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Short Communication

Genetic Diversity and Population Structure of Old World Camelids *(Camelus dromedaries* and *Camelus Bactrianus)* in Iran Using Mitochondrial DNA

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Abstract

This study was conducted to investigate the genetic diversity of Iranian camels using mitochondrial DNA. A fragment of 1052 bp cyt-b was amplified and sequenced in 120 individuals from four camel populations. The results revealed that there are 30 mutations for Bactrian camels and 9 mutations for dromedaries that lead to nine and six mtDNA haplotypes, respectively. Nucleotide diversity was calculated as 0.0015 and 0.0121 for dromedaries and Bactrian camel populations respectively. We found high haplotype diversity in Bactrian camel populations (0.886±0.145) and low genetic differentiation among dromedary populations. Phylogeny analysis showed different cluster for camelids.

Keywords: *Genetic diversity, Mitochondrial DNA, Haplotype, Phylogenetic analysis, Camelids*

Mitokondrial DNA Kullanılarak İran'daki Eski Dünya Develerinin *(Camelus dromedaries* ve *Camelus Bactrianus)* Genetik Farklılık ve Popülasyon Yapısının Araştırılması

Öz

Bu çalışma mitokondrial DNA kullanılarak İran develerinin genetik farklılığını araştırmak amacıyla yapılmıştır. Dört deve popülasyonundan toplam 120 devede 1052 bp cyt-b segmenti amplifiye edilmiş ve sekans analizi yapılmıştır. Elde edilen sonuçlar, çift hörgüçlü develerde 30 mutasyonun tek hörgüçlü develerde ise 9 mutasyonun olduğunu ve sırasıyla dokuz ve altı mtDNA haplotipinin bulunduğunu göstermiştir. Nükleotid farklılığı, tek ve çift hörgüçlü develer için sırasıyla 0.0015 ve 0.0121 olarak hesaplandı. Çift hörgüçlü deve popülasyonunda yüksek haplotip çeşitliliği (0.886±0.145) gözlemlenirken tek hörgüçlü deve popülasyonunda düşük genetik farklılaşma tespit edildi. Filogenetik analiz develer için farklı topluluklar gösterdi.

Anahtar sözcükler: *Genetik farklılık, Mitokondrial DNA, Haplotip, Filogenetik analiz, Deve*

INTRODUCTION

Bactrian and dromedary camels are two extant domesticated species of old world camels *(Camelus, Camelini)*. These species live in harsh and desert environments of Africa and Asia [1]. Characteristics such as withstand high temperature >42°C, resistance to dehydration, high salt tolerance and producing heavy-chain antibodies [2], make camels as an excellent target species to scientific studies. Also, they are economically important, as they provide milk, meat, draft

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and wool to the poor farmers from harsh climatic regions with a severe shortage of water.

Livestock genetic diversity is rapidly declining globally as specialization in animal breeding and the harmonizing effect of globalization advance. While biodiversity of livestock population could provide greater range of options for meeting future challenges including climate changing, new or resurgent disease threats, new knowledge of human nutrition requirements and changing market conditions or changing social necessary. Better characterization of locally adapted breeds will be a key for genetic diversity maintenance or exchange of genetics source [3].

In many domesticated animals, regional separation and different breeding schemes have created multiple subdivisions [4]. Evaluation of genetic variability is essential factor for conservation of genetic resources, support to future breeding options, to design rational breeding strategies for optimal use and conservation of available genetic variability [5]. Genetic characterization of native breeds is a powerful tool for the evaluation of worldwide genetic diversity. Mitochondrial DNA (mtDNA) has been commonly used for revealing genetic diversity, phylogeographical, population genetics and evolutionary studies. Cytochrome b (Cyt-b) gene region is part of mtDNA that play an important role as marker for the characterization of different genetic resources [6]. These markers have been widely applied to the analysis of phylogenetic relationships among mammals at species and breed level.

Several studies have investigated the genetic characteristics of different breeds and populations using mitochondrial DNA sequences. However, there is not such information available for camel populations in Iran. The purpose of this study was to provide insights on the genetic diversity and population structure information of Bactrian and dromedary camels using mitochondrial markers.

