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Genetic characterisation of two humped camel of India (*Camelus bactrianus*)

PRIYANKA BANERJEE[1], JYOTI JOSHI[2], UPASNA S[3], NAZIR GANAI[4] and R K VIJH[5]

National Bureau of Animal Genetic Resources Karnal, Haryana 132001 India
and
Sher-e- Kashmir University of Agricultural Sciences and Technology, Chatha, Jammu and Kashmir 192001 India ,

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ABSTRACT

Microsatellite data was generated on 17 bactrian camels using 27 markers. The data analysis revealed a fair degree of heterozygosity existing in these bactrian camel which are very few in number (149). The mean heterozygosity and effective number of alleles were 0.56 and 3.89 respectively. The F statistics also revealed normal rate of inbreeding in this stock. This was attributed to free flow of bactrian camel germplasm between valley and Tibetan plateau till recent times. The population constancy was also established using inter and intralocus tests. Effective conservation measures are suggested owing to small number of individuals and systematic mating plan is advocated.

Key words: *Camelus bactrianus*, Genetic characterization, Microsatellite

The livestock census of India 2007 (http: //dahd.nic.in) pegs the number of two humped camels (*Camelus bactrianus*) to be 149 in the Nubra valley of Jammu and Kashmir in the Ladakh region. The two humped camel is a native of the Gobi desert and occur only in Mongolia and Xinjiang province of China, but is also reported from South Russia, Iran and Afghanistan. These camels were a vital means of transport on the 6,400-km historic 'silk route'. The small bactrian population in India is the group of camels which remained in India after Chinese aggression. In fact, the single-humped dromedarian (*Camelus dromedarius)* camel found in Rajasthan, Arabia and North Africa is generally believed to have evolved from the double – humped bactrian species (Mason 1984). Very little work has been carried out on these camels especially at molecular genetics level (http: // www.nrccamel.res.in). A small head count of these camel pose several critical questions about their conservation. Meager information is available on bactrian camel (Jianlin *et al.* 2004, HongWei *et al.* 2009, Mishra *et al.* 2009) and is perhaps the least worked out domestic livestock owing to its geographical distribution. In this research paper, we report demographic and population genetic parameters based on 17 bactrian camel utilizing 27 microsatellite markers to suggest breeding strategies.

Present address: [1,2] Senior Research Fellow (priyankabnrj @gmail.com, jyotijoshi111@gmail.com), [3]Research Associate (upasna30@gmail.com), [5]Principal Scientist (rameshkvijh@ gmail.com). [4]Associate Professor, Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha, J&K. (drnazirahmad@gmail.com).

MATERIALS AND METHODS

The blood samples (6–8 ml) from 10 bactrian camels of Nubra valley of Ladakh and 7 samples from Jammu and Kashmir Government Livestock Farm Leh were collected. The blood samples were collected using EDTA-coated vacutainer tubes. The genomic DNA was isolated following the standard protocol involving Proteinase K digestion and phenol chloroform extraction (Sambrook *et al.* 2001). The set of primers for polymerase chain reaction (PCR) amplification of microsatellite loci were synthesized and the forward primers were labeled with different fluorescent dyes (Table 1). The PCR amplification was carried out in 25 µl reaction volume consisting of 50 ng genomic DNA, 1.5 mM $MgCl_2$, 200 mM dNTPs, 5 pmol of each primer and 1 U of Taq polynerse. The PCR reaction was carried out in Eppendorf PCRs. The thermocycling conditions utilized were initial denaturation at 95°C for 5 min, followed by 30 cycles of 45 s at 94°C, 45 s at annealing temperature and 1 min at 72°C. The final extension at 72°C was prolonged for 10 min. The samples were analyzed using DNA sequencer with Liz 500 as internal lane standard. The data was collected and analyzed using GeneMapper (Ver 4.0) software.

The basic population genetic parameters for the 27 microsatellite loci - number of alleles, effective number of alleles, observed and expected heterozygosity (Table 2) were estimated from the software POPGENE (Yeh *et al*. 1999).

