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Microsatellite analysis generates hope for sustainability of two dwindling camel populations of Rajasthan

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ABSTRACT

The declining camel population in the country is a matter of major concern for the conservation biologist, policy makers and the state governments. In the present study, diversity status of two declining camel populations of India, viz. Mewari and Jalori was established using 25 microsatellite markers. Analysis of genotype data showed that sufficient amount of genetic variation is maintained in these camel populations. A total of 174 alleles were detected in Mewari and 155 in Jalori camel. Highest number of alleles (17) was observed at CMS58 locus in Mewari and CVRL01 and YWLL08 in Jalori camel. The mean observed number and effective number of alleles across all the loci was 9.67±0.94, 4.52±0.46 and 8.61±0.86, 4.41±0.46 for Mewari and Jalori, respectively. Difference in the observed and expected number of alleles in both the populations suggested presence of several low frequency alleles in these populations. In accordance with high allelic diversity, estimate of observed heterozygosity (Ho) was also high (0.68±0.04 and 0.71±0.04 for Mewari and Jalori, respectively). Nine loci in Mewari and 10 in Jalori deviated from HWE. Observed and expected heterozygosity were of similar magnitude and correspondingly F_{IS} analysis identified no significant heterozygote deficiency. Both the populations did not suffer from any recent genetic bottleneck. Distinctness of two populations was supported by the observation that all the Jalori and Mewari animals except one were assigned to their own populations. Bayesian approach also concluded that the two populations were distinct. Abundant genetic variation maintained in these camel populations provides important inputs for the decision-making process regarding their conservation and improvement.

Key words: Bottleneck, Genetic diversity, India, Jalori, Mewari, Microsatellite, Polymorphism

Camel, an important component of desert ecosystem, is primarily reared for carting, agricultural operation and transportation in addition to the secondary utility of milk and hair production (Saini *et al.* 2006). Camel population of India is 7th in the world (19th Livestock census 2012). India mainly possesses dromedary camel (*Camelus dromedarius*), which are confined to the north-western part of the country. There are 9 registered breeds of indigenous camel, viz. Jaisalmeri, Jalori, Bikaneri, Kutchi, Malvi, Marwari, Kharai, Mewari and Mewati (NBAGR 2016). Out of these, Bikaneri, Jaisalmeri, Kutchi and Mewari are major breeds on the basis of population numbers.

Camel husbandry is a major economic activity of the rural people, especially in the Rajasthan which is home for 80% of India's camels. The shrinkage of range lands and its deterioration in terms of number of trees, herbs and shrubs has created considerable economic pressure on traditional camel herders. They are forced to shift camel rearing system from extensive to semi-intensive and to intensive system which is not economical.

Camel population is declining continuously and the current population is 0.40 million (19th Livestock Census 2012). Camel population in the state had fallen from 4,21,836 in 2007 to 3,25,713 in 2012, registering a drop of 22.79%. Mewari and Jalori are two such camel populations with declining number (Mehta and Dahiya 2017). Genetic diversity is equated with adaptive potential and hence is important for the long-term persistence of such populations (Kahilainen *et al.* 2014). Genetic variation reflects evolvability and adaptive potential of the population in the long-term due to its link with effective population size (Lanfear *et al.* 2014). Consequently, reduced intra-population genetic diversity is related to increased risk of extinction in natural populations. Since the ultimate goal of conservation is to ensure the long-term persistence of populations, estimation of existing genetic diversity is the first step for planning any such program.

Microsatellites are powerful DNA markers for quantifying genetic variations within and between populations of a species. Microsatellites are codominant in

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nature, highly polymorphic, easily typed, and Mendelian inherited (Sheriff and Alemayehu 2018). PCR for microsatellites can be automated for identifying simple sequence repeat polymorphism. Most of the microsatellites are non-coding, and therefore variations are independent of natural selection. These properties make them ideal genetic markers for conservation genetics and livestock management. Microsatellite marker analysis provides essential information for formulating meaningful conservation strategies for populations. This along with the other technologies like programmed breeding and assisted reproductive technologies can be integrated into a package for conserving genetic diversity and rehabilitation of the natural populations of camel.

