

## Molecular Assessment of Genetic Diversity in Dromedaries and Bactrian Camel Using Microsatellite Markers

N. Hedayat-Evrigh<sup>1\*</sup>, S. R. Miraei-Ashtiani<sup>2</sup>, M. Moradi Shahrebabak<sup>2</sup>, R. Khalkhali Evrigh<sup>3</sup>, and K. Pourasad<sup>2</sup>

### ABSTRACT

Dromedary and Bactrian camels are two species of camel in Iran that have ecological adaptation to cold and hot desert area, respectively. They play an important role in the life and food security of nomadic tribes. The present study was conducted to investigate genetic diversity of 180 Iranian camels using microsatellite markers. In a panel of 20 microsatellite markers, we observed 214 alleles with a mean number of 10.7 alleles per locus. All loci exhibited  $PIC$  values more than 0.7. The genetic differentiation values ( $F_{ST}$ ) per locus was different from 0.01 to 0.039 with an average of 0.021 across all loci. The estimate of genetic differentiation level between all Iranian camel populations in this study was low ( $F_{ST}$ : 0.008-0.021). High gene flow between populations was also observed. Phylogenetic tree illustrated that the highest genetic distance was between Bactrian and dromedary camel from YaD. However, the results of the present microsatellite analyses showed close genetic relationship in the studied populations. All of the population-locus combinations showed significant deviations ( $P < 0.01$ ) from Hardy-Weinberg equilibrium.

**Keyword:** Genetic conservation, Gene flow, Hardy-Weinberg equilibrium, Phylogenetic tree

### INTRODUCTION

Camel is one of the most successful animals in the evolution process. During their evolution, camels have acquired features that made them the best choice for living in harsh environments. Camels are cheap for keeping due to their ability to eat low-quality food and turn it into meat and milk in desert regions. Based on FAO definition, food security depends on the quantity and quality of food produced, as well as on its availability all the times (FAO, 2015). Camels are responsible for supporting a significant part of food security in arid climates.

Iran is located in one of the most arid regions in the world and over 84 percent of the country is arid and semi-arid (Lehane, 2014). Accordingly, it seems that in the near future, camel could be the dominant domestic animal in Iran. However, camel's breeding and husbandry in Iran is traditional, and little efforts have been made to convert them as farm animals.

Genetic diversity is a basic factor in carrying out breeding schemes and genetic conservation programs (Sharma *et al.*, 2015). Reduced populations of some species have raised conservation concerns in many regions in the world. Camel is one of the species that are faced with worldwide unkindness in recent years. Sharp decrease in camel population in

<sup>1</sup> Department of Animal Science, Faculty of Agricultural and Natural Resource, University of Mohagheh Ardabili, Ardabil, Islamic Republic of Iran.

\* Corresponding author; e-mail: nhedayat@uma.ac.ir

<sup>2</sup> Department of Animal Science, Faculty of Agriculture and Natural Resources, University of Tehran, Karaj, Islamic Republic of Iran.

<sup>3</sup> Department of Animal Science, Sari Agricultural Sciences and Natural Resources University, Sari, Mazandaran, Islamic Republic of Iran.



some countries such as India (Prasad *et al.*, 2015), Mongolia (Chuluunbat *et al.*, 2014) and Iran is not the good sign for future of this unique species. It is worth to mention that there are less than 150 Bactrian camels in Iran and this valuable animal is known as endangered species (Khademi, 2017). Modernization and changes in the lifestyle of people, as well as categorizing camels as “animals of primitive societies” (Yagil *et al.*, 1994) lead to decline in camel’s product acceptance and, consequently, lower camel husbandry. Small populations lose genetic diversity as a result of genetic drift and inbreeding. Conservation of genetic variation is important due to its role in empowering the populations for response to environmental changes as well as its correlation with reduced population fitness (Reed *et al.*, 2003). Assessment of genetic variation within and between species is necessary to understand population structure, local adaptation, and differentiation between populations (Bos *et al.*, 2008). Since some countries, especially Iran, have both species of camels, it might be expected to observe hybrid animals. Therefore, it is possible to investigate the hybridization between the mentioned species using genetic markers. Microsatellite marker due to its random distribution across the genome, codominance and highly polymorphic (Putman *et al.*, 2014) are ideal for estimation of genetic diversity and the relationship among the livestock breeds and species (Hampton *et al.*, 2004). They are also useful for evaluation of genetic distance and detection of population bottlenecks (Putman *et al.*, 2014). The aim of this study was to assess the genetic relationships between Iranian camels population using microsatellite markers polymorphisms.

## MATERIALS AND METHODS

### Sampling and Genomic DNA Extraction

We used four geographic subpopulations of camels including one area for Bactrian camels (ArB) from Ardabil Province in the northwest

of Iran (cold area) and three for dromedary camels in the center of Iran (hot area) including: (1) Trod Station (TrD) (camels collected from all over Iran and this station is the gene bank of dromedary camels in country); (2) Semnan Province (SeD) (that was a local herd in north of Dashte-e Kavir), and (3) Yazd Station (YaD) (in the center of Iran).