Table 1. Name of locus, primer sequences, allele size range, repeat motif and number of allele observed

Locus	Primer Forward	Primer Reverse	Min Size	Max Size	Acession Number	No. of Alleles recorded	Type of repeats
CMS121	CAAgAgAACTggTgAggATTTTC	AgTTgATAAAAATACAgCTggAAAg	147	173	AF329159	13	(TG)24
CMS13	TAgCCTgACTCTATCCATTTCTC	ATTATTTggAATTCAACTgTAAgg	236	254	AF329158	9	(AC)27
CMS58	AATATACATCCTCCCAACTggT	TTATTTCTCTTAACCCCTCTCTAA	82\	114	AF329142	11	(AC)18
LCA18	TCCACCCATTTAgACACAAgC	TAggAAgCTCCAAgAAgAAAAgAC	211	233	AF060097	11	(GT)12 T (CA)4
LCA63	TTACCCAgTCCTTCgTggg	ggAACCTCgTggTTATggAA	184	226	AF091123	14	(GT)8 (GC)4 AC (GT)8
VOLP08	CCATTCACCCCATCTCTC	TCgCCAgTgACCTTATTTAgA	142	148	AF305230	4	(TG)10TCCG(TG)2 TCCG(TG)5
CMS50	TTTATAgTCAgAgAgAgTgCTg	TgTAgggTTCATTgTAACA	164	190	AF329149	13	(GT)27
CVRL04	CCCTACCTCTggACTTTg	CCTTTTTgggTATTTTCAg	156	180	AF217604	10	(GT)19
CVRL05	CCCTACCTCTggACTTTg	gCCACTggTCCCTgTCATT	151	179	AF217605	12	(GT)25
LCA90	TATAACCCTggTCTCgCCAA	CCAAgTAgTATTCCATTATgCg	221	259	AF142660	10	(AC)13
VOLP10	CTTTCTCCTTTCCTCCCTACT	CgTCCACTTCCTTCATTTC	242	268	AF305231	11	(TG)2TA(TG)7TA (TG)7
YWLL44	CTCAACAATgCTAgACCTTgg	gAgAACACAggCTggTgAATA	101	111	GU723276	6	AC=TG repeats
CMS16	ATTTTgCAATTTgTTCgTTCTTTC	ggAgTTTATTTgCTTCCAACACTT	181	205	AF329157	7	(TG)34
CVRL01	gAAgAggTTggggCACTAC	CAggCAgATATCCATTgAA	188	244	AF217601	21	(GT)27(GC)6 i(GT)9
CVRL06	TTTTAAAAATTCTgACCAggAgTCTg	CATAATAgCCAAAACATggAAACAAC	182	208	AF217606	8	(TA)5(CA)35 (TA)4
LCA66	gTgCAgCgTCCAAATAgTCA	CCAgCATCgTCCAgTATTCA	232	246	AF091125		(CA)13
VOLP67	TTAgAgggTCTATCCAgTTTC	TggACCTAAAAgAgTggAC	144	180	AF305237	15	(TG)5(G)4(TG)9CG (TG)7
YWLL09	AAgTCTAggAACCggAATgC	AgTCAATCTACACTCCTTgC	144	176	Lang *et al.* 1996	13	-
CVRL02	TgTCACAAATggCAAgAT	AgTgTACgTAgCAgCATTATTT	205	215	AF217602	6	(AT)15(CA)16
CVRL07	AATACCCTAgTTgAAgCTCTgTCCT	gAgTgCCTTTATAAATATgggTCTg	280	314	AF217607	12	(GT)14(AT)14
CVRL08	AATTCCTgTgATTTTATACACA	CATgTCATgAAAgCTACAgTA	191	209	AF217608	5	(CA)10(GA)5
YWLL08	ATCAAgTTTgAggTgCTTTCC	CCATggCATTgTgTTgAAgAC	110	178	Lang *et al.* 1996	25	-
YWLL38	ggCCTAAATCCTACTAgAC	CCTCTCACTCTTgTTCTCCTC	175	189	Lang *et al.* 1996	8	-
VOLP03	AGACGGTTGGGAAGGTGGTAA	CGACAGCAAGGCACAGGA	148	168	AF305228	2	(TG)13
VOLP32	GGAATGGCTTGAAAGGAATG	CGAGCACCTGAAAGAAGACC	183	223	AF305234	-	(GT)19
LCA37	TAATTACCTCCCCCACCACA	TGGACCCAGGACTTGAAATG	143	183	AF060105	-	(CA)22
LCA77	GAGCCTTTTCTTCTTTCTCACG	GGGCAAGAGAGACTGACTGG	206	246	AF091129	-	(CA)(TG)