Various authors have reported microsatellite sequences and polymorphisms in camel for population genetic analysis (Eltanany *et al.* 2015). Limited studies describing the genetic variation in indigenous camel include Bikaneri, Kutchi, Jaisalmeri and Mewari (Vijh *et al.* 2007). Additionally, a study by Mehta *et al.* (2014) reported genetic bottleneck in all these four breeds based on microsatellite markers. Thus, in this study genetic variation, inbreeding depression and genetic bottleneck that reduce survival, reproduction, and ultimately fitness of a population was established for Jalori camel of Rajasthan. Mewari camel was included to pin point current scenario of diversity as its population decline is continuing at an alarming rate.

MATERIALS AND METHODS

Blood sample collection: Blood samples of 48 animals each belonging to Mewari and Jalori were collected randomly from their breeding tract. The breeding tract of Mewari camel extends in east from 76°73’ to 72°80’ longitude and in north from 22°55’ to 25°42’ latitude with average annual rainfall ranging from 60 to 80 cm. It encompasses the *Mewar* area, i.e. Udaipur, Chittorgarh, Rajsamand, Pratapgarh, Dungapur, Banswara, Bhilwara districts, and *Hadoti* area, i.e. Kota, Bundi, Barane and Jhalawar districts of Rajasthan. The geographical distribution of Jalori breed encompasses chiefly the Jalori and Sirohi disctricts of Rajasthan. It extends in east from 72°58’ to 71°3’ longitude and in north from 24°22’ to 25°22’ latitude with average annual rainfall ranging from 40 to 58 cm. All efforts were made to collect blood samples from true to the breed type animals distributed throughout the breeding tract. The samples were collected in EDTA containing vacutainer tubes and transported to lab at 4°C in the shortest possible time. DNA was extracted from whole blood using standard phenol-chloroform method (Sambrook *et al.*1989). The resulting DNA strands were spooled out and washed twice with ice cold 70% ethanol to remove excess salts. DNA was re-dissolved in 300–450 µl of TAE buffer (pH 8.0). The quality and concentration of DNA were checked on 0.6% agarose gel as well as by nanodrop spectrophotometer.

Microsatellite markers and genotyping: Twenty five microsatellite loci spread across the camelid genome were selected for microsatellite genotyping (Table 1). These markers have previously been demonstrated to be polymorphic in different Indian camel breeds (Vijh *et al.* 2007, Mehta *et al.* 2014). PCR was performed in a total reaction volume of 10 µl for all the selected loci in 96 well plates containing 10–20 ng of genomic DNA and 0.2 µM of each primer. To reduce the possibility of cross contamination and variation in the amplification reactions, master mix containing all PCR reagents including the *DreamTaq* polymerase enzyme, 0.2 mM of each dNTP and 2 mM of $MgCl_2$ except DNA template and primers was used. The amplification program was performed using the thermocycler under following conditions: initial denaturation at 94°C for 5 min, 40 cycles of denaturation at 94°C for 2 min, primer annealing at specific annealing temperature of microsatellite primer-pair (Table 1) for 45 sec, and extension at 72°C for 1 min. Final extension was performed at 72°C for 10 min. Fluorescently-labelled DNA fragments were analyzed on Applied Biosystems 3130XL Genetic Analyzer. Each PCR reaction consisted of GeneScan® LIZ 500 molecular weight standard. The fragment sizes were estimated by GeneScan analysis software (Applied Biosystems, USA) and extraction of allele size was done with the Gene Mapper 3.0 software. The extracted data was analyzed to estimate genetic diversity.

Diversity estimation and statistical analysis: Observed (Na) and effective numbers of alleles (Ne), observed (Ho) and expected heterozygosity (He), and heterozygote deficit (F_{IS}) per locus across breeds and markers was estimated by GenAlEx 6.5 software (Peakall and Smouse 2006). The tests for deviation from Hardy-Weinberg equilibrium (HWE) were derived. Analysis of Wright’s fixation indices (F_{IS}, F_{IT} and F_{ST}) were measured according to Weir and Cockerham (1984). Population assignment was performed using multilocus genotypes of individuals as implemented in GenAlEx 6.5. STRUCTURE software (Pritchard 2009) was used to study the breed structure and stratifications using genotype data. An admixture model was applied with correlated allele frequencies with K=2. There were 20 runs for each K value used. The number of iterations in each run was 10,000 in Burn-in, followed by 50,000 iterations of Markov Chain Monte Carlo length (MCMC). The clustering pattern was graphically displayed using DISTRUCT software (Rosenberg 2004).