Blood samples were collected from Jugular vein using 4 mL vacutainer tubes containing EDTA ( $1 \text{ mg mL}^{-1}$ ) of 180 camels including 140 *Camelus dromedarius* (50, 55, and 35 samples from YaD, TrD, and SeD, respectively) and 40 *Camelus bactrianus* (from Ardabil Province) (Figure 1) according to guidelines of the Iranian Council of Animal Care (1995). Genomic DNA was purified using Genomic DNA Mini Kit (Real Biotech Corporation, RBC, South Korea).

### Microsatellite Markers, PCR Amplification, and Genotyping

All test animals were genotyped using 20 microsatellite markers (FAO, 2011) (Table 1). Amplification was carried out in a total volume of 25  $\mu\text{L}$  containing 1.2 mM  $\text{MgCl}_2$ , 0.2 mM dNTP, 1.5 U Taq DNA polymerase, 0.15  $\mu\text{M}$  of forward and reverse primers (Invitrogen) and 100 ng genomic DNA. Thermo cycling profile consisted of initial denaturation at  $94^\circ\text{C}$  for 5 minutes, followed by 35 cycles of  $94^\circ\text{C}$  for 40 seconds, annealing at  $51\text{-}64^\circ\text{C}$  for 40 seconds and extension at  $72^\circ\text{C}$  for 40 seconds, with a final extension step at  $72^\circ\text{C}$  for 5 minutes.

The PCR amplified products (microsatellite loci) were genotyped by 8% PolyAcrylamide Gel Electrophoresis (PAGE) using the manual sequencer (Bio-Rad: Sequi-Gen@ GT), and colored with silver nitrate (Benbouza *et al.*, 2006).

### Statistical Analyses

The basic parameters for each locus and population including allele frequencies, effective Number of alleles ( $N_e$ ), observed



**Figure 1.** Map of Iran and sampling areas. Bactrian camels (ArB), Trods Station (TrD), Semnan Province (SeD) and Yazd Station (YaD).

**Table 1.** Characteristics of primers used in current study.

Loci	Forward 5' to 3'	Backward 5' to 3'	Accession No. N	Annealing T <sub>m</sub>
CMS16	<i>ATTTGCAATTTGTTTCGTTCTTTC</i>	<i>GGAGTTTATTTGCTTCCAACACTT</i>	AF329157	60
CMS50	<i>TTTATAGTCAGAGAGAGTGCTG</i>	<i>TGTAGGGTTTCATTGTAACA</i>	AF329149	58
CVRL01	<i>GAAGAGGTTGGGGCACTAC</i>	<i>CAGGCAGATATCCATTGAA</i>	AF217601	57
CVRL02	<i>TGTCACAAATGGCAAGAT</i>	<i>AGTGTACGTAGCAGCATTATTT</i>	AF217602	58
CVRL06	<i>TTTTAAAAATTCTGACCAGGAGTC</i>	<i>CATAATAGCCAAAACATGGAAACAAC</i>	AF217606	62
CVRL07	<i>AATACCCTAGTTGAAGCTCTGTC</i>	<i>GAGTGCCTTTATAAATATGGGTCTG</i>	AF217607	55
LCA37	<i>AAACCTAATTACCTCCCCCA</i>	<i>CCATGTAGTTGCAGGACACG</i>	AF060105	54
LCA56	<i>ATGGTGTTTACAGGGCGTTG</i>	<i>GCATTACTGAAAAGCCCAGG</i>	AF091122	57
LCA63	<i>TTACCCAGTCCTTCGTGGG</i>	<i>GGAACTCGTGGTTATGGAA</i>	AF091123	64
LCA65	<i>TTTTCCCTGTGGTTGAAT</i>	<i>AACTCAGCTGTTGTCAGGGG</i>	AF091124	55
LCA66	<i>GTGCAGCGTCCAAATAGTCA</i>	<i>CCAGCATCGTCCAGTATTCA</i>	AF091125	58
LCA70	<i>TTCTGATGTATGGCATAGCGA</i>	<i>TGGGGGTAAAGAGCAGGATAA</i>	AF091127	58
LCA77	<i>TGTTGACTAGAGCCTTTTCTTCTT</i>	<i>GGGCAAGAGAGACTGACTGG</i>	AF091129	56
VOLP03	<i>AGACGGTTGGGAAGGTGGTA</i>	<i>CGACAGCAAGGCACAGGA</i>	AF305228	62
VOLP32	<i>GTGATCGGAATGGCTTGAAA</i>	<i>CAGCGAGCACCTGAAAGAA</i>	AF305234	59
VOLP67	<i>TTAGAGGGTCTATCCAGTTTC</i>	<i>TGGACCTAAAAGAGTGGAC</i>	AF305237	51
YWLL08	<i>ATCAAGTTTGAGGTGCTTTCC</i>	<i>CCATGGCATTGTGTTGAAGAC</i>	FAO2011	56
YWLL38	<i>GGCCTAAATCCTACTAGAC</i>	<i>CCTCTCACTCTTGTCTCCTC</i>	FAO 2011	60
YWLL44	<i>CTCAACAATGCTAGACCTTGG</i>	<i>GAGAACACAGGCTGGTGAATA</i>	FAO 20011	62
VOLP10	<i>CTTCTCCTTCTCCTACT</i>	<i>CGTCCACTTCTTCATTTC</i>	AF305231	58



Number of alleles (No), expected (He) and observed (Ho) heterozygosity, Number of migrants (Nm) and Wright's  $F$ -statistics ( $F_{IS}$ ,  $F_{ST}$ , and  $F_{IT}$ ) within the camel populations were calculated by using GenALex version 6.5 (Peakall and Smouse, 2012) (Table 2). Deviations from Hardy-Weinberg Equilibrium (HWE) were also calculated by using GenAlex 6.5 and Polymorphic Information Content (PIC) values for each locus and population were calculated by Excel microsatellite toolkit 3.3.1. The allelic frequency data were used to compute unbiased estimates of genetic distance for small sample sizes (Nei, 1987).