The F_{IS} values were estimated using GENEPOP software version 4.0 (Raymond *et al*. 1995) by 2 methods of Weir and Cockerham (1984) and Robertson and Hill (1984). The values were estimated using the hypothesis of heterozygote deficiency (H_1) invoking Markov chain with 10000 Dememorization steps, 20 Batches and 5000 iterations per batch. The inter-individual genetic distance was calculated using the software POPULATIONS version 1.2.31 (Olivier 1999) and the tree was constructed using the software Tree Explorer version 2.12.

The severe reduction of effective population size was tested using the Bottleneck software version 1.2.02 (Piry *et al.* 1999). We utilized 3 quantitative tests for each population to know if they were in mutation drift equilibrium. The tests applied were Sign Rank Test, Standardized differences test and Wilcoxon rank test as implemented in the software Bottleneck (Piry *et al.* 1999). The tests were primarily utilized to test the significance between the expected heterozygosity from the number of alleles and observed heterozygosity. We utilized 3 models of microsatellite evolution, viz. infinite allele model (IAM), two phase model (TPM) and stepwise mutation model (SMM).

The data of allele frequency were classified into 10 allelic frequency classes and used for plotting a frequency histogram (Luikart *et al.* 1998). The 10 allelic frequency classes were 0.001–0.100, 0.101–0.200, 0.201–0.300, 0.301–0.400, 0.401–0.500, 0.501–0.600, 0.601–0.700, 0.701–0.800, 0.801–0.900 and 0.901–1.00. The low frequency allelic classes were 0.001– 0.100 and high frequency allelic classes were 0.901–1.000 the rest were termed as intermediate classes. The above classification system was arbitrary but suited graphical qualitative assessment of allele frequency distributions. The classification of the data in large number of allelic classes might have defied the very purpose of meaningful assessment of the distribution of allele frequencies. This graphical method concluded that a population might have been recently bottlenecked if fewer alleles are in low frequency class than in one or more intermediate frequency classes, thus the mode of the distribution get shifted (Maryuma and Feurst 1985).

The k and g tests of Reich and Goldstein (1998) exploits differences between the expected distributions of alleles in populations at mutation drift equilibrium and populations that have recently expanded. These tests were conducted for expansion of the bactrian population.

RESULTS AND DISCUSSION

The 27 microsatellite markers utilised in the present study were highly polymorphic. The mean number of alleles was 6.44 and the number of alleles ranged from 3–15 alleles. The effective number of alleles was 3.90, which is quite low and may be attributed to large number of alleles being at low frequency. The mean observed heterozygosity was 0.56 and expected heterozygosity was calculated as 0.72. The significant difference in the 2 values is due to inbreeding or random mating of individuals leading to loss of heterozygosity (Fig. 1).

Where in Na is the number of alleles, Na Freq means the number of alleles with a frequency of greater than 5% (4.74), Ne is the effective number of alleles and I is the Shannon index.

