Livestock Science 181 (2015) 85–88

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Short communication

Molecular characterization of camel breeds of Gujarat using microsatellite markers

A.C. Patel [a], T.K. Jisha [a], Disha Upadhyay [a], Rakesh Parikh [a], Maulik Upadhyay [a], Riddhi Thaker [a], S. Das [b], J.V. Solanki [a], D.N. Rank [a,b,*]

[a] Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand 388001, Gujarat, India
[b] Sahjeevan Trust, Vijay Nagar, Bhuj 370001, Gujarat, India

ARTICLE INFO

Article history:
Received 2 May 2014
Received in revised form
3 October 2015
Accepted 6 October 2015

Keywords:
Camel
Genetic variability
Microsatellite markers
Population differentiation

ABSTRACT

Camels are the economic backbone of many nomadic tribes of the world including India. Camel population in India is restricted to western part of the country and is represented by eight breeds. The western most state of the country, Gujarat, possesses two camel breeds, Kachchhi and Kharai inhabiting in the same area. Populations of both are facing severe decline which calls for their immediate conservation. In the present study we examined genetic variability and structure in Kachchhi and two populations of Kharai ($n=193$) using 27 microsatellite markers. A total of 138, 112 and 95 alleles were observed in Kachchhi, Kharai (K) and Kharai (A) respectively with the mean effective number of alleles per locus 2.818 ± 0.303; 2.373 ± 0.245 and 2.313 ± 0.224. The mean observed heterozygosity was 0.446 ± 0.039 for Kachchhi, 0.272 ± 0.040 for Kharai (K) and 0.423 ± 0.044 for Kharai (A), which was lower than expected heterozygosity 0.535 ± 0.045, 0.461 ± 0.051 and 0.474 ± 0.043 respectively. The average inbreeding coefficient (F_{IS}) 0.233 ± 0.037 was substantially high in both the breeds. The test for Hardy–Weinberg equilibrium showed significant deviations at most of loci. The mean multilocus F_{ST} value (0.237) suggested significant population differentiation. This was also supported by AMOVA (Analysis of Molecular Variance), Principal component analysis and Bayesian cluster analysis. The genetic distinctness of these camel breeds as revealed by microsatellite analysis may have significant impact on issues concerning conservation and biodiversity.

1. Introduction

Camels belong to the family Camiladae. Genus Camelus consists of *Camelus dromedarius*, dromedary (one hump) camel and *Camelus bactrianus*, Bactrian (two humped) camel. India inhabits mainly dromedary camels and its distribution is restricted to the western part of the country particularly in Rajasthan and Gujarat states with eight recognized camel breeds (www.nbagr.res.in/regcamel.html). Camels in India are mainly reared by landless nomadic or semi-nomadic tribes as an ancestral business. India has experienced recently a sharp decline in camel population. According to recent Livestock Census, the population of Kachchhi camel has declined from 10,477 in 2003 to 8575 in 2007 and that of Kharai just 2173 in 2007 thus, registering approximately 20 percent decline in Kachchhi camel population in last four years. Hence their conservation assumes national priority. Characterization at morphological and genetic level is the first step towards formulating breeding policies and prioritizing the breeds for conservation in an effective and meaningful way. Recent studies have established the usefulness of microsatellite loci as genetic tools for the study of dromedary and Bactrian camelids (Mburu et al., 2003).

Gujarat possesses two breeds, Kachchhi and Kharai (a recently recognized breed). Kachchhi breed inhabit Kachchh and Banaskantha, dry and semi-arid districts of north Gujarat. Kharai (meaning saline adapted) mainly thrive on mangroves and marine vegetation. They are restricted to coastal areas of Kachchh district (Kharai K). A small population of Kharai camel was translocated in the past (300–400 years before) at Aliabet (an erstwhile Narmada river delta) in Bharuch district (Kharai A). Though both, Kachchhi and Kharai camels share many characters they are not only morphologically distinct but differ with respect to milk and wool quality (Anonymous, 2011).

The present study was undertaken to evaluate the genetic diversity and to estimate its relationship among camel population using 27 microsatellite markers.