All genetic distances and the un-weighted pair-group method with an arithmetic mean (UPGMA) algorithm were implemented in the phylogenetic analysis using parsimony to construct phylogenetic trees based on microsatellite molecular markers calculated by Population 1.2.32 (Langella, 2011).

applied to determine population subdivisions and genetic differentiation using the STRUCTURE version 2.3.4 (Falush et al., 2012). Based on the Markov Chain Monte Carlo (MCMC) method, the program was run 10 times for each defined  $K$  ( $K=2$  to  $K=10$ ) genetic population. The results were obtained using admixture and correlated allele frequencies model based on  $10^5$  iterations burn-in period with individual admixture alpha set to a uniform prior with value 1.0 and continued by  $10^6$  iterations. (Druml et al., 2012). The number of subpopulations ( $K$ ) was identified based on maximum likelihood and delta  $K$  ( $\Delta K$ ) values.

## RESULTS

### Genetic Diversity in Iranian Camel Populations

Allelic data for 180 camels including 140 *Camelus dromedarius* and 40 *Camelus bactrianus* was generated by genotyping 20

#### Microsatellite Bayesian Cluster Analyses

A Bayesian clustering approach was

**Table 2.** Wright's  $F$ -statistics ( $F_{IS}$ ,  $F_{ST}$ , and  $F_{IT}$ ) and Number of migrants (Nm) in populations using microsatellite markers

Loci	$N_m$	$F_{ST}$ (P value)	$F_{IT}$	$F_{IS}$ (P value)
CVRL01	25.317	0.010(0.26)	0.225	0.217(0.048)
VOLP32	13.964	0.018(0.01)	0.017	0.035(0.734)
YWLL08	17.249	0.014(0.035)	0.069	0.055(0.971)
CMS16	19.646	0.013(0.073)	0.026	0.014(0.827)
CMS50	6.177	0.039(0.002)	0.212	0.180(0.062)
VOLP03	19.497	0.013(0.067)	0.089	0.077(0.983)
CVRL02	6.753	0.035(0.001)	0.119	0.087(0.827)
CVRL06	25.317	0.010(0.002)	0.225	0.217(0.049)
CVRL07	16.078	0.015(0.072)	0.113	0.099(0.942)
LCA37	16.720	0.016(0.224)	0.115	0.099(0.926)
LCA56	6.753	0.041(0.015)	0.215	0.188(0.098)
LCA63	19.625	0.017(0.034)	0.029	0.016(0.918)
LCA65	6.978	0.045(0.165)	0.125	0.097(0.955)
LCA66	7.471	0.032(0.006)	0.120	0.091(0.980)
LCA70	6.277	0.039(0.002)	0.210	0.185(0.0964)
LCA77	21.107	0.012(0.003)	0.105	0.094(0.994)
YWLL38	6.220	0.012(0.002)	0.212	0.089(0.994)
VOLP67	25.565	0.010(0.017)	0.241	0.234(0.034)
YWLL44	25.105	0.010(0.243)	0.237	0.229(0.044)
VOLP10	8.159	0.030(0.015)	0.108	0.081(0.984)
Mean (SE)	15.444 (1.622)	0.021 (0.003)	0.133 (0.017)	0.115 (0.017)

specific microsatellite loci. A total of 214 alleles with a mean number of 10.7 alleles ( $N_o$ ) were observed per locus. The average observed gene diversity ( $H_o$ ) was 0.74 [ranged from 0.61 (LCA70) to 0.867 (VOLP32)]. The average expected Heterozygosity ( $H_e$ ) across 20 loci was 0.861 [ranging from 0.787 (LCA70) to 0.828 (YWLL08)]. The comparison of average expected ( $H_e$ ) and observed ( $H_o$ ) values showed no significant differences among four populations. Moreover, the mean  $H_o$  values were 0.718, 0.752, 0.737, and 0.754, and  $H_e$  values were 0.835, 0.872, 0.863, and 0.872 for ArB, TrD, SeD and YaD, respectively. All loci exhibited  $PIC$  values more than 0.7, indicating high levels of polymorphism (Table 3). The highest and lowest  $PIC$  values were obtained for locus YWLL44 (0.887) and CMS50 (0.779), respectively. We observed three private alleles in YaD population for LCA66, YWLL44 and VOLP10 loci, other alleles were shared between populations.

### Heterozygosity and Test for Disequilibrium

The heterozygosity estimates of individual loci are presented in Table 4. The entire population-locus combinations were tested for deviations from Hardy-Weinberg Equilibrium (HWE). All of the population-locus combinations showed significant deviations ( $P < 0.01$ ) from HWE. Also, HWE test carried out for each markers in separate population (Supplementary Table 1) showed significant deviation ( $P < 0.05$ ) for all loci in

all populations. This result might be because of population subdivision, nonrandom mating, co-ancestry (sampling of siblings) or null alleles.

