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Original article

Identification of a new Indian camel germplasm by microsatellite markers based genetic diversity and population structure of three camel populations

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ABSTRACT

Camel invokes fascinating chapter of Indian desert history and is integral component of its ecosystem. Camel population has reached a crisis point after three decades of decline (75%) causing major concern to the policy makers. >28% of Indian camel is not yet characterized. It is imperative to describe country's camel germplasm and its existing diversity for designing conservation plan. One such population is Sindhi, distributed along border with Pakistan. Twenty five microsatellite markers being valuable tool for estimating genetic diversity were selected to elucidate genetic variability and relationship of Sindhi with two registered camel breeds of India- Marwari and Kharai. The standard metrics of genomic diversity detected moderate variability in all the three populations. A total of 303 alleles with a mean of 8.116 ± 0.587 alleles per locus were found in total of 143 animals. Sindhi population had intermediate allelic diversity with 8.522 ± 1.063 alleles per locus. Corresponding values in Marwari and Kharai were 8.783 ± 0.962 and 7.043 ± 1.030, respectively. Genetic variability within the breeds was moderate as evidenced by the mean observed heterozygosity of 0.556 ± 0.025. Sindhi camel population harbors higher genetic variability (Ho = 0.594) as compared to the two registered camel breeds (Marwari, 0.543 and Kharai, 0.531). Mean expected heterozygosity under Hardy-Weinberg equilibrium was higher than the observed values across the three camel groups, indicating deviations from assumptions of this model. In fact, average positive F value of 0.084 to 0.206 reflected heterozygote deficiency in these populations. These Indian camel populations have not experienced serious demographic bottlenecks in the recent past. Differences among populations were medium and accounted for 7.3% of total genetic variability. Distinctness of three camel populations was supported by all the approaches utilized to study genetic relationships such as genetic distances, phylogenetic relationship, correspondence analysis, clustering method based on Bayesian approach and individual assignment. Sindhi camel population was clearly separated from two registered breeds of Indian camel. Results conclude Sindhi to be a separate genepool. Moderate genetic diversity provides an optimistic viewpoint for the survival of severely declining indigenous camel populations with appropriate planning strategies for conserving the existing genetic variation and to avoid any escalation of inbreeding.

1. Introduction

Dromedary camel (*Camelus dromedarius*) inhabits various desert regions of the world including India. Their main habitat is arid and semi arid zones of India (Mehta, 2014). It provides mobility in the desert; are good draught animals; can survive continuous spells of hot and arid conditions; and, during drought and famine offer nutritious milk when other livestock perish. However, camel is fast vanishing from Indian desert landscape mainly due to

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intense destruction and fragmentation of natural habitats, infrastructural development and transport modernization leading to their reduced economic importance (Prasad et al., 2015). Indian camel has registered a drop of 37% from the last census in 2012 and 75% since 1992. The population was 0.4 million in 2012 reducing drastically to 0.25 million in 2019 (20th Livestock census, 2019). Given the current scenario, it may soon figure in the IUCN Red list as a critically endangered species (Meena, 2018). Hence, there is an urgent need for planning conservation of country's camel genetic resource.

Molecular characterization of genetic diversity is helpful in the planning of conservation programmes and in understanding existing variability in a population, its differentiation, structure, origin and domestication. Genetic characterization of livestock requires a basic understanding of the variation both within and between populations. Genetic diversity is important not only to meet present requirements, but is especially important for future. It forms the basis on which evolutionary forces act upon and ultimately shape the trajectory of variation within populations through time. Moreover, livestock breeding utilizes within and between populations variability to improve the traits of interest. Forthcoming era may require a shift in emphasis from high-input to low-input production systems and will favor diverse breeds and different characteristics within breeds. Increasing emphasis is given now-a-days to animal welfare, environmental protection, distinctive product quality, human health and climate change which are often met by local breeds. Conservation of local breeds or populations is possible only if there is a clear understanding of the genetic structure and diversity as well as the relationship between their genetic makeup and performance characteristics.

Characterization of new camel population and establishing their uniqueness from the registered breeds has been subject of study during the last two decades in India. These studies are related to morphometric characterization of camel germplasm along with generation of molecular information on population variability. The efforts lead to registration of 9 breeds of indigenous camel. Four of these are major breeds, Jaisalmeri, Bikaneri, Kutchi and Mewari based on population size (NBAGR, 2019). Nondescript camel constitutes 28.22% in the total camel population which may encompass one or more of lesser known populations. There is a need for characterization of lesser known populations and to establish their distinctness from the registered camel breeds before decisions can be taken about what to conserve. One such population is Sindhi that exists in the interior parts of the country. Animals of this population have short, less curved and smaller neck (Khanvilkar et al., 2009). Literature on phenotypic or genetic characterization of this population is lacking. Similar is the situation of Marwari camel though it is a registered breed (INDIA_CAMEL_1700_MARWARI_02003). Only 1,073 animals of this breed have been reported (19th Livestock census, 2012). Additionally, Kharai is the latest breed to be registered in India (INDIA_CAMEL_0400_KHARAI_02009). Kharai camel is also referred as Sindhi, adding to the confusion pertaining to the Sindhi germplasm in India (Mehta et al., 2015). Small population of all the three groups of indigenous camel calls for sincere efforts to ensure conservation of these unique and valuable germplasm. For example, Kharai camel eats saline mangrove plants and swims long distance in sea. All the three populations having less numbers are more likely to experience severe population fluctuations concomitant with environmental stochasticity which reduces long-term effective population sizes and ultimately the accumulation of genetic diversity (Sonsthagen et al., 2017). Moreover, there was no corroborative evidence to confirm the distinct genetic status of Sindhi population till now.

Microsatellite markers are useful in establishing existing diversity and population structure within and between breeds or populations due to their high degree of polymorphism, which makes them extremely informative and provides very high discriminating power (Sharma et al., 2006). These have been extensively used in livestock species, however, genetic characterization studies on camels has been poorly recorded (Musthafa, 2015). Limited studies have elucidated the genetic variation in indigenous camel of India using microsatellite markers (Mehta et al., 2007; Vijh et al., 2007; Prasad et al., 2015; Sharma et al., 2018). This study was undertaken to get an in-depth description of the current status of the genetic diversity and population structure as well as estimating genetic distinctness of two registered camel breeds (Marwari and Kharai) and a lesser known camel population (Sindhi) of India. The results can guide more efficient germplasm resources utilization, conservation and breeding strategies development. This will also facilitate registration of Sindhi camel as a breed.