The values obtained in Table 2 for observed heterozygosity (0.56) and effective number of alleles (3.89) were very similar

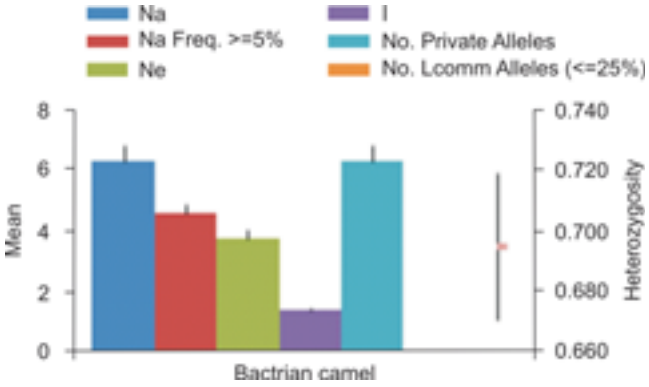
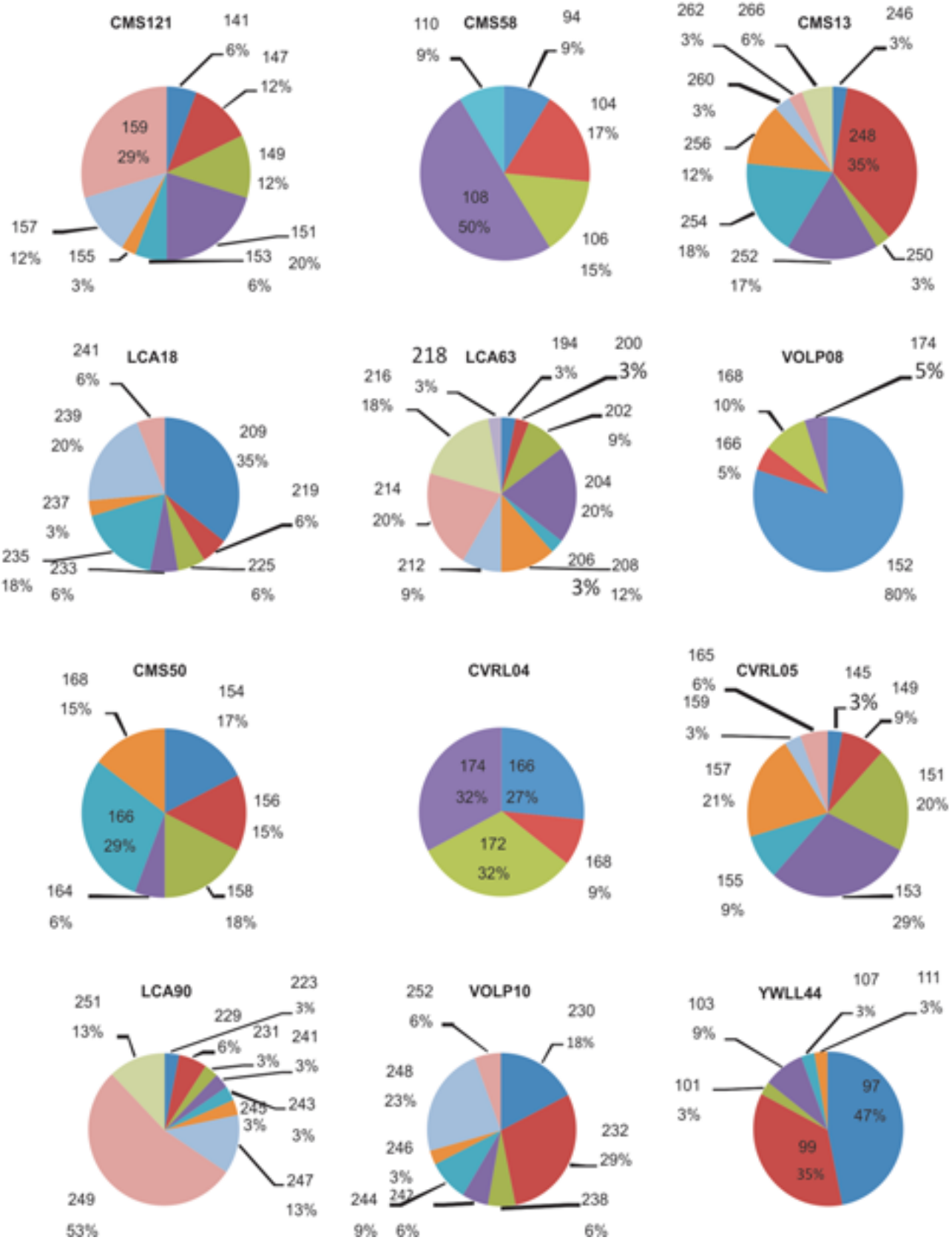