Bottleneck events in the population were tested by two methods. The bottleneck hypothesis was investigated using Bottleneck v1.2.02 (http://www.ensam.inra.fr/URLB). The bottleneck compares heterozygosity expected at HWE to the heterozygosity expected at mutation drift equilibrium. The first method consisted of three excess heterozygosity tests developed by Cornuet and Luikart (1996) like sign test, standardized differences test and a Wilcoxon sign rank test. The probability distribution was established using 1,000 simulations based on allele frequency and heterozygosity under three models namely infinite allele model (IAM), stepwise mutation model (SMM) and two-

Table 1. Characteristics of the primers for 25 microsatellite loci tested in Mewari and Jalori camel

Locus	Sequence (5'-3')	Dye	Annealing temperature (T_m)	Observed size range (bp)
LCA 77	F-TGTTGACTAGAGCCTTTTCTTCTTT R-GGGCAAGAGAGACTGACTGG	PET	55°C	208–224
VOLP 32	F-GTGATCGGAATGGCTTGAAA R-CAGCGAGCACCTGAAAGAA	FAM	60°C	165–241
VOLP 03	F-AGACGGTTGGGAAGGTGGTA R-CGACAGCAAGGCACAGGA	HEX	60°C	128–174
CVRL 06	F-TTTTAAAAATTCTGACCAGGAGTCTG R-CATAATAGCCAAAACATGGAAACAAC	PET	60°C	182–202
CVRL 07	F-AATACCCTAGTTGAAGCTCTGTCCT R-GAGTGCCTTTATAAATATGGGTCTG	HEX	60°C	274–306
CMS13	F-TAGCCTGACTCTATCCATTTCTC R-ATTATTTGGAATTCAACTGTAAGG	NED	58°C	226–252
LCA63	F-TTACCCAGTCCTTCGTGGG R-GGAACCTCGTGGTTATGGAA	HEX	58°C	170–242
LCA18	F-TCCACCCATTTAGACACAAGC R-TAGGAAGCTCCAAGAAGAAAAGAC	FAM	60°C	191–233
YWLL 44	F-CTCAACAATGCTAGACCTTGG R-GAGAACACAGGCTGGTGAATA	PET	60°C	89–107
LCA 37	F-AAACCTAATTACCTCCCCCA R-CCATGTAGTTGCAGGACACG	PET	55°C	99–173
CVRL08	F-AATTCCTGTGATTTTATACACA CATGTCATGAAAGCTACAGTA	PET	60°C	171–211
VOLP08	F-CCATTCACCCCATCTCTC R-TCGCCAGTGACCTTATTTAGA	FAM	58°C	106–172
CMS50	F-TTTATAGTCAGAGAGAGTGCTG R-TGTAGGGTTCATTGTAACA	NED	60°C	150–190
CVRL05	F-CCTTGGACCTCCTTGCTCTG R-GCCACTGGTCCCTGTCATT	HEX	60°C	151–191
CVRL04	F-CCCTACCTCTGGACTTTG R-CCTTTTTGGGTATTTTCAG	FAM	58°C	160–174
CMS 58	F-AATATACATCCTCCCAACTGGT R-TTATTTCTCTTAACCCCTCTCTAA	NED	58°C	80–138
CVRL 01	F-GAAGAGGTTGGGGCACTAC R-CAGGCAGATATCCATTGAA	HEX	58°C	178–236
YWLL 08	F-ATCAAGTTTGAGGTGCTTTCC R-CCATGGCATTGTGTTGAAGAC	FAM	60°C	102–170
CMS 16	F-ATTTTGCAATTTGTTCGTTCTTTC R-GGAGTTTATTTGCTTCCAACACTT	NED	60°C	181–189
YWLL 38	F-GGCCTAAATCCTACTAGAC R-CCTCTCACTCTTGTTCTCCTC	HEX	60°C	173–189
YWLL 09	F-AAGTCTAGGAACCGGAATGC R-AGTCAATCTACACTCCTTGC	FAM	60°C	127–221
VOLP67	F-TTAGAGGGTCTATCCAGTTTC R-TGGACCTAAAAGAGTGGAG	PET	58°C	82–186
VOLP 10	F-CTTTCTCCTTTCCTCCCTACT R-CGTCCACTTCCTTCATTTC	PET	58°C	208
LCA66	F-GTGCAGCGTCCAAATAGTCA R-CCAGCATCGTCCAGTATTCA	FAM	58°C	208–314
LCA 90	F-TATAACCCTGGTCTCGCCAA R-CCAAGTAGTATTCCATTATGCG	FAM	58°C	–