* Corresponding author at: Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand388001, Gujarat, India. Fax: +91 2692 261486.
E-mail address: dnrank@gmail.com (D.N. Rank).

http://dx.doi.org/10.1016/j.livsci.2015.10.007
1871-1413/

2. Materials and methods

Blood samples were collected from the Jugular vein in 9 ml capacity vacutainer (EDTA, K3) from non-related animals of Kachchhi breed ($n=75$), Kharai (K) ($n=64$) and Kharai (A) populations ($n=54$). Not more than five samples were collected per herd (22 herds per breed were sampled). The collected blood samples were brought to the laboratory on ice. Genomic DNA was extracted manually (John et al., 1991) or by using Kit (HiPurATM Mammalian Genomic DNA Purification Spin Kit, HiMedia).

PCR amplification of DNA samples, using 27 microsatellite markers was carried out in seven multiplex panels. Samples were genotyped by capillary electrophoresis on automated DNA sequencer (ABI PRISM® 310 Genetic Analyzer) using GSLiz500 as size standard. Further analysis of the samples was done using Gene mapper 4.0 version.

2.1. Statistical analysis

Allele frequency, observed and effective number of alleles, observed and expected heterozygosity estimates and Pair wise Nei's genetic distance, mean number of migrants (Nm) were computed using GenAlEx6.41 software. *F*-statistics (F_{IS}, F_{IT}, and F_{ST}) and Allelic Richness (AR) were computed using FSTAT v 2.9.3.2 program (Goudet, 2002) with Jackknifing procedure applied over loci in deriving their significance levels. AR was calculated using a rarefied sample size of 40 diploid individuals per breed. Polymorphism Information Content (PIC) was measured using SAS software. A Fisher's exact test was performed to determine possible deviations from the HWE and genotype linkage disequilibrium for all pairs of loci using GENEPOP v 4.1.4 (Rousset, 2008). The same software was also used for finding the frequency of private alleles. Pair wise distance matrix based on the proportion of shared alleles with individuals as taxonomic unit was utilized to construct neighbor-joining tree using PHYLIP version 3.5 (Felsenstein, 1993). Pair-wise chord distances between individual animals were utilized to perform principal component analysis using SPSS version 13.0.

3. Results

3.1. Microsatellite polymorphism and alleles

Our observation on 193 samples showed that except two loci (YWLL40 and YWL08) all other loci were polymorphic in nature. The number of alleles per polymorphic locus ranged from 2 to 15 in Kachchhi breed, 2 to 13 in Kharai population (K) and 2 to 10 in Kharai population (A). Out of 27 markers, 14 markers in Kachchhi, 11 markers in Kharai (K) and 5 markers in Kharai (A) showed high polymorphism (having more than 4 observed alleles). In the pooled population (all population together), the number of alleles in polymorphic markers ranged from 2 (YWLL29, YWLL36, LCA56) to 19 (CVRL01).

AR is a major decisive factor to measure genetic diversity, and this parameter is of key relevance especially in conservation programs (Foulley and Ollivier, 2006). AR over pooled population per locus was measured at between 2 (LCA56, YWLL29 and YWLL36) and 13.94 (CVRL01) and 5.795 across all loci. A Mean frequency of 0.0846 was obtained for private alleles.

3.2. Diversity estimation

Table 1 depicts various genetic parameters estimated for the three populations based on 25 polymorphic markers. The mean observed heterozygosity (Ho) for Kachchhi (0.446 ± 0.039), Kharai (K) (0.272 ± 0.040) and Kharai (A) (0.423 ± 0.044) where less than

Table 1
Genetic diversity indices across 25 microsatellite markers in camel breeds.