Fig. 1. Allelic patterns in double humped camel.

Table 2. Table showing number of alleles, effective number of alleles, observed and expected heterozygosity and F_{IS} values for all 27 loci.

Locus	Na	Ne	Obs_Het	Exp_Het	Fis
CMS121	8	5.61	0.94	0.85	–0.15
CMS13	9	4.82	0.76	0.82	0.03
CMS58	5	3.14	0.76	0.70	–0.12
LCA18	8	4.70	0.59	0.81	0.25
LCA63	10	6.72	0.76	0.88	0.10
VOLP08	4	1.53	0.20	0.36	0.42
CMS50	6	5.12	0.88	0.83	–0.10
CVRL04	4	3.48	0.47	0.73	0.34
CVRL05	8	5.21	0.88	0.83	–0.09
LCA90	9	3.10	0.56	0.70	0.17
VOLP10	8	5.21	0.82	0.83	–0.02
YWLL44	6	2.81	0.35	0.66	0.45
CMS16	5	2.82	0.70	0.68	–0.09
CVRL01	15	9.32	0.76	0.92	0.14
CVRL06	4	1.95	0.56	0.50	–0.15
LCA66	4	2.74	0.41	0.65	0.35
VOLP67	7	3.68	0.71	0.75	0.03
YWLL09	6	2.95	0.29	0.68	0.56
CVRL02	3	2.11	0.00	0.54	1.00
CVRL07	4	2.25	0.41	0.57	0.26
CVRL08	5	3.50	0.82	0.74	–0.15
YWLL08	8	5.12	1.00	0.83	–0.24
YWLL38	4	2.53	0.44	0.63	0.28
VOLP03	5	3.85	0.70	0.78	0.05
VOLP32	12	5.45	0.76	0.84	0.06
LCA37	4	3.28	0.38	0.72	0.46
LCA77	3	2.24	0.00	0.57	1.00
Mean	6.44	3.90	0.59	0.72	0.15