2. Materials and methods

2.1. Sample collection, DNA extraction and quantification

Blood samples were collected from 143 randomly selected and unrelated animals of 3 camel populations of India over a period of December 2018 to January 2019. These samples represented 48 Marwari, 47 Kharai and 48 Sindhi camels. The animals were selected from herds located in their respective breeding tracts (Fig. 1). Distribution area was earmarked after extensive survey done in an Indian Council of Agricultural Research (ICAR) funded project-Network project on Animal Genetic Resources. True to the breed type animals were selected. Breeding tract encompasses Pali (25.7543° N, 73.5594° E) and Sirohi (24.7467° N, 72.8043° E) districts of Rajasthan for Marwari camel, Jaisalmer (26.9157° N, 70.9083° E) and Barmer (25.7521° N, 71.3967° E) districts of Rajasthan for Sindhi camel and Jamnagar (22.4707° N, 70.0577° E), Bharuch 21.7051° N, 72.9959° E, Ahmedabad 23.0225° N, 72.5714° E, Ananad (22.5645° N, 72.9289° E), Dwarika (28.5823° N, 77.0500° E) and Vadodara (22.3072° N, 73.1812° E) districts of Gujrat for Kharai camels. Unrelated samples were ensured by interviewing owners and selecting only 2–3 samples per village in order to minimize genetic relationship and to maximize sample representativeness.

Blood samples (8–10 ml) were collected by veterinarians from the jugular vein of animal using vacutainer tubes having K2-EDTA (ethylenediaminetetraacetic acid) anticoagulant. Samples were transported to the laboratory in an ice-box and stored at −80 °C until used for DNA extractions. DNA was isolated 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 Tris-acetate-EDTA (TAE) buffer (pH 8). The quality and concentration of DNA were checked on 0.6% agarose gel as well as by nanodrop spectrophotometer. An intact band on the gel and 260/280 ratio of 1.8–1.9 indicated good quality of extracted DNA.

2.2. Microsatellite loci amplification

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; Sharma et al., 2018). Forward primer of each primer pair was labeled with a fluorescent dye. PCR was performed in a total reaction volume of 10 μL using 96 well plates. Each well consisted of 10–20 ng of genomic DNA and 0.2 uM of primer. To reduce the possibility of cross contamination and variation in the amplification

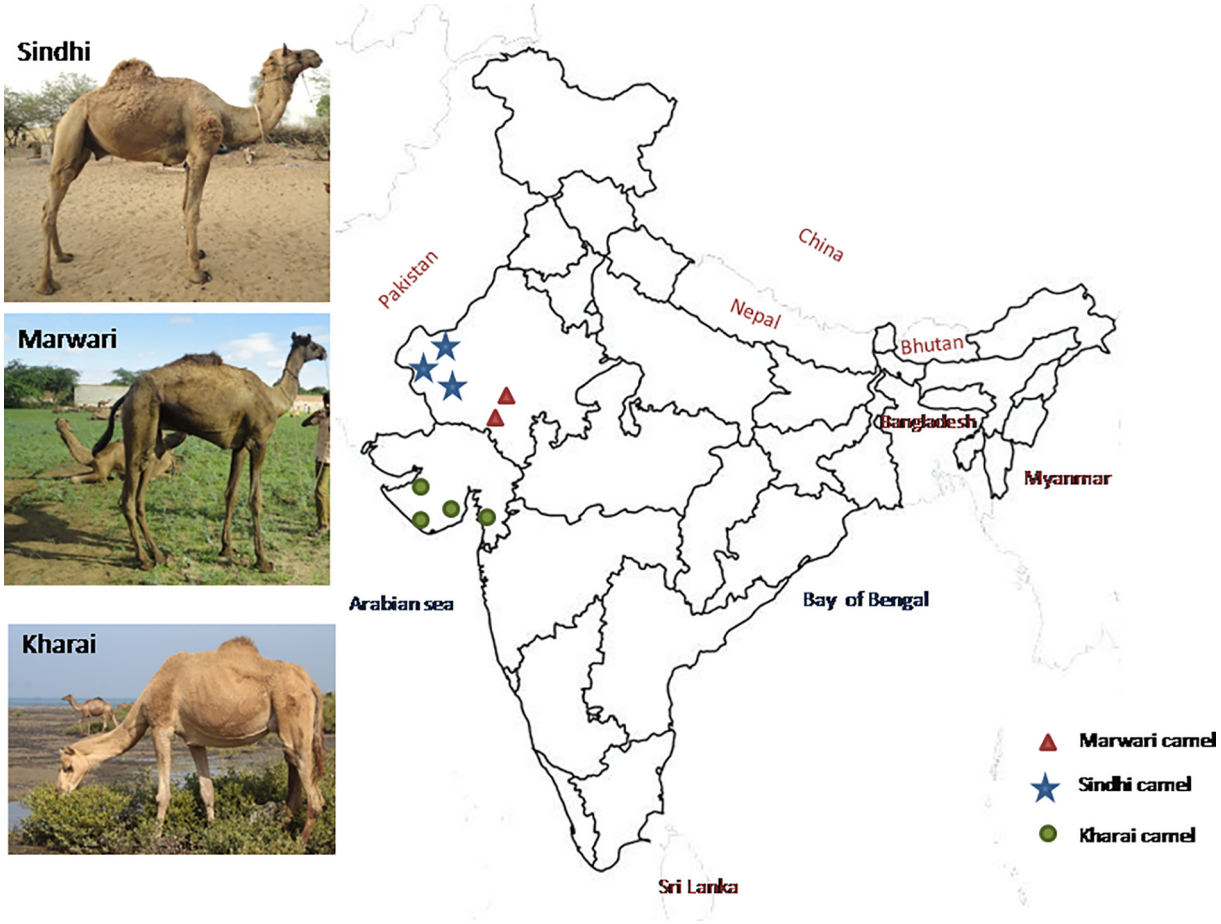

Fig. 1. Representative animal and sampling sites for Marwari, Sindhi and Kharai camel, marked on map of India.

reactions, master mix containing all PCR reagents except DNA template and primers was used. It consisted of *DreamTaq* polymerase enzyme, 0.2 mM of each dNTP and 2 mM of $MgCl_2$. Amplification was carried out using 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 s, and extension at 72 °C for 1 min. Final extension was performed at 72 °C for 10 min. Amplification products were electrophoresed in 2% agarose gel treated with ethidium bromide for later visualization of DNA bands under ultraviolet light.

2.3. Microsatellite marker genotyping

Fluorescently-labelled DNA fragments were analyzed by capillary electrophoresis on Applied Biosystems 3130XL Genetic Analyzer. The genotyping reaction consisted of 1 μL PCR products, 8.9 μL of Hi-Di formamide and 0.1 μL of GeneScan® LIZ 500 size standard. 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.

2.4. Statistical analysis

2.4.1. Diversity estimation

The genotype data was analyzed using GenAlEx 6.5 software (Peakall and Smouse, 2012) to calculate allele frequencies at each locus for each population, average number of allele per population; observed (Na) and effective numbers of alleles (Ne) and heterozygosity values; observed (Ho) and expected (He), Shannon information index (I) as well as heterozygote deficit (F_{IS}) per locus across breeds and markers. Average values were expressed as Mean ± SE from values at each locus. Chi-square tests of deviations from Hardy-Weinberg equilibrium (HWE) were derived.