Breed	**Kachchhi**					**Kharai-K**					**Kharai-A**				
Locus	**Na**	**Ne**	**Ho**	**He**	**PIC**	**Na**	**Ne**	**Ho**	**He**	**PIC**	**Na**	**Ne**	**Ho**	**He**	**PIC**
VOPL03	7	1.424	0.713	0.298	0.2874	3	1.598	0.391	0.374	0.3149	3	2.016	0.444	0.504	0.3860
LCA66	7	4.233	0.560	0.764	0.7263	4	2.512	0.000	0.602	0.5488	4	1.955	0.500	0.489	0.4381
LCA66	5	3.954	0.760	0.747	0.7010	5	3.021	0.469	0.669	0.6090	4	3.323	0.759	0.699	0.6426
YWLL44	4	1.977	0.440	0.494	0.4149	3	1.530	0.422	0.346	0.3075	3	1.776	0.389	0.437	0.3933
VOPL08	4	1.538	0.360	0.350	0.3113	3	1.208	0.188	0.172	0.1619	3	1.366	0.315	0.268	0.2367
VOPL32	2	1.785	0.467	0.440	0.3432	5	2.370	0.000	0.578	0.4941	2	1.946	0.463	0.486	0.3680
YWLL59	2	1.676	0.453	0.403	0.3219	2	1.806	0.422	0.446	0.3466	2	1.647	0.389	0.393	0.3157
YWLL38	6	2.910	0.600	0.656	0.5885	6	2.264	0.359	0.558	0.5111	4	2.918	0.642	0.657	0.5919
VOPL67	5	2.127	0.107	0.530	0.4272	1	1.000	0.000	0.000	0.000	3	1.699	0.100	0.411	0.3448
LCA59	3	1.764	0.427	0.433	0.3501	2	1.580	0.422	0.367	0.2997	2	1.480	0.333	0.324	0.2718
LCA56	2	1.540	0.293	0.351	0.2891	2	1.753	0.344	0.430	0.3374	2	1.670	0.222	0.401	0.3207
YWLL29	2	1.609	0.400	0.378	0.3068	2	1.882	0.000	0.469	0.3589	2	1.857	0.426	0.461	0.3550
YWLL36	2	1.301	0.240	0.231	0.2044	1	1.000	0.000	0.000	0.0000	2	1.624	0.259	0.384	0.3103
VOPL10	8	3.806	0.507	0.737	0.6978	7	3.463	0.267	0.711	0.6616	4	3.500	0.615	0.714	0.6637
LCA33	3	2.166	0.440	0.538	0.4677	3	1.768	0.234	0.434	0.3466	4	3.772	0.519	0.735	0.6856
CMS13	8	4.193	0.693	0.762	0.7293	6	4.229	0.571	0.764	0.7263	4	2.078	0.444	0.519	0.4580
CMS121	8	3.513	0.622	0.715	0.6861	8	3.789	0.641	0.736	0.6954	4	1.852	0.444	0.460	0.4274
LCA90	7	4.558	0.693	0.781	0.7472	5	3.101	0.136	0.678	0.6144	4	2.721	0.667	0.633	0.5584
CMS50	9	7.080	0.627	0.859	0.8429	8	4.288	0.317	0.767	0.7317	10	5.982	0.926	0.833	0.8121
LCA18	6	3.754	0.587	0.734	0.6935	2	1.906	0.365	0.475	0.3623	5	1.457	0.296	0.314	0.2838
CMS16	4	1.987	0.459	0.497	0.3977	2	1.117	0.079	0.105	0.0994	2	1.097	0.560	0.088	0.0844
CVRL04	3	2.851	0.676	0.649	0.5761	6	2.842	0.453	0.648	0.6028	4	2.855	0.593	0.650	0.5798
CVRL07	9	4.103	0.400	0.756	0.7186	13	6.344	0.180	0.842	0.8246	5	3.954	0.389	0.747	0.7083
CVRL05	7	1.970	0.465	0.492	0.4620	4	2.419	0.541	0.587	0.5449	5	1.771	0.444	0.435	0.4035
CVRL01	15	6.271	0.597	0.841	0.8228	9	3.269	0.548	0.694	0.6493	8	4.148	0.778	0.759	0.7199
Mean	**5.185**	**2.818**	**0.446**	**0.535**	**NA**	**4.222**	**2.373**	**0.272**	**0.461**	**NA**	**3.593**	**2.313**	**0.423**	**0.474**	**NA**
SE	**0.618**	**0.303**	**0.039**	**0.045**	**NA**	**0.566**	**0.245**	**0.040**	**0.051**	**NA**	**0.378**	**0.224**	**0.044**	**0.043**	**NA**

Na-Observed number of alleles; Ne-Effective number of alleles; Ho-Observed heterozygosity; He-Expected heterozygosity; PIC-Polymorphism Information Content; NA-Not Applicable