### Microsatellite Bayesian Cluster Analyses

The STRUCTURE software was used to determine the population structure and degree of admixture. All runs showed a meaningful pattern of distinct population based on each  $K$ . However, the highest log likelihood scores was obtained for  $K=6$  in dromedary camel, while it was  $K=3$  for Bactrian camel (Figure 2). The log likelihood values for  $K=2-10$  is shown in supplementary Table 2.

### Genetic Differentiation between Populations and Phylogenetic Analysis

The genetic differentiation values ( $F_{ST}$ ) per locus was different from 0.01 (CVRL01) to 0.039 (LCA70) with an average of 0.021 across all the loci based on microsatellite markers. Based on  $F_{ST}$  analysis, highest and lowest genetic differentiations were observed between Bactrian camels and YaD dromedaries ( $F_{ST}=0.021$ ) and between TrD and of SeD dromedaries ( $F_{ST}=0.008$ ), respectively (Table 5).

With Nei's  $D_{XY}$  distance, minimum value was observed for Trod Station-YaD (0.112) and maximum for ArB-YaD (0.258) pair

**Table 3.** Genetic variation in 20 microsatellite loci in Iranian camels.

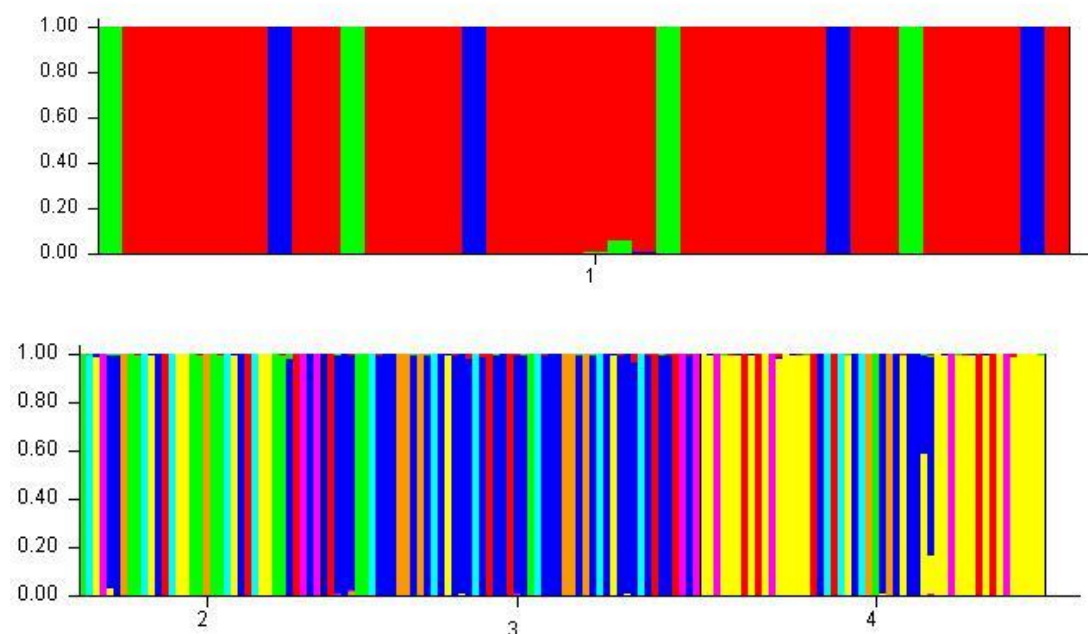
Population	Microsatellite information				
	A/Locus <sup>a</sup>	A <sup>b</sup>	Ho <sup>c</sup>	He <sup>d</sup>	F <sub>IS</sub>
ArB	10.1±0.53	6.59±0.51	0.718±0.030	0.835±0.012	0.140±0.029
TrD	10.6±0.51	7.33±0.51	0.752±0.031	0.872±0.010	0.138±0.032
YaD	11.2±0.29	7.38±0.35	0.754±0.029	0.872±0.008	0.135±0.031
SeD	10.9±0.51	7.17±0.38	0.737±0.027	0.873±0.006	0.156±0.026

<sup>a</sup> Mean number of Alleles per Locus; <sup>b</sup> Effective Allele; <sup>c</sup> Observed Heterozygosity, <sup>d</sup> Expected Heterozygosity.