2.4.2. Relationships and genetic differentiation among populations

In order to study the relationships and the genetic differentiation among tested populations, different F statistics estimates, Principal coordinate analysis (PCoA), analysis of molecular variance (AMOVA) and genetic distances were applied. PCoA for the microsatellite markers, Nei's genetic distance (Nei, 1972), and Cavalli-Sforza Chord distance was estimated by GenAlEx 6.5. The dendrograms of phylogenetic trees were built from different distance matrices and were visualized by MEGA4 (Tamura et al., 2007) using the neighbor-joining (NJ) and unweighted pair group method with arithmetic mean (UPGMA) approaches.

2.4.3. Population structure and individuals assignment

Population assignment was performed using multilocus genotypes of individuals as implemented in GenAlEx 6.5. We used the STRUCTURE software (Pritchard, 2009) to study the relationships among the three camel populations and to assign samples into clusters using the Bayesian method under an admixture model. Length of the burn-in period was 20,000 with 50,000 MCMC repetitions. Different K values between $K = 2$ and $K = 6$, where K is the number of tested clusters, were applied. Runs for each K were repeated 20 times. The software CLUMP (Jakobsson and

Table 1
Characteristics of 25 microsatellite polymorphic loci used for camel diversity estimation.

Marker	Primer Sequence; (5′→3′) forward, (3′→5′) reverse	Size range (bp)	Fluorescent dye	GenBank accession number	Annealing temperature (T_m)^{0}C	Observed Size range (bp)		
						Marwari	Kharai	Sindhi
LCA77	F: 5′TGTTGACTAGAGCCTTTTCTTCTTT3′ R: 3′GGGCAAGAGAGACTGACTGG5′	206–246	PET	AF091129	55	212–236	218–234	210–264
VOLP32	F: 5′GTGATCGGAATGGCTTGAAA3′ R: 3′CAGCGAGCACCTGAAAGAA5′	182–223	FAM	AF305234	60	175–235	173–227	–
LCA63	F: 5′TTACCCAGTCCTTCGTGGG3′ R: 3′GGAACCTCGTGGTTATGGAA5′	182–236	HEX	AF091123	58	172–226	172–222	186–236
YWLL44	F: 5′CTCAACAATGCTAGACCTTGG3′ R: 3′GAGAACACAGGCTGGTGAATA5′	101–111	PET	GU72326	60	93–131	109–113	87–127
VOLP08	F: 5′AGACGGTTGGGAAGGTGGTA3′ R: 3′CGACAGCAAGGCACAGGA5′	142–148	FAM	AF305234	58	146–150	146–150	144–150
CMS13	F: 5′TAGCCTGACTCTATCCATTTCTC3′ R: 3′ATTATTTGGAATTCAACTGTAAGG5′	236–254	NED	AF329158	58	244–260	246–258	242–262
CVRL04	F: 5′CCCTACCTCTGGACTTTG3′ R: 3′CCTTTTTGGGTATTTTCAG5′	156–180	FAM	AF217604	58	148–178	158–178	158–176
CVRL05	F: 5′CCTTGGACCTCCTTGCTCTG3′ R: 3′GCCACTGGTCCCTGTCATT5′	151–179	HEX	AF217605	60	151–181	157–181	159–181
VOLP67	F: 5′TTAGAGGGTCTATCCAGTTTC3′ R: 3′TGGACCTAAAAGAGTGGAG5′	100–220	PET	AF305237	58	82–190	80–190	150–192
LCA66	F: 5′GTGCAGCGTCCAAATAGTCA3′ R: 3′CCAGCATCGTCCAGTATTCA5′	232–246	FAM	AF091125	58	230–244	230–244	230–246
CVRL08	F: 5′AATTCCTGTGATTTTATACACA3′ R: 3′CATGTCATGAAAGCTACAGTA5′	191–209	PET	AF217608	60	211–227	211–229	203–229
LCA90	F: 5′TATAACCCTGGTCTCGCCAA3′ R: 3′CCAAGTAGTATTCCATTATGCG5′	221–256	FAM	AF142660	58	211–247	211–245	231–245
CVRL01	F: 5′GAAGAGGTTGGGGCACTAC3′ R: 3′CAGGCAGATATCCATTGAA5′	180–244	HEX	AF217601	58	162–246	162–246	198–248
YWLL08	F: 5′ATCAAGTTGAGGTGCTTTCC3′ R: 3′CCATGGCATTGTGTTGAAGAC5′	110–178	FAM	*	60	112–174	94–166	180–190
CMS50	F: 5′TTTATAGTCAGAGAGAGTGCTG3′ R: 3′TGTAGGGTTCATTGTAACA5′	164–190	NED	AF329149	60	150–192	150–206	–
CVRL06	F:5′TTTTAAAAATTCTGACCAGGAGTCTG3′ R:3′CATAATAGCCAAAACATGGAAACAAC5′	182–208	PET	AF217606	60	168–204	180–220	178–204
VOLP03	F: 5′GCCAAAATAGGCTTACCCTTG3′ R: 3′CCCGCTTCATCTATTGGAAA5′	148–168	HEX	AF305228	60	138–172	134–174	136–186
CMS58	F: 5′AATATACATCCTCCCAACTGGT3′ R: 3′TTATTTCTCTTAACCCCTCTCTAA5′	92–124	NED	AF329142	58	76–144	98–144	80–144
YWLL09	F: 5′AACTCTAGGAACCGGAATGC3′ R: 3′ACGCAATCTACACTCCTTGC5′	125–215	FAM	*	60	145–171	139–171	145–161
CVRL07	F: 5′AATACCCTAGTTGAAGCTCTGTCCT3′ R: 3′GAGTGCCTTTATAAATATGGGTCTG5′	280–314	HEX	AF217607	60	276–306	276–300	276–316
LCA37	F: 5′AAACCTAATTACCTCCCCCA3′ R: 3′CCATGTAGTTGCAGGACACG5′	143–183	PET	AF060105	55	125–189	133–189	133–189
CMS16	F: 5′ATTTTGCAATTTGTTCGTTCTTTC3′ R: 3′GGAGTTTATTTGCTTCCAACACTT5′	181–205	NED	AF329157	60	181–209	185–191	185–203
LCA18	F: 5′TCCACCCATTTAGACACAAGC3′ R: 3′TAGGAAGCTCCAAGAAGAAAAGAC5′	211–238	FAM	AF060097	60	227–245	227–233	227–247
YWLL38	F: 5′GGCCTAAATCCTACTAGAC3′ R: 3′CCTCTCACTCTTGTTCTCCTC5′	175–189	HEX	GU72325	60	175–191	181–191	181–191
VOLP10	F: 5′CTTTCTCCTTTCCTCCCTACT3′ R: 3′CGTCCACTTCCTTCATTTC5′	222–268	PET	AF305231	58	212–270	212–266	214–274

*Lang et al. (1996).

Rosenberg, 2007) was used to align multiple replicates for each *K* in order to facilitate the interpretation of clustering results. The DISTRUCT application (Rosenberg, 2004) was used to graphically display the results. The best number of clusters was determined depending on ΔK value (Evanno et al., 2005), which was calculated and plotted using Structure Harvester application (Earl and vonHoldt, 2011).

