alleles (Na)	Effective alleles (Ne)	Allele size range (bp)	Observed Het (Ho)	Nei's Het Expected (He)	PIC	Shannon diversity Index (I)	FIS value	Chi-square Probability	G-square Probability
VOPL03	5	1.43	143-171	0.1757	0.3007	0.2887	0.6683	0.4157	0.000	0.000
YWLL40	1	1.00	172	0	0	0	0	-	-	-
LCA66	5	3.97	237-245	0.5676	0.7486	0.711	1.4757	0.2419	0.000	0.000
LCA63	4	3.84	214-222	0.7568	0.7400	0.692	1.3651	-0.0226	0.668	0.659
YWLL44	3	1.92	109-113	0.4459	0.4798	0.402	0.7819	0.0706	0.118	0.396
VOPL08	3	1.50	143-149	0.3514	0.3346	0.297	0.5955	-0.0499	0.747	0.546
VOPL32	2	1.76	262-264	0.4730	0.4334	0.34	0.6250	-0.0912	0.466	0.461
YWLL59	2	1.68	104-106	0.4595	0.4065	0.324	0.5965	-0.1303	0.286	0.274
YWLL38	5	2.83	178-190	0.6081	0.6472	0.576	1.1337	0.0604	0.005	0.013
VOLP67	4	2.08	148-158	0.1081	0.5205	0.411	0.8114	0.7923	0.000	0.000
LCA59	3	1.74	106-110	0.4324	0.4255	0.346	0.6635	-0.0163	0.000	0.006
LCA56	2	1.54	133-135	0.2973	0.3539	0.291	0.5389	0.1600	0.149	0.164
YWLL29	2	1.61	208-210	0.4054	0.3817	0.309	0.5696	-0.0622	0.164	0.627
YWLL08	1	1.00	155	0	0	0	0	-	-	-
YWLL36	2	1.30	109-113	0.2432	0.2337	0.206	0.3960	-0.0406	0.767	0.760
VOPL10	7	3.70	248-264	0.5000	0.7304	0.689	1.5087	0.3154	0.000	0.000
Mean	**3.1875**	**2.0606**		**0.3640**	**0.4210**		**0.7331**	**0.1354**		
SD	**1.6820**	**0.9840**		**0.2149**	**0.2291**		**0.4496**			

number of alleles in dromedary camel than llamas and alpacas, where numbers of alleles for most of these microsatellites are reported to be much higher (Lang *et al,* 1996; Obreque *et al,* 1998; Penedo *et al,* 1999; Sarno *et al,* 2000).

Present investigation includes 4 micro-satellites not recommended by FAO-DAD (LCA66, VOLP67, YWLL29 and YWLL36) however, surprisingly two of them (LCA66, VOLP67) were the most polymorphic among the investigated.

As a measure of deviation from HW equilibrium the Chi-square and likelihood ratio test were performed which showed a total of 6 loci with P-value indicating deviation from HW expectations at the level of 5% or lower. The FIS values for these marker loci were positive except LCA59 (-0.0163). The mean FIS value of 0.1354 indicates sizable level of inbreeding in this breed.

The observed heterozygosity is based on the number of heterozygous individuals in the population under investigation while, the expected heterozygosity depends on the number of alleles and their frequency in a population at a particular locus. Although the range is wide, the mean observed and expected heterozygosity were 0.364 and 0.421, respectively.

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 0.206 to 0.711 with PIC more than 0.50 at only 4 loci. The PIC values reported in New World Camelids are relatively higher due to more number of alleles at these loci (Lang *et al,* 1996; Obreque *et al,* 1998; Penedo *et al,* 1999).

Camelid breeds are less explored with respect to molecular investigations and there are only few published reports on camel genetic diversity using microsatellites. The low MNA and narrow allele size range observed in the present investigation indicates lower genetic variability of the breed. The less genetic variation observed in this breed we believe, is not the characteristics of this breed but is probably due to choice of less polymorphic markers as several other studies have also reported comparable number of alleles and other parameters like size range, mean number of alleles (MNA), % Heterozygosity etc in the Indian and other dromedary. Indian camels have shown higher estimates of genetic variability when other series of microsatellite were used (Mehta SC, personal communication).

Since the population of Kachchhi camel breed has gone down drastically, genetic effects of reduction in population size require evaluation. The BOTTLENECK program was used to test for genetic bottleneck in the recent breeding history of this breed (Cornuet and Luikart, 1996). Under the assumption of the stepwise mutation model (SMM), the most suitable model for microsatellite evolution, neither the sign and standardised differences tests nor the Wilcoxon Signed Rank Test revealed any significant result ($p > 0.05$, Table 3). These findings indicated the absence of genetic bottleneck in the investigated population, and the population can be considered in mutation drift equilibrium. However, the typical L-like distribution of the allele frequencies (Fig 1) was not observed.

Table 3. Mutation-drift equilibrium test under SMM mutation models in Kachchhi camel population.

Sign Test	Standardisation Differences Test	Wilcoxon Rank Test
Hee = 7.56	T2 =0.150	P (One tail for H deficiency): 0.82123
Hd =6	P =0.44032	P (One tail for H excess): 0.19550
He =8		P (two tail for H excess or deficiency): 0.39099
P =0.51647		

Hee = Expected number of loci with heterozygosity excess. Hd = heterozygosity deficiency. He = heterozygosity excess.