CMS121
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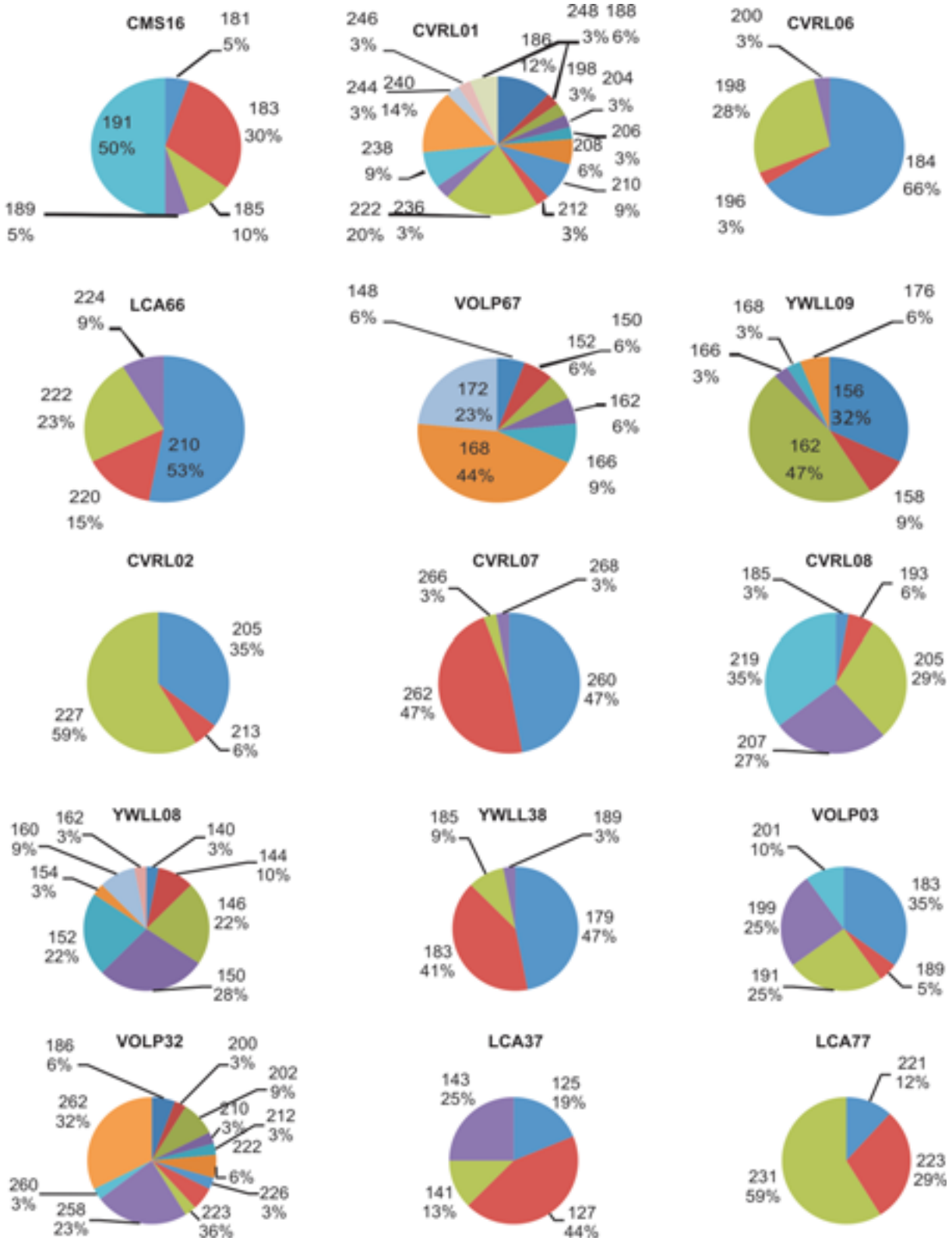

Fig. 2. Pie chart of different microsatellite loci showing their frequency and allele size.

to those reported by HongWei *et al.* (2009) for Chinese and Mongolian bactrian camel, which were 0.61 and 3.55 for observed heterozygosity and effective number of alleles respectively. Jianlin *et al.* (2004) reported that there was not much difference between the Chinese and Mongolian bactrian camel for the two parameters. The large differences in the observed and expected number of alleles are attributed to the fact that large number of alleles are at low frequency. The values were also very similar to those obtained for Indian dromedarian camel for the 4 breeds of camel (Vijh *et al.* 2007). Locus wise allele frequencies are given in the Fig. 2. The contribution of each allele for all loci and their frequency along with their allele size is depicted in the pie chart. The allele size with maximum frequency covers a larger proportion and relative value of each allele at a locus is depicted in pie charts.

The deviation from Hardy Weinberg equilibrium was calculated using GENEPOP software version 4.0. The probability values signifying deviation from Hardy Weinberg Equilibrium are presented in the Table 3.

Robertson and Hill (1984) reported a lower variance under the null hypothesis. The tests revealed that out of the 27 loci analysed only 5 loci deviated from Hardy-Weinberg equilibrium and these are LCA77, LCA37, VOLP32, CVRL02 and YWLL09.

Table 3. Values for F_{IS}, Weir and Cockerham (1984) and Robertson and Hill (1984).