-, not amplified

phase model of mutation (TPM). The second method was the graphical representation of the mode-shift indicator (Luikart and Cornuet 1998) originally proposed by Luikart *et al.* (1998). Loss of rare alleles in bottlenecked populations was detected when one or more of the common allele classes have a higher number of alleles than the rare allele class.

RESULTS AND DISCUSSION

Genetic variation within populations: Microsatellite locus LCA90 did not amplify with DNA samples of both the breeds. Further, microsatellite loci which were amplified in all the 48 samples of both the breeds and had minimum of four alleles were only considered for analyses of genetic diversity as well as for the distinction of two camel breeds. Thus LCA77, VOLP10, VOLP67, LCA66, YWLL09 and CVRL08 were also excluded from the original list of 25 markers (Table 1). Remaining 18 microsatellites were considered for further analyses. These results are in accordance with the observations of Mehta *et al.* (2014) that out of 40 microsatellite loci, only 20 were polymorphic in Rajasthan camel breeds. The parameters and indices of genetic diversity among the marker loci and within two populations of Mewari and Jalori are presented in Table 2. Total number of observed alleles (Na) was 174 in Mewari and 155 in Jalori camel. Correspondingly, much higher mean number of alleles (MNA) was recorded in Mewari (9.67±0.94) as compared to the Jalori camel (8.61±0.86). Private alleles were also recorded in both the populations but their frequency was less than 5%. Mewari and Jalori showed almost similar mean values of the expected number of alleles (Ne), with means of 4.52±0.46 and 4.41±0.46, respectively. Among loci, CMS58 displayed the highest (17) and VOLP08 displayed the lowest (4) number of observed alleles in Mewari camel. Expected number of alleles (Ne) varied from 1.51 (VOLP08) to 9.2 (YWLL08). A different set of markers occupied the highest and lowest position in Jalori camel with CVRL01 and YWLL08 having highest (17) and CVRL04 having the lowest (4) observed number of alleles. Whereas, the scenario for expected number of alleles was similar to that observed in Mewari camel (Table 2). Large number of alleles at low frequency was responsible for the significantly less effective numbers of alleles than the number of actually observed alleles (Table 2).

Among loci, VOLP08 and YWLL08 displayed the lowest and highest estimates, respectively for polymorphism index in both the breeds (Table 2).The mean value of Shannon's information index (I) was 1.69±0.11 in Mewari and 1.61±0.10 in Jalori with YWLL08 emerging as the most informative marker (I>2.4). The observation is in accordance with the previously published report on four breeds (Bikaneri, Kutchi, Jaisalmeri and Mewari) of Indian dromedary camel (Vijh *et al.* 2007), where VOLP08 was least polymorphic and locus YWLL08 was most polymorphic with 25 alleles. Large values of I for selected markers in both the breeds confirmed that this set of markers can potentially be used for diverse genetic applications such as linkage mapping, individual identification and parentage