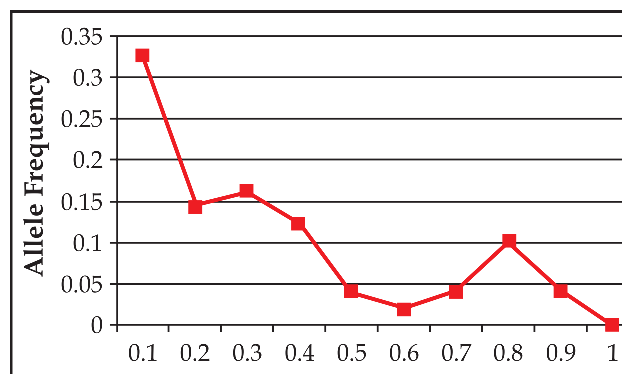

Fig 1. Kachchhi camel population showing normal L shaped curve under bottleneck analysis.

The present study contributes to the knowledge of population structure and assessment of existing genetic diversity in the Kachchhi camel population. Further, genetic analysis of other Indian camel and their comparisons need to be carried out to determine the phylogenic evolutionary relationships and genetic distances among the indigenous camel breeds.

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References

Al-Swailem AM, Shehata MM, Al-Busadah KA, Fallatah MH and Askari E (2009). Evaluation of the genetic variability of microsatellite markers in Saudi Arabian camels. International Journal of Food, Agriculture and Environment 7:636-639.

Arranz JJ, Bayon Y and San-Primitivo F (2001). Genetic variation at microsatellite loci in Spanish sheep. Small Ruminant Research 39:3-10.

Bjornstad G and Roed KH (2001). Breed demarcation and potential for breed allocation of horses assessed by microsatellite markers. Animal Genetics 32:59-65.

Cornuet JM and Luikart G (1996). Descripion and power analysis of two tests for detecting recent population bottleneck from allele frequencies data. Genetics 144: 2001-2014.

Das, sabyasachi, Mehta, Umesh and Patel, Jatin (2011). Kachchhi and Khariya camel: present status, challenges and future prospects. pp. 17-23. In Proceedings of the Swarnim Gujarat Camel Seminar on Technology Interventions to Enhance Camel Productivity organized by Anand Agricultural University at Bhuj on 13th March, 2011, Gujarat, India.

Fan B, Wang ZG, Li YJ, Zhao XL, Liu B, Zhao SH, Yu M, Li MH, Chen SL, Xiong TA and Li K (2002). Genetic variation analysis within and among Chinese indigenous swine populations using microsatellite markers. Animal Genetics 33:422-427.

Gautam L, Mehta SC, Gahlot RS and Gautam K (2004). Genetic characterisation of Jaisalmeri camel using microsatellite markers. Indian Journal of Biotechnology 4:457-59.

Goldstein DB and Pollock DD (1997). Launching microsatellite: A review of mutation processes and method of phylogenetic inferences. Journal of Heredity 88:335-42.

Ivankovic A, Kavar T, Caput P, Mioc B, Pavic V and Dovc V (2002). Genetic diversity of three donkey populations in Croatian coastal region. Animal Genetics 33b:169-177.

Jianlin H, Mburu D, Oching J, Kaufmann B, Rega JEO and Hanotte O (2000). Application of New World Camelidae microsatellite primers for amplification of polymorphic loci in Old World Camelids. Animal Genetics 31:404-419.

John SWM, Weitzner G, Rozen R and Scriver CR (1990). A rapid procedure for extracting genomic DNA from leukocytes. Nucleic Acids Research 19(2):408.

Karima FM, Hassan AIR, Sekena HA, Mohamed AEM and Dalia MH (2011). Genetic variations between camel breeds using microsatellite markers and RAPD techniques. Journal of Applied Biosciences 39:2626-2634.

Lang KDM, Wang Y and Plante Y (1996). Fifteen polymorphic dinucleotide microsatellites in llamas and alpacas. Animal Genetics 27:293.

Livestock census (Department of Agricultural Research and Education, Ministry of Agriculture, Government of India), 2007.

Mburu DN, Ochieng JW, Jianlin H, Kaufmann B, Kuria SG, Rege JEO and Hanotte O (2001). Genetic diversity and relationship of indigenous Kenyan camel breeds: preliminary results. In: The Challenges of Drought to Livestock Production in Kenya (Ed. S.A. Abdulrazak, D. Mwangi and E.A. Mukisira), pp. 151–4. Proceedings of the Animal Production Society of Kenya. Symposium Held at Egerton University, Njoro, 7–8 March 2001, APSK, Nairobi, Kenya.

Mehta SC, Goyal A and Sahani MS (2007). Genetic differentiation of Indian camel breeds using random oligonucleotide primers. Indian Journal of Biotechnology 6:336-339.

Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Obreque V, Coogle L, Henney PJ, Bailey E, Mancilla R, García-Huidobro J, Hinrichsen P and Cothran EG (1998). Characterisation of 10 polymorphic alpaca dinucleotide microsatellites. Animal Genetics 29:461-2.

Penedo MCT, Caetano AR and Cordova K (1999). Eight microsatellite markers for South American camelids. Animal Genetics 30:161-168.

Sarno RJ, David VA, Franklin WL, Obrien SJ and Johnson WE (2000). Development of microsatellite markers in the guanaco, *Lama guanicoe*: utility for South American camelids. Molecular Ecology 9:1919-1952.

Sasse J, Mariasegaram M, Babu R, Kinne J and Wernery U (2000). South American camelid microsatellite amplification in *Camelus dromedarius*. Animal Genetics 31:33

Vijh RK, Tantia MS, Mishra B and Bharani ST (2007). Genetic diversity and differentiation of dromedarian camel of India. Animal Biotechnology 18(2):81-90.

Yeh FC, Boyle T, Rongcal Y, Ye Z and Xian JM (1999). POPGENE version 3.31, a Microsoft Windows based free ware for population genetic analysis. University of Alberta, Edmonton.