**Table 4.** Diversity measures in four Iranian Camel populations across 20 microsatellite loci.

	No <sup>a</sup>	Ne <sup>b</sup>	Ho <sup>c</sup>	He <sup>d</sup>	PIC <sup>e</sup>
CMS16	10±2.91	7.671±0.25	0.861±0.025	0.884±0.012	0.847
CMS50	7±2.81	4.725±0.57	0.639±0.029	0.807±0.030	0.779
CVRL01	11.25±2.08	7.453±0.94	0.678±0.018	0.881±0.013	0.869
CVRL02	10±2.18	6.478±0.25	0.743±0.040	0.875±0.007	0.86
CVRL06	12.25±2.89	7.793±0.25	0.668±0.042	0.875±0.004	0.869
CVRL07	12±2.97	7.963±0.64	0.765±0.015	0.877±0.004	0.844
LCA37	11.75±2.34	7.863±0.67	0.758±0.021	0.868±0.006	0.876
LCA56	8±1.74	5.71±0.50	0.629±0.032	0.797±0.005	0.845
LCA63	11±1.84	8.671±0.84	0.841±0.026	0.864±0.002	0.87
LCA65	10±1.94	6.478±0.50	0.733±0.043	0.845±0.005	0.812
LCA66	10.25±2.04	6.592±0.57	0.755±0.039	0.847±0.017	0.861
LCA70	8.5±1.67	5.884±0.45	0.61±0.022	0.787±0.010	0.856
LCA77	11.5±2.32	7.998±0.28	0.783±0.043	0.888±0.011	0.876
VOLP03	11±2.12	5.406±0.49	0.744±0.015	0.828±0.008	0.807
VOLP10	10.25±2.04	6.749±0.50	0.794±0.027	0.879±0.005	0.865
VOLP32	9±1.89	6.682±0.25	0.867±0.032	0.866±0.008	0.85
VOLP67	13±2.49	7.689±0.85	0.661±0.036	0.88±0.002	0.868
YWLL08	12±2.72	8.849±0.75	0.828±0.032	0.899±0.007	0.887
YWLL38	12±2.76	7.844±0.50	0.778±0.038	0.888±0.003	0.806
YWLL44	13.25±2.96	7.842±0.84	0.667±0.029	0.882±0.005	0.87
mean	10.7±1.06	7.1±0.22	0.74±0.014	0.861±0.005	0.851

<sup>a</sup> Observed Number of alleles; <sup>b</sup> Effective Number of alleles; <sup>c</sup> Observed Heterozygosity; <sup>d</sup> Expected Heterozygosity, <sup>e</sup> Polymorphic Information Content.



**Figure 2.** Bayesian clustering (180 animals) using 20 markers for Bactrian (upper) and dromedary camels (lower). The clustering was obtained from STRUCTURE, for a model admixture and correlated allele frequencies between populations. The colored segment shows the individuals estimation proportion of membership (average across 10 run in k= 3 and k= 6)

(Table 5). The phylogenetic analysis with Population software confirmed the result of genetic distance analysis that was obtained by GenAlex software. The phylogenetic tree presented in Figure 3 illustrates the distance between populations, in a way that the distance between the ArB-YaD dromedaries and between the ArB-TrD dromedaries are more than the distance between the ArB and SeD dromedaries.

## DISCUSSION

In Iran, several modern domestic dromedary and Bactrian camel populations and subpopulations with distinct morphological features were divided due to geographical and artificial separation by human. Although proper environmental conditions for Bactrian (cold regions) and dromedary camels (hot regions) are different, but, because of diverse climate zones, both species of camel live in Iran. Since 2009, Iranian camels' population has experienced a sharp decline (FAOSTAT, 2014) and concerns about the loss of genetic diversity are rising.

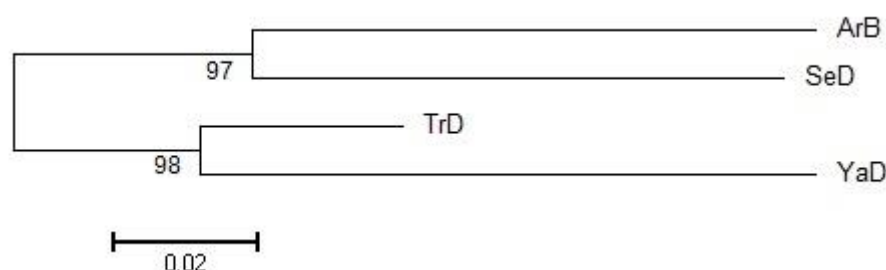
This study seems to be the first comprehensive study about Iranian camels using microsatellite marker and some complementary analyses to the investigation of genetic diversity across camel populations.

## Genetic Diversity Analysis

The results of microsatellite markers analyses indicated high diversity within populations. High genetic variation among the dromedaries might be due to high levels of cross-breeding between different breeds or migration from one place to another. In fact, migration increases the chance of cross-breeding and heterozygosity, however, continued migration decreases the genetic differentiation between subpopulations. These results also showed a close genetic relationship, relatively low distance, and common ancestor among Iranian dromedary camels and high gene flow between populations (Supplementary Table 3). The mean of the number of effective migrants in the studied population was 15.44 (Table 2). High gene flow in Iranian camels may be

**Table 5.**  $F_{ST}$  (upper) (P value) and  $D_{XY}$  (lower) between populations with microsatellite markers.

population	ArB	SeD	TrD	YaD
ArB	-	0.018(0.004)	0.017(0.001)	0.021(0.001)
SeD	0.195	-	0.008(0.255)	0.011(0.003)
TrD	0.191	0.095	-	0.009(0.165)
YaD	0.251	0.142	0.112	-



**Figure 3.** Neighbor Joining Tree constructed between sampled populations of Iranian camels in this study. Bactrian camels (ArB), Trod Station (TrD), Semnan Province (SeD) and Yazd Station (YaD).



explained by their rearing and management system. Most of the herders in two large desert regions of Iran (Dasht-e Kavir and Dashte-e Loot Deserts) leave their herds in the deserts for the whole year, all camel strains are grown in mixtures, and there is no strategy for breeding goals. Thus gene flow increased by crossing between various populations from various areas. Regarding genetic variability, analysis of 20 microsatellite markers in this study revealed that the observed number of alleles at each locus varied from 7 (CMS50) to 13.25 (YWLL44), indicating notable diversity in studied loci. An average number of alleles per locus between the four regional populations ranged from 10.1 to 11.2 for Bactrian and YaD dromedaries, respectively. Low mean effective number of alleles in our study (7.1) compared with the observed number of alleles (10.7) suggested that there were many alleles with low frequencies in Iranian camel populations. Effective number of alleles in our study was comparable with racing camels from Australia (10.59) (Spencer *et al.*, 2010), but reported  $N_e$  values for Kenyan camels (7) (Mburu *et al.*, 2003), Sudanese camels (4.15) (Eltanany *et al.*, 2015), Kachchhi (2.818) and Kharai-k (2.373) breeds in India (Patel *et al.*, 2015) were significantly lower than our calculated value for the mentioned parameter.