Table 2. Microsatellite diversity estimates in two camel breeds

	Mewari camel								Jalori camel							
Marker	Na	Ne	I	Ho	He	F	χ^2	Prob	Na	Ne	I	Ho	He	F	χ^2	Prob
VOLP32	15	6.95	2.31	0.73	0.86	0.15	122.907	0.112	6	2.55	1.23	0.65	0.61	-0.06	34.279	0.003**
VOLP03	13	4.15	1.84	0.93	0.76	-0.23	43.458	0.999	8	5.55	1.82	0.96	0.82	-0.17	68.246	0.000***
CVRL06	6	2.54	1.14	0.38	0.61	0.38	29.664	0.013*	6	2.74	1.18	0.81	0.64	-0.28	17.827	0.272
CVRL07	10	5.29	1.93	0.84	0.81	-0.04	35.299	0.85	10	6.54	2.02	0.9	0.85	-0.06	56.208	0.122
CMS13	8	3.88	1.62	0.65	0.74	0.12	24.273	0.667	7	3.51	1.5	0.65	0.72	0.09	57.096	0.000***
LCA63	6	3.84	1.49	0.64	0.74	0.13	43.669	0.000***	8	5.5	1.83	0.58	0.82	0.29	104.858	0.000***
LCA18	10	5.59	1.96	0.48	0.82	0.41	150.465	0.000***	8	4.35	1.67	0.94	0.77	-0.23	50.579	0.006**
YWLL44	8	2.71	1.37	0.48	0.63	0.24	105.4	0.000***	5	2.48	1.16	0.54	0.6	0.09	10.739	0.378
LCA37	8	4.34	1.65	0.73	0.77	0.06	41.753	0.046*	8	4.46	1.69	0.53	0.78	0.32	85.063	0.000***
VOLP08	4	1.51	0.68	0.54	0.34	-0.62	5.604	0.469	6	1.58	0.79	0.53	0.37	-0.46	66.981	0.000***
CMS50	14	6.22	2.1	0.81	0.84	0.03	70.437	0.946	11	4.69	1.85	0.93	0.79	-0.19	29.806	0.998
CVRL05	13	3.82	1.83	0.81	0.74	-0.1	77.553	0.493	12	5	1.96	0.93	0.8	-0.17	36.292	0.999
CVRL04	7	3.26	1.39	0.77	0.69	-0.11	60.174	0.000***	4	2.87	1.17	0.4	0.65	0.39	56.404	0.000***
CMS58	17	7.48	2.36	0.83	0.87	0.05	193.424	0.001***	8	4.36	1.65	0.7	0.77	0.1	54.509	0.002**
CVRL01	6	4.09	1.59	0.47	0.76	0.38	37.708	0.001***	17	7.41	2.3	0.51	0.86	0.41	287.492	0.000***
YWLL08	16	9.2	2.42	0.87	0.89	0.02	136.305	0.147	17	9.36	2.46	0.87	0.89	0.02	142.985	0.324
CMS16	6	3.11	1.32	0.58	0.68	0.14	51.151	0.000***	6	3.06	1.28	0.6	0.67	0.1	9.234	0.865
YWLL38	7	3.43	1.42	0.7	0.71	0.02	23.366	0.325	8	3.45	1.42	0.67	0.71	0.05	28.587	0.434
Mean	9.67	4.52	1.69	0.68	0.74	0.06			8.61	4.41	1.61	0.71	0.73	0.01		
SE	0.94	0.46	0.11	0.04	0.03	0.06			0.86	0.46	0.1	0.04	0.03	0.06		

Na = No. of different alleles; Ne = No. of effective alleles = 1 / (Sum π^2); I = Shannon's Information Index = –1* Sum ($\pi \times$ Ln (π)); where π is the frequency of the ith allele for the population; Ho = Observed heterozygosity = No. of Hets / N; He = Expected heterozygosity = 1 - Sum π^2; uHe = Unbiased expected heterozygosity = (2N / (2N–1)) *He; F = Fixation Index = (He – Ho) / He = 1 – (Ho/He); *P<0.05, **P<0.01, ***P<0.001.

testing in camel populations. The observed and the expected heterozygosity on the basis of allele frequency also gave almost similar values for both the breeds. The mean observed heterozygosity (Ho) for all the 18 loci in Mewari camel was 0.68±0.04 while the mean expected heterozygosity (He) was 0.74±0.03. Similarly, Jalori camel had mean values of 0.71±0.04 and 0.73±0.04 for Ho and He, respectively (Table 2).