2.4.4. Bottleneck detection

Bottleneck events in the population were tested by two methods using Bottleneck v1.2.02 (http://www.ensam.inra.fr/URLB). The first method consisted of three excess heterozygosity tests developed by Cornuet and Luikart (1996) like sign test, standardized differences test and a Wilcoxon signed rank test. The probability distribution was established using 1000 simulations based on allele frequency and heterozygosity under three models namely infinite allele model (IAM), stepwise mutation model (SMM) and two-phase model of mutation (TPM). The genetic bottleneck test was reconfirmed through a Mode shift indicator test based on qualitative descriptive allele frequency distribution (Luikart and Cornuet, 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.

3. Results

3.1. Gene diversity within and among populations

All the microsatellite loci were amplified successfully in Marwari and Kharai camel, whereas twenty three loci, excluding VOLP32 and CMS50 amplified unambiguously in Sindhi camel population (Table 1). Accordingly, a common set of twenty three

Table 2
Genetic diversity indices for the three unique indigenous camel populations of India.

Breed/population	Marwari							Kharai							Sindhi						
Locus	N	Na	Ne	I	Ho	He	F	N	Na	Ne	I	Ho	He	F	N	Na	Ne	I	Ho	He	F
LCA63	48	8	4.331	1.633	0.750	0.769	0.025	45	5	3.482	1.349	0.822	0.713	−0.153	43	7	4.109	1.525	0.744	0.757	0.016
LCA77	48	7	2.653	1.168	0.271	0.623	0.565	46	2	1.044	0.105	0.043	0.043	−0.022	44	12	3.176	1.698	0.227	0.685	0.668
VOLP08	48	3	1.596	0.621	0.438	0.373	−0.171	47	3	1.542	0.574	0.404	0.352	−0.150	45	4	1.982	0.874	0.444	0.496	0.103
YWLL44	47	6	2.314	1.154	0.574	0.568	−0.012	41	3	1.718	0.725	0.366	0.418	0.125	43	8	6.663	1.971	0.674	0.850	0.206
CMS13	45	9	5.025	1.852	0.400	0.801	0.501	46	5	2.645	1.128	0.630	0.622	−0.014	46	7	3.367	1.437	0.565	0.703	0.196
CVRL04	43	10	4.796	1.788	0.698	0.792	0.119	46	5	3.062	1.242	0.630	0.673	0.064	47	8	3.725	1.601	0.851	0.732	−0.163
CVRL05	44	12	6.757	2.087	0.591	0.852	0.306	45	11	2.736	1.497	0.667	0.635	−0.051	48	6	3.313	1.351	0.688	0.698	0.015
LCA66	43	7	4.290	1.586	0.395	0.767	0.484	46	6	2.993	1.283	0.652	0.666	0.021	45	8	4.313	1.660	0.644	0.768	0.161
VOLP67	48	24	8.486	2.601	0.708	0.882	0.197	47	25	5.708	2.405	0.787	0.825	0.046	47	18	8.925	2.460	0.872	0.888	0.018
CVRL01	46	16	7.222	2.228	0.804	0.862	0.066	45	12	6.279	2.110	0.667	0.841	0.207	48	18	6.898	2.288	0.833	0.855	0.025
CVRL08	45	5	2.081	0.917	0.156	0.520	0.701	41	4	1.799	0.750	0.268	0.444	0.396	45	5	1.485	0.675	0.333	0.326	−0.021
LCA90	46	5	2.689	1.102	0.696	0.628	−0.108	45	5	2.550	1.090	0.578	0.608	0.050	48	5	2.963	1.164	0.688	0.663	−0.038
YWLL08	45	13	6.164	2.056	0.844	0.838	−0.008	47	13	6.594	2.118	0.872	0.848	−0.028	48	4	2.349	0.975	0.646	0.574	−0.125
CMS58	48	10	3.534	1.604	0.667	0.717	0.070	45	6	3.497	1.451	0.600	0.714	0.160	45	10	2.260	1.297	0.467	0.558	0.163
CVRL06	43	6	2.856	1.337	0.302	0.650	0.535	46	4	1.363	0.526	0.261	0.266	0.020	47	4	2.535	1.067	0.468	0.605	0.227
CVRL07	47	11	3.735	1.671	0.468	0.732	0.361	44	8	3.959	1.573	0.409	0.747	0.453	47	10	5.185	1.844	0.702	0.807	0.130
VOLP03	48	6	2.052	1.004	0.542	0.513	−0.057	46	9	2.098	1.213	0.587	0.523	−0.121	46	14	4.692	1.931	0.696	0.787	0.116
YWLL09	46	4	1.617	0.688	0.130	0.382	0.658	39	4	1.416	0.602	0.231	0.294	0.214	45	4	1.923	0.852	0.511	0.480	−0.065
CMS16	48	6	1.875	0.914	0.188	0.467	0.598	47	4	2.260	0.961	0.255	0.557	0.542	47	5	2.414	1.031	0.191	0.586	0.673
LCA18	43	5	3.522	1.351	0.628	0.716	0.123	45	4	2.516	1.064	0.800	0.602	−0.328	41	5	3.349	1.345	0.585	0.701	0.165
LCA37	48	8	2.727	1.357	0.854	0.633	−0.349	46	8	2.342	1.238	0.609	0.573	−0.062	46	8	2.584	1.260	0.717	0.613	−0.170
VOLP10	47	12	4.240	1.773	0.660	0.764	0.137	38	10	4.039	1.714	0.447	0.752	0.405	41	22	9.313	2.588	0.439	0.893	0.508
YWLL38	47	9	3.537	1.557	0.723	0.717	−0.009	46	6	4.182	1.536	0.630	0.761	0.171	48	4	3.028	1.179	0.667	0.670	0.005
Mean	46.130	8.783	3.830	1.480	0.543	0.677	0.206	44.739	7.043	3.036	1.228	0.531	0.586	0.084	45.652	8.522	3.937	1.481	0.594	0.682	0.122
SE	0.394	0.962	0.392	0.105	0.046	0.031	0.061	0.527	1.030	0.319	0.115	0.046	0.043	0.044	0.443	1.063	0.447	0.108	0.038	0.029	0.048

N, Number of animals; Na, Number of observed alleles; Ne, number of effective alleles; I, Shannon information index for polymorphism content; Ho observed heterozygosity; He, expected heterozygosity; F, heterozygote deficiency/ Inbreeding coefficient

Table 3
F-Statistics and estimates of gene flow (Nm) for all populations across 20 loci.