Average heterozygosity of useful markers to measure genetic diversity in population must be more than 0.3 (Takezaki *et al.*, 1996). Observed heterozygosity for Iranian Bactrian camels in the present work (0.718) was in accordance with previously reported value (0.714) by Shah-Karami *et al.* (2012), as well as observed and expected heterozygosity values for Bactrian camels in our study (0.718 and 0.835, respectively) were higher than Mongolian Bactrian (0.522 and 0.546, respectively) reported by Chuluunbat *et al.* (2014). Also, mean value for  $H_o$  in Iranian dromedary populations (0.748) was higher than those reported for Australian (0.455) (Spencer *et al.*, 2010), Tunisian (0.46) (Ahmed *et al.*, 2010) and Saudi Arabian (0.665) (Mahmoud *et al.*,

2012) dromedaries. The high genetic diversity in the studied population could be explained by the lack of targeted breeding schemes to improve specific traits. By the way, these results are of substantial interest for conservation point of view, especially for Bactrian population, because this species is in the endangered condition in the country. Genetic variation at 20 microsatellite loci in Iranian camels is presented in Table 3, all values for  $H_e$  were more than  $H_o$ . Several factors can cause higher expected heterozygosity compared with observed heterozygosity in a population, including inbreeding, the presence of null allele, and lack of neutrality relative to selection, with selection in homozygotes (Maudet *et al.*, 2002).

Overall, analysis of genotype markers revealed that the lowest  $PIC$  value was 0.779 for CMS50 and the highest value was 0.887 for YWLL08 loci. Range of  $PIC$  value in our work was higher than the reported  $PIC$  range for Jaisalmeri camels (0.268 – 0.588) (Gautam *et al.*, 2004).  $PIC$  is a valuable indicator for informativeness of markers, and it does not depend on the mode of inheritance of the trait being linked (Guo and Elston, 1999). In the current study, high  $PIC$  value for all loci indicated that selected markers are appropriate for the study of the genetic variation of Iranian camel populations as well as the usefulness of them in the planning of the breeding programs, however, it needs further research to distinguish strains within the breed to design proper breeding strategies.

The estimate of genetic differentiation level between all Iranian camel populations in this study was low ( $F_{ST}$ : 0.008-0.021). Results for dromedary populations revealed that lowest differentiation was between SeD-TrD (0.008) and highest between YaD-SeD (0.011). Nevertheless, despite the expectation of high differentiation between Bactrian camels and dromedaries, our observation was relatively low in this study. However, in contrast with dromedary populations, Bactrian camel showed high values for pairwise genetic distance



estimates as compared to other populations ( $F_{ST}$ : 0.018-0.021 versus 0.008-0.011). These results were confirmed by the phylogenetic tree that was constructed using microsatellite markers information (Figure 3). The difference in genetics between populations was calculated by the mean  $F_{ST}$  value of 0.021, indicating that 97.9% of the total genetic variation related to differences among individuals within populations and only 2.1% corresponded to differences between populations. Mean  $F_{ST}$  value in the present study was lower than Indian camels (0.213) reported by Prasad *et al.* (2015), Australian wild dromedary camels (0.042) reported by Spencer *et al.* (2015), and Tunisian camels (0.09) reported by Ahmed *et al.* (2010). The result of cluster analyses performed with the STRUCTURE indicated a weak genetic structure in this study, which corresponds to strong gene flow between the studied populations (Figure 2).

### CONCLUSIONS

This study, which to our knowledge is the first detailed analysis of population structure and genetic diversity of Iranian camels, revealed that there are weak genetic structures, high level of genetic variability and gene flow in the studied populations. The genetic variation among the Iranian dromedaries and Bactrian camels might be attributed to higher levels of cross-breeding, migration, and lack of artificial breeding strategies. Results of the present microsatellite analysis suggest a close genetic relationship between populations. There is extensive gene flow between the YaD and TrDal, and between TrD and Semnan Province populations. Microsatellite markers can be powerful tools in designing breeding programs. Data suggests that Iranian dromedaries and Bactrian camels could retain valuable information and resources for future agriculture. It appears that some of the genetic material may no longer be retained in other breed lines found elsewhere in the world. Further investigation

will be planned mainly to characterize presumably different ecotypes genetically to help implement conservation and genetic improvement programs for camels in Iran.

It is not easy to evaluate genetic relationships among Iranian livestock, especially camels, because the definition of breeds is basically based on the farm holding ethnic groups or geographical areas. In this situation, the use of suitable outgroups is required to obtain good assessments for genetic relationships of Iranian camels.