Locus	P-value	Weir and Cockerham estimate	Robertson and Hill estimate
CMS121	0.25	-0.12	0.03
CMS13	0.35	0.07	0.03
CMS58	0.88	-0.09	-0.11
LCA18	0.03	0.28	0.18
LCA63	0.08	0.13	0.08
VOLP08	0.06	0.46	0.06
CMS50	0.77	-0.07	-0.07
CVRL04	0.04	0.37	0.28
CVRL05	0.82	-0.06	-0.06
LCA90	0.12	0.20	0.06
VOLP10	0.46	0.01	0.00
YWLL44	0.03	0.48	0.15
CMS16	0.73	-0.03	-0.05
CVRL01	0.01	0.17	0.09
CVRL06	0.79	-0.12	-0.04
LCA66	0.12	0.38	0.16
VOLP67	0.32	0.06	0.01
YWLL09	0.00	0.58	0.47
CVRL02	0.00	1.00	1.06
CVRL07	0.15	0.29	0.12
CVRL08	0.72	-0.12	-0.05
YWLL08	1.00	-0.21	-0.11
YWLL38	0.15	0.31	0.13
VOLP03	0.39	0.11	0.02
VOLP32	0.00	0.09	0.24
LCA37	0.00	0.49	0.57
LCA77	0.00	1.00	1.06

In bactrian camel, the sample size was 17 i.e., 34 haploid genomes. Out of 27 loci, the expected number of loci with heterozygote excess was 15.84 under IAM, 16.15 under TPM and 16.06 under SMM. The number of loci with heterozygosity excess observed in the study was 22, 16 and 11 loci respectively for the 3 mutational models. The probability values were 0.01089 (IAM), 0.54863 for (TPM) and 0.03800 for (SMM) respectively. The sign test revealed bottleneck in the population of bactrian camel under IAM while the null hypothesis of mutation drift equilibrium is accepted under TPM and rejected for SMM due to heterozygotic deficiency. Similar results were obtained for the standardized difference test in which the T2 values were calculated. The T2 values were 2.43, 0.13 and –3.16 and null hypothesis of mutation drift equilibrium is rejected for IAM (>1.645 (table value)) while it is accepted for TPM and rejected due to heterozygotic deficiency in SMM. The Wilcoxon rank test for bactrian camel also reveals similar results the probability values being 0.00325 (<0.05) i.e. accepting null hypothesis of mutation drift equilibrium. The mode shift reveals no distortion of allelic frequencies distribution and the graphic representation is normal L-shaped distribution (Fig. 3).

The analysis of data on the basis of microsatellite loci revealed that there is not any severe reduction in the effective population size of the bactrian camel in India though the population size has been very small. The geographic distribution must have provided room for bactrian camel for flow of germplasm across international borders which has of late been restricted. Under the circumstances the effective population size of bactrian has not been too small especially when the total population of bactrian camel is only 149 heads. It is presumed that these camels must be the remnants of a migratory herd and must have been heterogeneous and no bottleneck was detected. Thus the bactrian camels of India are at mutation drift equilibrium.

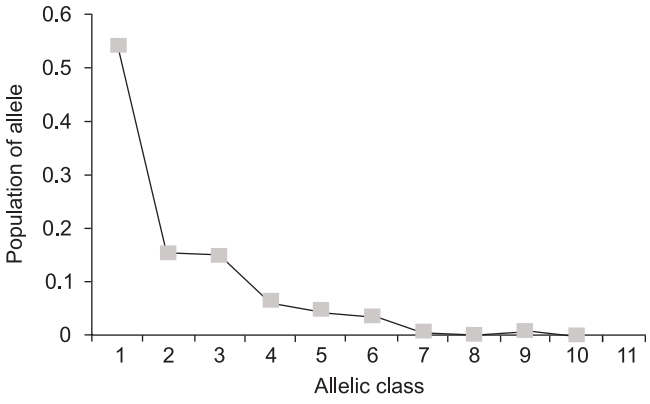

Fig. 3. L-shaped graph revealing no Mode-shift.