Diversity parameters estimated for Mewari camel were different from the previously reported values (Vijh *et al.* 2007, Mehta *et al.* (2014). As per the diversity estimates published by Mehta *et al.* (2014), least genetic variation was observed in the Mewari camel as compared to the other three camel breeds, viz. Bikaneri, Jaisalmeri and Kacchi of India. The observed number of alleles ranged from two to five in Mewari breed with an average of 3.1±0.19. The observed heterozygosity ranged from 0.14 to 0.83 with the average expected heterozygosity of 0.51±0.03. Maximum number of alleles observed for a locus (17) as well as MNA (9.67±0.94) was much higher in the Mewari population in the present study (Table 2). Differences in the number of alleles and their frequencies were expected due to the difference in the microsatellite markers used as well as the technique of genotyping. Results of Mehta *et al.* (2014) were based on 6% Urea polyacrylamide gel electrophoresis and silver staining whereas, automated genotyping on a DNA sequencer was employed in current investigation. Our estimations are closer to that reported by Vijh *et al.* (2007), where automated allele genotyping was done. They reported substantial heterozygosity in the four Indian camel breeds with mean heterozygosity of 0.58, 0.57, 0.56, and 0.60 for Bikaneri, Jaisalmeri, Kutchi, and Mewari camel breeds, respectively.

MNA in Mewari (9.67±0.94) and Jalori (8.61±0.86) correspond to the 9.27 alleles per locus in Saudi Arabian camel populations namely, viz. Magaheem, Maghateer, Sofr and Shual (Mahmoud *et al.* 2012). The MNA in camel ecotypes of Sudan was 8.58±0.91 (Eltanany *et al.* 2015). The MNA in Mewari and Jalori camel was much higher than that is found within four Saudi Arabian camel populations (Mahmoud *et al.* 2012, 2013), Tunisian (Ould Ahmed *et al.* 2010) and Egyptian dromedary populations (Karima *et al.* 2011) which may be attributed to the fewer loci in their studies. The heterozygosity values in Mewari (0.68±0.04) and Jalori (0.71±0.04) camel were also comparable to the dromedary population of other countries such as Saudi Arabian camels (0.605–0.665) (Mahmoud *et al.* 2012) and South African (0.60) and Sudanese camel (0.68) (Nolte *et al.* 2005). Much lower estimates have been described for Tunisian camels (0.460) (Ould Ahmed *et al.* 2010), and Australian camels (0.455) (Spencer and Woolnough 2010).

Nine loci in Mewari and 10 in Jalori population deviated (P<0.01) from HWE, respectively (Table 2). Migration, mutation, non random mating, genetic drift and both artificial and natural selection are factors that are known to cause deviations from HWE. Inbreeding and loss of genetic diversity are expected to be encountered in small and/or declining populations. However, non random mating was ruled out by the absence of heterozygote deficiency in the two populations. On the contrary, 5 loci in Mewari and 8 in Jalori presented negative values due to the heterozygote excess at these loci (Table 2). Significant (P<0.05) heterozygosity deficiency was not recorded in both the breeds as F value was only 0.06±0.06 for Mewari and 0.01±0.06 for Jalori camel. This intriguing observation may be attributed to the availability of sufficient males in their population. The male to female ratio of camel in the country has not changed significantly despite the 22.55% reduction in their population as it was 1:1.3 in 2007 and 1:1 in 2012 (19th Livestock Census 2012).

Table 3. F-Statistics estimates for each locus in two Indian camel breeds

Locus	F_{IS}	F_{IT}	F_{ST}
VOLP32	0.061	0.181	0.128
VOLP03	–0.196	–0.084	0.094
CVRL06	0.041	0.105	0.067
CVRL07	–0.050	–0.023	0.026
CMS13	0.105	0.120	0.017
LCA63	0.217	0.257	0.051
LCA18	0.104	0.129	0.028
YWLL44	0.168	0.176	0.009
LCA37	0.190	0.191	0.001
VOLP08	–0.533	–0.532	0.001
CMS50	–0.074	–0.035	0.036
CVRL05	–0.135	–0.122	0.012
CVRL04	0.129	0.194	0.075
CMS58	0.070	0.122	0.056
CVRL01	0.396	0.439	0.072
YWLL08	0.022	0.022	0.000
CMS16	0.122	0.123	0.001
YWLL38	0.033	0.033	0.000
Mean	0.037	0.072	0.037
SE	0.046	0.047	0.009