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### REFERENCES

1. Ahmed, M. O., Salem, F. B., Bedhief, S., Rekik, B. and Djemali, M. 2010. Genetic Diversity in Tunisian Dromedary (*Camelus dromedarius*) Populations Using Microsatellite Markers. *Livest. Sci.*, **132(1)**: 182-185.
2. Benbouza, H., Jacquemin, J. M., Baudoin, J. P. and Mergeai, G. 2006. Optimization of a Reliable, Fast, Cheap and Sensitive Silver Staining Method to Detect SSR Markers in Polyacrylamide Gels. *Biotechnol. Agron. Soc.*, **10(2)**: 77-81.
3. Bos, D. H., Gopurenko, D., Williams, R. N. and DeWoody, J. A. 2008. Inferring Population History and Demography Using Microsatellites, Mitochondrial DNA, and Major Histocompatibility Complex (MHC) Genes. *Evol.*, **62(6)**: 1458-1468.
4. Chuluunbat, B., Charruau, P., Silbermayr, K., Khorloojav, T. and Burger, P. 2014. Genetic Diversity and Population Structure of Mongolian Domestic Bactrian Camels (*Camelus bactrianus*). *Anim. Genet.*, **45(4)**: 550-558.
5. Druml, T., Salajpal, K., Dikic, M., Urosevic, M., Grilz-Seger, G. and Baumung, R. 2012. Genetic Diversity, Population Structure and Subdivision of Local Balkan Pig Breeds in Austria,



- Croatia, Serbia and Bosnia-Herzegovina and Its Practical Value in Conservation Programs. *Genet. Sel. Evol.*, **44(1)**: 1-9.
6. Eltanany, M. Sidahmed, O. E. and Distl, O. 2015. Assessment of Genetic Diversity and Differentiation of Two Major Camel Ecotypes (*Camelus dromedarius*) in Sudan Using Microsatellite Markers. *Arch. Anim. Breed.*, **58(2)**: 269.
  7. Falush, D., Hubisz, M., Stephens, M., Pritchard, J., Donnelly, P., Wen, W., Trienis, M. and Melsted, P. 2012. *The Program Structure Software*. <http://pritch.bsd.uchicago.edu/structure.htm>
  8. FAO-STAT. 2014. *Food and Agricultural Organization, Statistical Databases*.
  9. FAO. 2011. *Molecular Genetic Characterization of Animal Genetic Resources*. FAO Animal Production and Health Guidelines.
  10. FAO, I. WFP. 2015. The State of Food Insecurity in the World 2015. Meeting the 2015 international hunger targets: taking stock of uneven progress." Food and Agriculture Organization Publications, Rome.
  11. Gautam, L., Mehta, S. C., Gahlot, R. S. and Gautam, K. 2004. Genetic Characterisation of Jaisalmeri Camel Using Microsatellite Markers. *Ind. J. Biotechnol.*, **3**: 457-479.
  12. Guo, X. and Elston, R. 1999. Linkage Information Content of Polymorphic Genetic Markers. *Hum. Hered.*, **49(2)**: 112-118.
  13. Hampton, J. O., Spencer, P., Alpers, D. L., Twigg, L. E., Woolnough, A. P., Doust, J., Higgs, T. and Pluske, J. 2004. Molecular Techniques, Wildlife Management and the Importance of Genetic Population Structure and Dispersal: A Case Study with Feral Pigs. *J. Appl. Ecol.*, **41(4)**: 735-743.
  14. Iranian Council of Animal Care. 1995. *Guide to the Care and Use of Experimental Animals*. Vol. 1., Isfahan University of Technology, Isfahan, Iran.
  15. Khademi, T. G. 2017. A Review of Genetic and Biological Status of Iranian Two-Humped Camels (*Camelus bactrianus*), a Valuable Endangered Species. *J. Entomol. Zool. Stud.*, **5(4)**: 906-909.
  16. Langella, O. 2011. Populations 1.2.32 – Population Genetic Software. CNRS UPR9034. <http://bioinformatics.org/populations/>.
  17. Lehane, S. 2014. *The Iranian Water Crisis*. Strategic Analysis Paper, Future Directions International International Pty Ltd.: Perth, Australia, 11.
  18. Mahmoud, A., Alshaikh, M., Aljumaah, R. and Mohammed, O. 2012. Genetic Variability of Camel (*Camelus dromedarius*) Populations in Saudi Arabia Based on Microsatellites Analysis. *Afr. J. Biotechnol.*, **11(51)**: 11173-11180.
  19. Maudet, C., Luikart, G. and Taberlet, P. 2002. Genetic Diversity and Assignment Tests among Seven French Cattle Breeds Based on Microsatellite DNA Analysis. *J. Anim. Sci.*, **80(4)**: 942-950.
  20. Mburu, D. N., Ochieng, J. W., Kuria, S. G., Jianlin, H., Kaufmann, B., Rege, J. E. O. and Hanotte, O. 2003. Genetic Diversity and Relationships of Indigenous Kenyan Camel (*Camelus dromedarius*) Populations: Implications for Their Classification. *Anim. Genet.*, **34(1)**: 26-32.
  21. Nei, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York, 512 PP.
  22. Patel, A., Jisha, T., Upadhyay, D., Parikh, R., Upadhyay, M., Thaker, R., Das, S., Solanki, J. and Rank, D. 2015. Molecular Characterization of Camel Breeds of Gujarat Using Microsatellite Markers. *Livest. Sci.*, **181**: 85-88.
  23. Peakall, R. and Smouse, P. E. 2012. GENALEX 6: Genetic Analysis in Excel: Population Genetic Software for Teaching and Research. *Mol. Ecol. Resour.*, **6(1)**: 288-295.
  24. Prasad, S., Ali, S. A., Banerjee, P., Joshi, J., Sharma, U. and Vijh, R. 2015. Population Genetic Structure of the Camel, *Camelus dromedarius* Based on Microsatellite Loci: Knock-on Effect for Conservation. *Biotech. Res. Comm.*, **8(2)**: 153-160.
  25. Putman, A. I. and Carbone, I. 2014. Challenges in Analysis and Interpretation of Microsatellite Data for Population Genetic Studies. *Ecol. Evol.*, **4(22)**: 4399-4428.
  26. Reed, D. H. and Frankham, R. 2003. Correlation between Fitness and Genetic Diversity. *Conserv. Biol.*, **17(1)**: 230-237.
  27. Shah-karami, S., Afraz, F., Mirhoseini, S. Z., Banabazi, M. H., Asadzadeh, N., Asadi, N., Hemmati, B., Qanbari, A. and Razavi, K. 2012. Genetic Diversity in Iranian Bactrian Camels (*Camelus bactrianus*)