Locus	Fis	Fit	Fst	Nm
LCA63	−0.035	−0.017	0.017	14.153
CMS13	0.249	0.290	0.055	4.334
CVRL04	0.008	0.057	0.049	4.823
CVRL05	0.110	0.182	0.082	2.808
LCA66	0.231	0.264	0.042	5.682
VOLP67	0.087	0.120	0.036	6.714
CVRL01	0.099	0.138	0.043	5.551
CVRL08	0.413	0.431	0.031	7.751
LCA90	−0.033	−0.005	0.027	8.923
YWLL08	−0.045	0.114	0.152	1.393
CMS58	0.128	0.218	0.103	2.170
CVRL06	0.322	0.388	0.096	2.345
CVRL07	0.309	0.319	0.014	17.532
VOLP03	−0.001	0.043	0.044	5.416
YWLL09	0.245	0.550	0.405	0.368
CMS16	0.606	0.624	0.047	5.116
LCA18	0.003	0.083	0.080	2.881
LCA37	−0.198	−0.165	0.028	8.632
VOLP10	0.358	0.413	0.086	2.659
YWLL38	0.059	0.086	0.028	8.705
Mean	0.146	0.207	0.073	5.898
SE	0.043	0.045	0.018	0.950

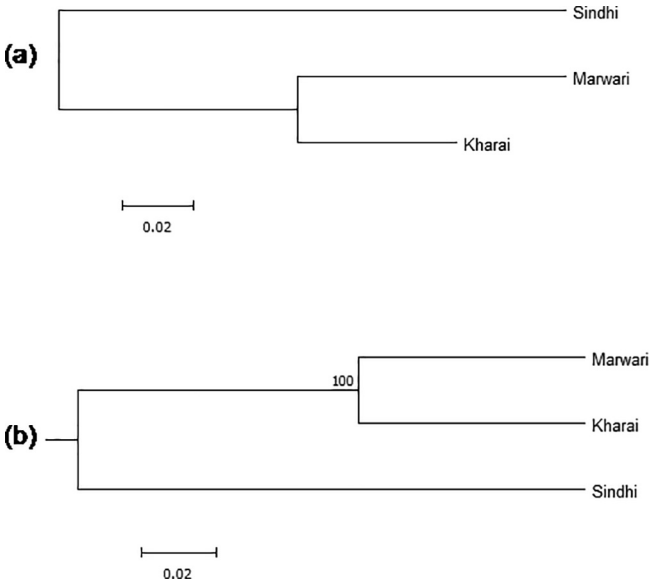

Fig. 2. Dendrogram depicting genetic relationships among three camel populations based on Nei's genetic distance a) neighbor-joining (NJ) tree b) unweighted pair group method with arithmetic mean (UPGMA).

microsatellite markers was utilized for subsequent comparative analysis. A total of 303 alleles with an overall mean of 8.116 ± 0.587 alleles per locus were observed in 143 individuals of two registered camel breeds of India (Marwari and Kharai) and one population in the need of characterization, Sindhi.

The parameters and indices of genetic diversity within three populations are listed in Table 2. Marwari and Sindhi showed almost similar mean values of the observed (N_a) and expected (N_e) number of alleles. Mean number of observed alleles was less in Kharai (7.043 ± 1.030) as compared to the Marwari (8.783 ± 0.962) and Sindhi camel (8.522 ± 1.063). Mean values of the expected number of alleles in Kharai (3.036 ± 0.319) was also less as compared to Marwari and Sindhi, 3.83 ± 0.392 and 3.937 ± 0.447, respectively. Private alleles were also recorded in all the populations but their frequency was<5%.

Among loci, VOLP67 displayed the highest (24) and LCA77 displayed the lowest (2) number of observed alleles in Marwari camel. Expected number of alleles (Ne) varied from 1.569 (VOLP08) to 8.486 (VOLP67). Just like Marwari, VOLP67 displayed the highest number of observed alleles (25) in Kharai population, whereas LCA77 exhibited only 2 alleles. A different set of markers occupied the highest and lowest position in Sindhi camel with VOLP 10 having highest (22) and VOLP08, YWLL08, CVRL06 and YWLL09 having the lowest (4) observed number of alleles.

The mean value of Shannon's information index (I) was 1.397 ± 0.064 across all the loci and populations. It had lower value in Kharai (1.228 ± 0.115) as compared to Sindhi (1.481 ± 0.108) and Marwari (1.480 ± 0.105) camel. VOLP67 and VOLP10 emerged as the most informative markers (I > 2.4). The observed and the expected heterozygosity calculated on the basis of allele frequency varied within a narrow range across the three populations (Table 2). Both the values were highest for Sindhi (Ho, 0.594 ± 0.038 and He, 0.682 ± 0.029) followed by Marwari (Ho, 0.543 ± 0.046 and He, 0.677 ± 0.031) and were least Kharai (Ho, 0.531 ± 0.046 and He, 0.586 ± 0.043). Significant (P < 0.05) heterozygote deficiency was observed in all the three camel populations. F value varied between 0.084 ± 0.044 (Kharai camel) and 0.206 ± 0.061 (Marwari camel). Maximum number of loci presenting negative values due to the heterozygote excess at these loci was observed to be 9 in Kharai camel followed by 7 in Marwari and 6 in Sindhi (Table 2).

3.2. Relationships and genetic differentiation among populations

To describe the level of heterogeneity within and between the studied Indian camel populations, F-statistics values were determined and are summarized in Table 3. The genetic differentiation (Fst) among the populations was of medium category. All the analyzed markers contributed towards Fst estimates with the maximum value of 0.405 (YWLL09) and not a single loci had value less than 0.01. Fst revealed that 7.3% of total genetic variance resulted from genetic differentiation between camel populations. The other 92.7% was due to the within population components of the genetic variance. A moderate global inbreeding coefficient (Fit = 0.207 ± 0.045) was attributed to significant within-population inbreeding (Fis = 0.146 ± 0.043) and medium differentiation between populations (Fst = 0.073 ± 0.018). The value of number of migrants (Nm) was also calculated. Highest gene flow was observed between Marwari and Kharai (Nm = 6.482) followed by Marwari and Sindhi (3.502) and least was between Kharai and Sindhi (3.273).

Pair-wise Fst coefficients between two populations are shown in Table 4 above diagonal. In summary, the pair-wise F_{ST} values were of similar magnitude between Sindhi and Marwari (0.067) and between Sindhi and Kharai (0.071). Minimum population differentiation was recorded between Marwari and Kharai (0.037). The Nei's genetic distance (D_A) data are presented below diagonal in Table 4. The largest distance was recorded between Sindhi and Marwari (0.376) followed by Sindhi and Kharai (0.307), while

Table 4
Pair wise population matrix of Nei's unbiased genetic distance (D_A) below diagonal and population differentiation (Fst) above diagonal.

Camel population	Marwari	Kharai	Sindhi
Marwari	–	0.037	0.067
Kharai	0.130	–	0.071
Sindhi	0.376	0.307	–

Table 5
Partitioning of molecular variance within and between camel populations and the level of population sub-structure (Fst) in three camel populations.

Source of variation	d. f.	Sum of squares	Variance components	Percentage of variation	Fst	Fis	Fit	Nm	P
Among populations	2	140.899	0.650	8	0.084*	0.209	0.275	2.717	0.001
Within populations									
Among individuals	140	1194.580	1.473	19					
Within individuals	143	799.000	5.587	73					
Total									
	285	2134.479	7.710	100					

* Significant deviation of pair-wise fixation index (F_{ST}) value through 99 permutations from zero ($P < 0.001$); d.f.: degree of freedom.