Genetic differentiation between camel populations: To describe the level of heterogeneity within and between the two camel breeds, F-statistics values were determined and are summarized in Table 3. A small global inbreeding coefficient ($F_{IT,}$ 0.072±0.047) was attributed to non-significant within-population inbreeding ($F_{IS,}$ 0.037±0.046) and low differentiation between populations ($F_{ST,}$ 0.037±0.009). All the analyzed markers showed low F_{ST} estimates with the maximum value of 0.128 (VOLP32) and as many as 6 loci having value less than 0.01. F_{ST}, revealed that only 3.7% of total genetic variance resulted from genetic differentiation between two camel breeds. The other 96.7% was due to the within population components of the genetic variance. Very low genetic distinction has also been reported among South African (Nolte *et al.* 2005), Saudi Arabian (Mahmoud *et al.* 2013) and Sudan dromedaries (Eltanany *et al.* 2015). However, Xiaohong *et al.* (2012) found a plausible genetic substructure among Bactrian Chinese and Mongolian camel populations according to

Fig. 1. Population assignment for individual animals of Mewari and Jalori camel

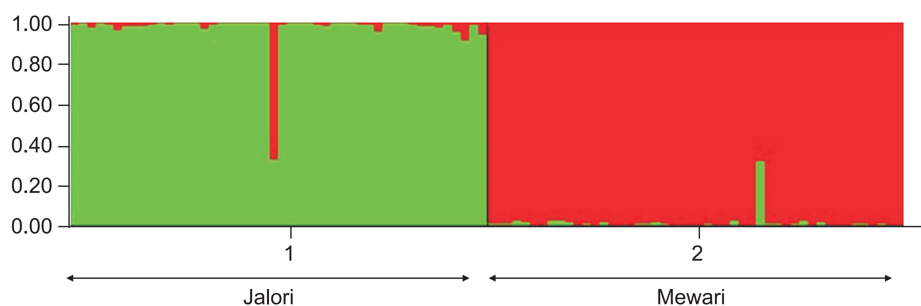

Fig. 2. Stacked vertical line plots of the estimated membership fractions of each individual analyzed for the k (2) inferred clusters (Individuals are grouped by population).

their natural geographic barriers.

Weak genetic differentiation ($F_{ST,}$ 0.037), between Mewari and Jalori camel populations, as well as the low F_{IS} values, may indicate genetic gain in these populations. This may be due to the gene flow, introgression and cross-breeding which may be prevalent under the field conditions. It was also reflected in the gain of genetic variation described by high heterozygosities observed within these populations. Nevertheless, occurrence of substantial gene flows and hybridization among Mewari and Jalori were not supported by further analysis. The overall accuracy of self-assignment was 98.6% for the data sets consisting of 18 microsatellite markers. All the animals of Mewari and Jalori were correctly assigned to their respective groups except a

Table 4. Test for null hypothesis for mutation drift equilibrium under three mutation models (IAM, TPM and SMM) using Sign rank, Standardized differences and Wilcoxon tests

Test/ Model		Mewari camel			Jalori camel		
		IAM	TPM	SMM	IAM	TPM	SMM
Sign rank test	Exp	14.23	14.28	14.11	13.08	13.07	13.07
(Number of loci with	Obs	17	11	3	20	11	4
heterozygosity excess)	P-value	0.17319	0.12459	0.00000*	0.00131*	0.24578	0.00009*
Standardized differences test	T_2-value	1.456	–1.847	–7.773	2.480	–0.686	–5.938
Wilcoxon rank test (one tail	P-value	0.07269	0.03234*	0.00000*	0.00658*	0.24635	0.00000*
for heterozygosity excess)	P-value	0.01147*	0.92425	1.00000	0.00013*	0.58814	0.99996

*Rejection of null hypothesis (P<0.05).