The k-test of Reich and Goldstein (1998) exploits differences between the expected distributions of alleles in populations at mutation drift equilibrium and populations that have recently expanded. The g-test of Reich and Goldstein (1998) which compares the between-loci variance in the number of repeats with a theoretical expectation derived assuming that the loci follow SMM and that the population size is stable. We performed both the k- and the g-tests. k-statistics were calculated for each locus, and the significance of the proportion of positive k values was based on a binomial distribution with the probability of a positive k set conservatively as 0.515 (Reich *et al.* 1999). Significance levels for the g-test were compared to the values given in Reich *et al.* (1999). The inter-locus test (g test) revealed the value to be 2.4864. This was higher than the fifth percentile cut off of g value (Reich *et al.* 1999). Thus the inter locus g test reveals the constancy of population size. The within locus test (k test) showed 12 loci to be with negative values while 17 loci had positive values. The probability value was 0.72, rejecting the hypothesis of expansion of population.

The inter-individual genetic distances were calculated using allele sharing method and tree constructed using neighbor joining algorithm (Saitou and Nei 1987). The 17 individuals analyzed grouped into 2 distinct clusters. The camels DH11-DH17 belonged to the camel farm at Ladakh presently maintained by Jammu and Kashmir Government. All these camels clubbed together from rest of the bactrian camels of Nubra valley (Fig. 4).

The data analysis revealed that there is good amount of heterozygosity in Indian bactrian camel, which can be exploited by developing conservation strategies and suitable breeding plans. Due to very small number of two humped camels, efforts need to be made to preserve the germplasm by declaring them as heritage animals. These animals are considered to be nuisance by the local population as they destroy the fields. Locals may be educated about the alternative usage of these animals for carting on roads and also as tourist attraction. More so these camels can be reared by army for using these camels for baggage as they are sure footed, adapted to extreme cold climatic conditions and can carry much more weight in area inaccessible by road compared to mules being utilized at present. As bactrian camels are very hardy animals, selection can be made for exploiting their milk production capabilities and utilizing their undercoats. Systematic breeding plans need to be implemented to avoid inbreeding and maintenance of its effective population size. Provision of veterinary help and vaccination are must to maintain these heritage camels of India. Scientific breeding plans need to be prepared to avoid inbreeding which may adversely affect survivability of these Bactrian camel of India.

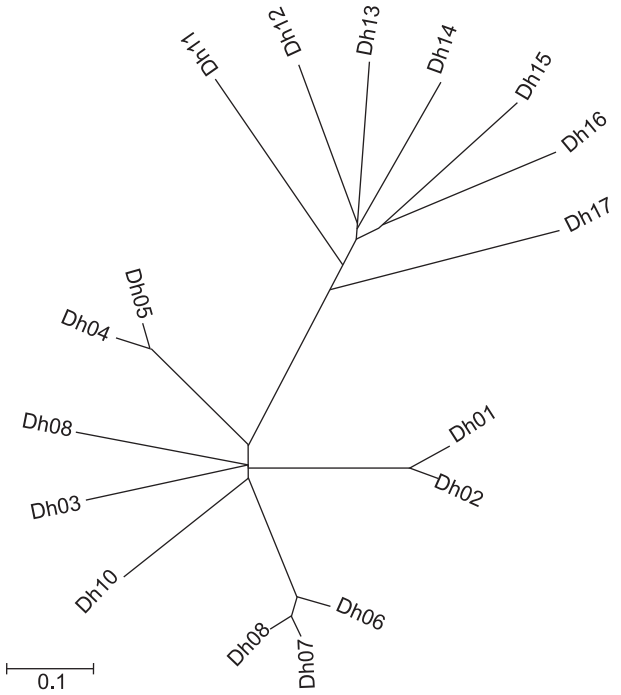

Fig. 4. Neighbor Joining tree using Allele sharing method in bactrian camels of India.

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