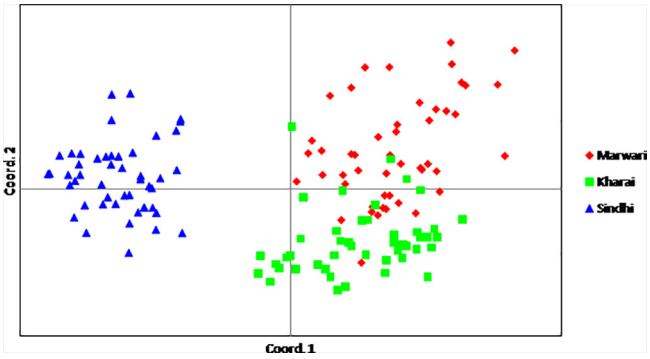

Fig. 3. Two-dimensional plot of the Principal coordinate analysis (PCoA) depicting relative position of Marwari, Kharai and Sindhi camel populations.

Marwari and Kharai were closest (0.130). Genetic relationship among populations was further confirmed by the reconstruction of phylogenetic tree. Both the NJ and the UPGMA trees were constructed using Nei's genetic distance (Fig. 2). Results illustrated separation of the three Indian camel populations. Marwari and Kharai breeds had the closest phylogenetic relationship whereas, Sindhi camel population was separated from both of these.

AMOVA revealed that 8.4% of total genetic variance resulted from genetic differentiation between three populations. The other 91.6% was due to the within population components of the genetic variance (Table 5). The pairwise fixation index (F_{ST}) value provided by AMOVA through 99 permutations differed significantly from zero at $P < 0.05$. The cluster pattern produced by the multivariate analysis using PCoA is illustrated in Fig. 3, in which Sindhi population clustered separately from the two registered camel breeds of India (Marwari and Kharai), which also formed separate groups.

3.3. Population structure and individuals assignment

The overall accuracy of self-assignment was very high (Fig. 4), to the tune of 99% as all the animals except two of Marwari breed were correctly assigned to their respective groups. Clustering using Bayesian approaches was performed on the entire data set with an increasing number of inferred clusters (K) from 2 to 6 and produced consistent results. Clustering assignment isolated the Sindhi camel from the other two camel breeds as early as 2 groups, and this group maintained its integrity through the analysis. For K equaled to 2, all the Kharai and Marwari animals were assigned to cluster 1, while all the individuals of Sindhi were assigned to cluster 2 (Fig. 5). When K equaled to 3, all the individuals of a population were assigned to their respective groups. Even if K was increased upto 6, all the individuals of Sindhi population were still assigned to their group, whereas, most of the Marwari followed by Kharai were assigned into multiple clusters instead of one. Evanno's test indicated that the most informative number of subpopulations was three (K = 3) (Supplementary Fig. S1).

3.4. Bottleneck analysis

According to this test, a population which experienced a bottleneck exhibits a significant heterozygosity excess ($P < 0.05$). The results for bottleneck analysis in the three camel populations have been presented in Table 6. Null hypothesis of existence of the populations at mutation–drift equilibrium on the basis of excess heterozygosity was rejected by Sign rank test under the assumption of step wise mutation model (SMM) for all the three

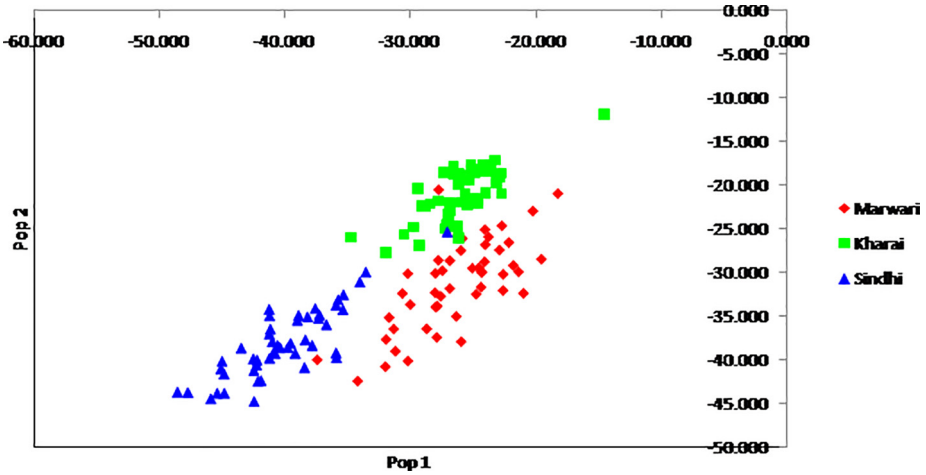

Fig. 4. Population assignment of Marwari, Kharai and Sindhi camel based on log likelihood ratio.

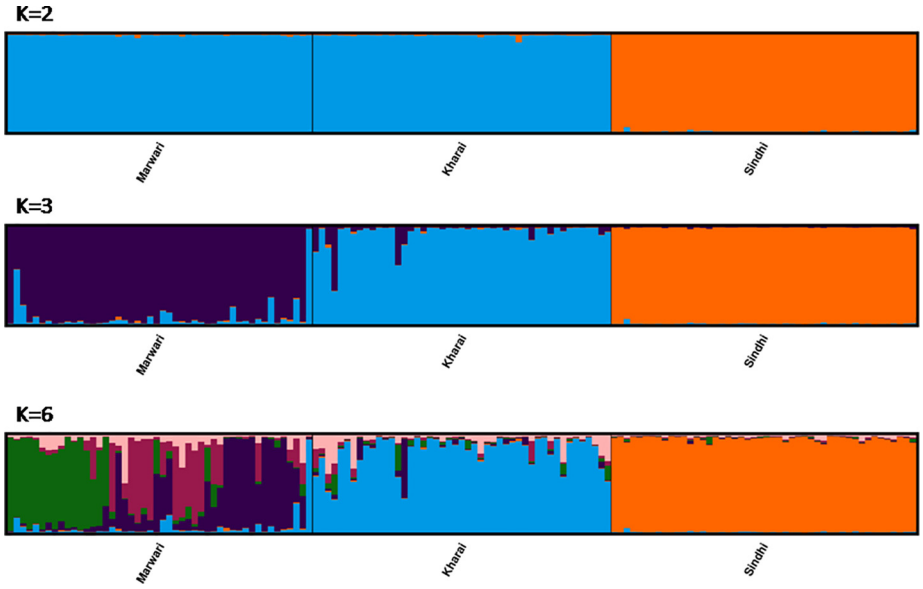

Fig. 5. Clustering assignment depending on the Bayesian method under an admixture model obtained by STRUCTURE software. Each individual is represented by a single column that is divided into segments whose size and color correspond to the relative proportion of the animal genome corresponding to a particular cluster. Populations are separated by black lines.