- Using, Microsatellite Markers. *Modern Genet. J.*, **7**: 249-258. (in Persian)
28. Sharma, R., Kishore, A., Mukesh, M., Ahlawat, S., Maitra, A., Pandey, A. K. and Tandia, M. S. 2015. Genetic Diversity and Relationship of Indian Cattle Inferred from Microsatellite and Mitochondrial DNA Markers. *BMC Genet.*, **16(1)**: 1.
29. Spencer, P., Hampton, J. O., Pacioni, C., Kennedy, M. S., Saalfeld, K., Rose, K. and Woolnough, A. P. 2015. Genetic Relationships within Social Groups Influence the Application of the Judas Technique: A Case Study with Wild Dromedary Camels. *J. Wildlife Manage.*, **79(1)**: 102-111.
30. Spencer, P., Wilson, K. and Tinson, A. 2010. Parentage Testing of Racing Camels (*Camelus dromedarius*) Using Microsatellite DNA Typing. *Anim. Genet.*, **41(6)**: 662-665.
31. Spencer, P. and Woolnough, A. 2010. Assessment and Genetic Characterization of Australian Camels Using Microsatellite Polymorphisms. *Livest. Sci.*, **129(1)**: 241-245.
32. Takezaki, N. and Nei, M. 1996. Genetic Distances and Reconstruction of Phylogenetic Trees from Microsatellite DNA. *Genet.*, **144(1)**: 389-399.
33. Yagil, R., Zagorski, O. and Van Creveld, C. 1994. Science and Camels Milk Production (Some Keys for Nutrition and Marketing/Dromadaires et Chameaux, Animauxlaitiers). *Actes du Colloque*, 24-26 Octobre 1994, Nouakchott, Mauritania, CIRAD.

### ارزیابی مولکولی تنوع ژنتیکی در شترهای تک کوهانه و دو کوهانه با استفاده از نشانگرهای ریز ماهواره‌ای

ن. هدایت-اوریق، س. ر. میرایی آشتیانی، م. مرادی شهر بابک، ر. خلخالی اوریق و ک. پوراسد

#### چکیده

شترهای تک کوهانه و دو کوهانه دو گونه از شترها در ایران می باشند که از نظر اکولوژیکی به ترتیب به زندگی در شرایط بیابانی گرم و سرد عادت کرده اند. آن‌ها نقش مهمی در زندگی و تامین امنیت غذایی ایلات عشایری بازی می کنند. مطالعه حاضر به منظور بررسی تنوع ژنتیکی ۱۸۰ نفر شترهای ایرانی با استفاده از نشانگرهای ماهواره‌ای انجام شد. در یک پانل از ۲۰ نشانگر ریز ماهواره‌ای، تعداد ۲۱۴ آلل با میانگین ۱۰/۷ آلل به ازای هر جایگاه مشاهده کردیم. همه جایگاه‌ها، مقدار PIC بالاتر از ۰/۷ را به نمایش گذاشتند. مقدار تمایز ژنتیکی ( $F_{ST}$ ) به ازای هر جایگاه از مقدار ۰/۰۱ تا ۰/۰۳۹ متفاوت و میانگین این معیار برای کل جایگاه‌ها ۰/۰۲۱ بود. در این مطالعه، برآورد سطح تمایز ژنتیکی بین تمام جمعیت‌های شتر ایران کم بود ( $F_{ST}$ : ۰/۰۰۸-۰/۰۲۱). همچنین جریان ژنی بالایی بین جمعیت‌ها مشاهده شد. درخت فیلوژنتیکی نشان داد که بیشترین فاصله ژنتیکی بین شترهای دو کوهانه و شترهای تک کوهانه ایستگاه یزد بود. با این حال، نتایج تجزیه و تحلیل ریز ماهواره‌ای حاضر، رابطه ژنتیکی



نزدیکی در جمعیت‌های مطالعه شده را نشان داد. تمامی ترکیبات جمعیت-لوکوس، انحراف معناداری ( $P < 0.01$ ) از تعادل هاردی-واینبرگ نشان دادند.