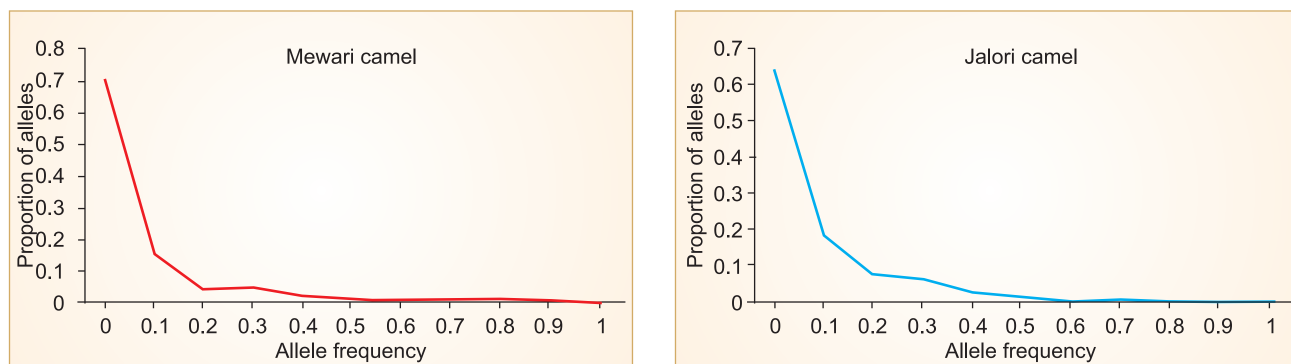

Fig. 3. Mode-shift test for bottleneck analysis.

single animal of Jalori. As a result, all the animals were clustered in two distinct groups (Fig. 1). Vijh *et al.* (2007) also reported that the assignment was 100% only for the Mewari animals among the four breeds. The STRUCTURE algorithm clustered the two breeds into distinct groups with no sign of admixture at K=2 (Fig. 2). Altogether these results confirm the distinct identity of Mewari and Jalori breeds.

Bottleneck: The microsatellite loci are probably the best markers available for detecting recent bottlenecks because of their generally high level of variability (Cornuet and Luikart 1996). The bottleneck tests for the departure from mutation drift equilibrium based on heterozygosity excess or deficiency. This does not require information on historical population sizes or level of genetic variations.

The results for bottleneck analysis in Mewari and Jalori are presented in Table 4. Null hypothesis of existence of the populations at mutation–drift equilibrium on the basis of excess heterozygosity was rejected by standardized differences test for Mewari and by standardized differences as well as sign test for Jalori camel. In sign test, only one model (SMM) depicted significant ($P<0.05$) difference in the expected and observed number of loci with heterozygosity access in Mewari population. Whereas, the difference was significant under SMM and IAM for Jalori camel. The standardized differences test revealed that the standardized differences between He and Hee were significantly ($P<0.05$) different in two mutation models for both the Mewari (TPM, SMM) and Jalori camel (IAM, SMM). In Wilcoxon test, the probability values for heterozygosity excess (P—one tail for He) were significant ($P<0.05$) only under IAM mutation model ($P<0.05$) in both the breeds. Moreover, the normal L shaped curve was observed in Mewari as well as Jalori camel populations (Fig. 3) on visualizing the allele frequency spectra by the qualitative graphical method of Luikart and Cornuet (1998). According to this approach, population bottlenecks are expected to cause a characteristic mode shift distortion in the distribution of allele frequencies at selectively neutral loci. So, the null hypothesis that the Mewari and Jalori populations are at mutation drift equilibrium was not rejected.

These observations are not in agreement with the previous results depicting genetic bottleneck in the recent past population dynamics of four breeds of Indian dromedary including Mewari (Mehta *et al.* 2014). Firstly, automated genotyping was done for generation of genotype data instead of the Urea-PAGE. Moreover, the present study is more rational as it was conducted only when a detailed study on its distribution was carried out to delineate the true picture of its distribution as well as breed characteristics under ICAR-Network Project on Animal Genetic Resources. This resulted in selection of animal from the entire distribution area of Mewar and Hadoti region. As a result, random sampling for diversity estimation and maximum diversity coverage was ensured. This observation was complemented by the large differences observed in the estimated diversity parameters of two studies.

Rajasthan camel became the first domestic animal to be declared as "State Animal" in India in an effort to plan strategies to prevent its continuous population decline. The structure and composition of the declining populations get distorted due to the disproportionate reduction in the animals of the two sexes, loss of elite animals, irrational breeding and associated factors. The microsatellite markers generated important information on the genetic variation and population structure and it is a significant step towards realizing the goals of managing conservation and biodiversity of Mewari and Jalori camel.

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