Table 6
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		Marwari camel			Kharai camel			Sindhi camel		
		I.A.M.	T.P.M.	S.M.M.	I.A.M.	T.P.M.	S.M.M.	I.A.M.	T.P.M.	S.M.M.
Sign rank test (Number of loci with heterozygosity excess)	Exp	14.86	14.71	14.77	14.27	14.57	14.65	13.48	13.55	13.57
	Obs	16	7	2	18	14	5	16	10	5
	P- value	0.40131	0.00177*	0.00000*	0.09349	0.48349	0.00009*	0.19699	0.09897	0.00031*
Standardized differences test	T2 value	1.168	−3.111	−10.050	0.539	−3.421	−8.976	1.719	−2.042	08.709
	P- value	0.12133	0.00093*	0.00000*	0.29493	0.00031*	0. 00,000*	0.04343*	0.02060*	0.00000*
Wilcoxon rank test (one tail for heterozygosity excess)	P- value	0.04257*	0.99631	1.00000	0.19049	0.82374	0.99996	0.04257*	0.99631	1.00000

* Rejection of null hypothesis ($p < 0.05$).

populations, and under TPM for Marwari camel. Two models (TPM and SMM) under standardized differences test gave consistent results against the mutation–drift equilibrium assumption. In addition, it varied under all the three models in Sindhi population. In Wilcoxon test, the probability values for heterozygosity excess (P-one tail for He) were significant ($P < 0.05$) under only one model (IAM) in Marwari and Sindhi populations. Whereas, the differences were insignificant for the Kharai camel under all the three models. This result was supported by mode-shift indicator test in which all the populations at equilibrium showed a normal 'L' shaped allele frequency distribution (Fig. 6).

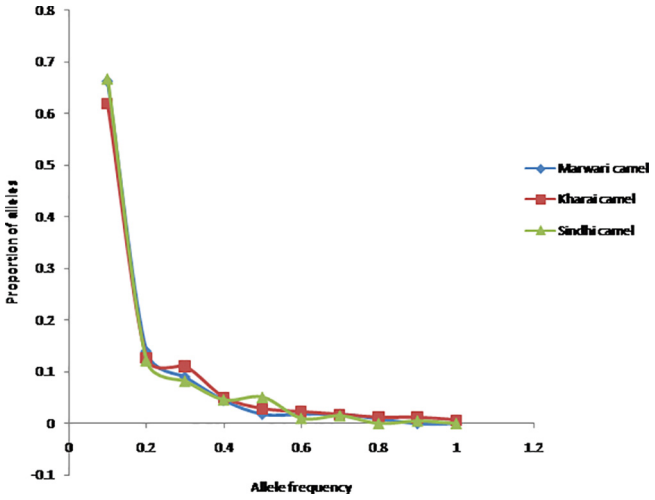

Fig. 6. Graphic representation of proportion of alleles and their distribution in three camel populations.

4. Discussion

Current study investigated genetic diversity and relationship of two Indian camel breeds, Marwari and Kharai with one unexplored population, Sindhi by utilizing microsatellite markers. It resulted in acquisition of original and comparable information confirming the genetic distinctness of Sindhi camel population. Diverse parameters to compute genetic diversity within and among populations were exploited such as mean number of alleles per locus (MNA), polymorphic information content or Shannon's information index, observed and expected heterozygosities, phylogenetic or tree building approach based on genetic distance and Baysian approach of population clustering.

4.1. Informativeness of selected microsatellites

Simple sequence repeat markers (SSR) have been extensively utilized as valuable genomic markers for estimating genetic diversity and divergence within and among populations (Wang et al.,

2019). Microsatellite loci which amplified in all the three camel populations and had minimum of four alleles were only considered for population relationship and differentiation analyses. As a result, LCA77, VOLP08 and YWLL44 were excluded from the original list of 25 markers in addition to VOLP32 and CMS50 that did not amplify in the Sindhi camel. Selection of loci displaying a large range of polymorphisms reduced the danger of overestimating genetic variability (Wimmers et al., 2000). Our results are in accordance with the observations of Mehta et al., 2014 that out of 40 microsatellite loci, only twenty were polymorphic in Rajasthan camel breeds. VOLP67 and VOLP10 loci were most polymorphic among 25 loci (Table 2) which was similar to the previously published report on Indian dromedary camel breeds (Bikaneri, Kutchi, Jaisalmeri, Jalori and Mewari) (Vijh et al., 2007; Sharma et al., 2018). These markers have also shown a high level of allelic richness and polymorphic information content in the studies carried out in Australia, Kenya, Saudi Arabia, Canary Islands, Egypt and Tunisia camels (Musthafa, 2015). I values were indicative of the high polymorphic nature of the microsatellites analyzed. Large values of I (1.397 ± 0.064) for the markers in present study once again confirmed that this set of markers can potentially be used for performing diverse population genetics applications such as linkage mapping, individual identification and parentage testing in camel populations.

4.2. Genetic diversity

Allelic richness is a major decisive factor to measure genetic diversity, and this parameter is of key relevance especially in conservation programs (Foulley and Ollivier, 2006). In this study, Marwari camel was most diverse, which had 202 alleles, closely followed by Sindhi with 196 alleles while Kharai camel breed showed the least diversity with a total of 162 alleles. Significantly less mean effective number of alleles in comparison to the mean observed number of alleles across all the three groups pointed towards large number of low frequency alleles in these populations. MNA observed in Marwari (8.78 ± 0.96) and Sindhi (8.52 ± 1.06) was higher than the Kharai (7.04 ± 1.03) camel. Overall, moderate gene diversity was detected across the pooled populations, per locus of these Indian camel groups (Na = 8.116 ± 0.587). It was less than the previously reported MNA in Mewari (9.67 ± 0.94) and Jalori (8.61 ± 0.86) camel of India (Sharma et al., 2018) and more than the MNA (5.185 ± 0.618) in Kachchhi camel breed of India (Patel et al., 2015). Similarly, higher gene diversity has been reported in camel ecotypes of other countries such as Sudan (8.58 ± 0.91) (Eltanany et al., 2015) and Saudi Arabian camel populations (9.27) namely; Magaheem, Maghateer, Sofr and Shual (Mahmoud et al., 2012). However there are reports of less diversity across some other camel breeds. The MNA in Marwari, Kharai and Sindhi camel in current study was much higher than that is found in Saudi Arabian (Mahmoud et al., 2013), Tunisian (Ahmed et al., 2010) and Egyptian dromedary populations (Karima et al., 2011), which may be attributed to the fewer loci investigated in their studies.

The diversity estimates computed for Marwari, Kharai and Sindhi in present investigation were not compared with the diversity estimates reported for Indian camel in most of the previously published papers, such as Mehta et al. (2014). Differences in the number of alleles and their frequencies were expected due to the variation in the microsatellite markers utilized as well as due to 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. Thus, results are compared with the compatible publications only.

The observed heterozygosity (Ho) values in Marwari (0.54 ± 0.05), Kharai (0.53 ± 0.05) and Sindhi (0.59 ± 0.04) camel were parallel to the observations of Vijh et al. (2007) for four Indian camel breeds having mean heterozygosity of 0.58, 0.57, 0.56, and 0.60 for Bikaneri, Jaisalmeri, Kutchi, and Mewari camel breeds, respectively. Lower estimate for the mean observed heterozygosity (Ho) has been recorded for Kachchhi camel breed (0.45 ± 0.04) by Patel et al. (2015). On the contrary, much higher estimates were observed for Mewari (0.68 ± 0.04) and Jalori (0.71 ± 0.04) camel of India (Sharma et al., 2018). The heterozygosity values reported in present paper for the three Indian camel populations were also found to be 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) camel. Much lower estimates have been described for Tunisian camels (0.460) (Ahmed et al., 2010), and Australian camels (0.455) (Spencer and Woolnough, 2010) whereas, higher estimates have been reported for Sudanese camel (0.68) (Nolte et al., 2005).

The inbreeding coefficient (F) may explain such moderate levels of genetic diversity. Heterozygote deficiency varied from 8.4% in Kharai to 20.6% in Marwari camel (Table 2), which represents the non random union of gametes. Accordingly, deviation from Hardy–Weinberg equilibrium (HWE), revealed that 9 loci in Kharai and Sindhi and 17 in Marwari were not in HWE ($P < 0.01$). Homozygosity excess might result from inbreeding, sampling biases, Wahlund effects, population bottlenecks, or the presence of null alleles. Latter four factors were not contributory in the present case thus, inbreeding seems to be the common phenomenon in these explored Indian camel populations. Inbreeding and loss of genetic diversity are expected to be encountered in small and/or declining populations. So, these high inbreeding values could be due to small population sizes, small numbers of breeding males or limited geographical dispersion of the three camel groups. Continuously declining camel population along with the absence of any official breeding plan is also contributing to the heterozygote deficiency.

4.3. Genetic differentiation

Four different approaches utilized to study genetic relationships (genetic distances, correspondence analysis, clustering methods and individual assignment) gave similar results. Sindhi camel population was clearly separated from two registered breeds of Indian camel. These results form the basis to claim the right of Sindhi to be registered as a separate breed of India.

F-statistics was summarized in fixation index as genetic differentiation (Fst), the global heterozygote deficit among three camel groups (Fit) and the heterozygote deficit within a group (Fis). All of the loci, except five, showed positive Fis values, with an average value of 0.149, indicating a deficiency of heterozygosity within the breeds. The average genetic differentiation between breeds (Fst) and total inbreeding (Fit) were 0.073 and 0.207, respectively (Table 3). Wright (1978) suggested that Fst value of 0.05–0.15 indicates the moderate genetic differentiation among the populations. Accordingly, moderate population differentiation in the indigenous camel groups investigated here, was unlike the very low genetic distinction reported among South African (Nolte et al., 2005), Saudi Arabian (Mahmoud et al., 2013) and Sudan dromedaries (Eltanany et al., 2015). Similar to our findings, Xiaohong et al. (2012) found a plausible genetic substructure among Bactrian Chinese and Mongolian camel populations due to natural geographic barriers. The Fst value was highest between Kharai and Sindhi populations, and it meant medium differentiation. No paired populations were in high level of differentiation.

Stable genetic flow among populations was evident from a high number of migrants from one population to another (Nm = 5.898 > 1.00). These values were intermediate with respect to other Indian camel breeds. Limited gene flow was recorded between

Jaisalmeri and Mewari camel (Nm = 1.29) and high between Jaisalmeri and Kutchi (Nm = 15.58) camel breeds of India (Vijh et al., 2007). Prevalent field conditions are facilitating higher gene flow among the three camel groups explored in present study. These include sharing of migratory routes, absence of breed/population specific breeding policy perpetuating crossbreeding or uncontrolled mating, altogether resulting in gene flow and introgression. The Bayesian analysis displayed a strong genetic structure among the three populations of Indian camel by unambiguously clustering them into three distinct genetic pools. The UPGMA and PCoA analysis also provided similar genetic clustering of the populations.

4.4. Demographic bottlenecks

The microsatellite loci are probably the best markers available for detecting recent bottlenecks because of their generally high level of variability as well as no need for information on historical population sizes or level of genetic variations (Cornuet and Luikart, 1996). There was no evidence of a genetic bottleneck in all the three camel populations. Similar observations have been reported for two other camel breeds (Mewari and Jalori) of India (Sharma et al., 2018). However, four breeds of Indian dromedary have been reported to suffer from genetic bottleneck in their recent past population dynamics (Mehta et al., 2014). The demographic bottleneck is found in a population with sudden decreases of population size due to habitat loss, alteration, fragmentation, and natural calamities. The bottleneck is responsible for reducing genetic diversity by decreasing heterozygosity due to random drift and inbreeding of the populations (Nei et al., 1975). Genetic bottleneck in the recent past is a significant aspect to consider for conservation because it leads to a reduction in genetic variability, inbreeding, expression of undesirable recessive alleles, and therefore diminishing the survival rates.

Absence of signs of a genetic bottleneck is interesting to note despite the declining populations of camel in the country. Our results can be considered to be rational as it was conducted only after detailed study on camel populations in the field 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 true to the breed type animals for the study from the entire distribution area. As a result, random sampling for diversity estimation and maximum diversity coverage was ensured. Moreover, automated genotyping was done instead of the Urea-PAGE for generation of genotype data. Besides in the population undergoing genetic bottleneck, the rare alleles are lost first leading to excess of observed heterozygosity (Allendorf, 1986) which was not the case for all the three populations under consideration (Fig. 5). Similar trend of higher expected than observed heterozygosity was also reported in other Indian camel breeds (Vijh et al., 2007; Banerjee et al., 2012; Patel et al., 2015; Sharma et al., 2018).

In conclusion, genetic relationship among an unregistered population Sindhi and two registered camel breeds of India was established. Altogether above results confirm the distinct identity of Sindhi camel. Sindhi population was considered to be a non-descript population, till now. It is distributed in districts adjoining international border with Pakistan. Sindhi camel has home tract in the Sindh area of Pakistan (Khanna et al., 2004). Considering historical evidence, it would be of further interest to analyze the relationship of the Sindhi population with the other Indian camel breeds as well as with Sindhi camel breed of neighboring country, Pakistan as several Indian camel breeds are believed to have been developed from Sindhi camel of Pakistan by selective breeding. Important clues regarding distinctness of Sindhi camel population can also be understood by considering the socio-historical context in which camel breeding is practiced in India. Pastoral communities in India have social practices which defy the sale of breeding stock for profit making, leading to a separate genepool (Köhler-Rollefson and Rathore, 2004). Regional situations are also responsible in sequestering camel populations. Altogether these could develop distinct breeds.

Indian autchthonous camel plays a role in the economic issues of marginal and disadvantaged area, and moreover they are of crucial importance in the context of climate change. It is important to conserve and manage its breeding. Breeding policies needs to be framed and implemented to avoid the loss of genetic originality of each camel breed. It may include establishing breeding farms to provide purebred males for reducing inbreeding in the population. They should be given priority in conservation.

5. Conclusion

Our results showed that the studied indigenous camel population had a robust structure, with a clear differentiation between the Sindhi, a non recognized population and two registered camel breeds of India. Moderate level of heterozygosity and allelic richness generates hope for conserving indigenous camel germplasm of India in spite of their small and declining populations. The data presented here can be utilized to frame conservation programmes directed towards reducing inbreeding and to minimize loss of genetic variability.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2020.04.046.

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