MATERIAL and METHODS

Sampling and Genomic DNA Extraction

Blood samples (4 mL) were taken from jugular vein of 120 camels including 85 dromedaries and 35 Bactrian camels. Dromedary camels belonged to three populations, Yazd station (40 camels), Trod station (28 camels) and Semnan herd (17 camels) that are located in the central deserts of Iran. Bactrian camels that were used for sampling in present study were from Bactrian camel research station in Ardabil province located in the northwest of Iran (as a gene bank for Bactrian camels in Iran). The camels that were sampled have no close relationship with each other. DNA was extracted using the RBC mini kit for mammalian blood (Real Biotech Corporation, RBC, South Korea).

Mitochondrial DNA Amplification and Sequencing

Two specific primers including Forward1:5'TCTAACCAC GACTAATGACAT3', backward: 5'TCCTTTTTCGGCTTACAA GACC3', forward2: 5' GAGAACACAGGCTGGTGAATA3' and backward2: 5'CTCAACAATGCTAGACCTTGG3' was used for amplification of the mitochondrial Cyt-b gene [7]. Amplification was carried out in a total volume of 25 µL containing 1.2 mM $MgCl_2$, 0.2 mM dNTP, 1.5U Taq DNA polymerase, 0.15 µM of forward and reverse primers (Invitrogen) and 100 ng genomic DNA.

The thermo cycling profile consisted of denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 40 s, annealing at 57 and 59°C (for primers respectively) for 40 s and extension at 72°C for 40 s, with a final extension step at 72°C for 5 min. PCR products were purified by Invitrogen Kit and were sequenced using forward primers with sanger sequencing method in the Macrogen company at south Korea (sequences of each haplotype deposited in GenBank with accession no. MG932682-MG932690).

Genetic Diversity Investigation

MEGA 6.0 software (based on the maximum likelihood algorithm) was used for reconstructing a phylogenetic tree and calculation of Tajima's D [8]. Tajima's D was calculated as a neutrality test. Alignment of sequenced fragments was performed using BioEdit software V.7.0.1 [9]. DnaSP 5.10 software was used to estimate nucleotide diversity (π) and haplotype variability (h) [10]. To inference of evolutionary history (among species) and genetic processes (within species), we used cyt-b sequences to show phylogenetic relationship and genetic variation among our samples and some other domestic animals. Genetic differentiation between all pairs of populations was assessed by pairwise F_{ST} values. For inferring intraspecific phylogenies, Median Joining Network analysis [11] was generated by Network V.4.6.1.1 (fluxus-engineering.com).

RESULTS

Genetic Diversity in Iranian Camel Populations

A fragment of 1052 bp of cyt-b gene was amplified. Alignment of cyt-b sequences revealed that there are 30 mutations included 3 singletons and 27 parsimony informative sites for Bactrian camels as well as 9 mutations for dromedary camels comprising 1 singleton and 8 parsimony informative sites *(Table 1)*. These results indicated that Bactrian camels are more variable in cyt-b gene in comparing with dromedary camels.

Nine and six mtDNA haplotypes were detected in Bactrian and dromedary camels respectively *(Table 1)*. There was one main haplotype (H1) in all populations of dromedary camel. Three haplotypes (H3, H4, and H5) were differed by single substitutions for the main haplotype. H2 and H6

Table 1. *Genetic variation based on cytochrome b gene in dromedary and Bactrian camel populations*

Population	Variable Site	Singleton Site	Parsimony Informative Site	Haplotypes	H_d	$\pi*10^{-1}$	K	Tajima D
Camelus dromedarus	8	1	9	6	0.362	0.014	0.785	-1.573
DC-Yazd	6	1	5	3	0.227	0.010	0.553	-1.949
DC-Trod	6	0	6	3	0.283	0.015	0.886	-1.291
DC-Semnan Herd	3	0	3	3	0.667	0.020	1.273	0.922
Camelus Bactrianus	30	3	27	9	0.886	0.121	5.341	-1.299

H_d: *Haplotype diversity;* ***π:*** *Nucleotide diversity;* ***k:*** *Average number of nucleotide difference;* ***DC:*** *Camelus dromedarus*

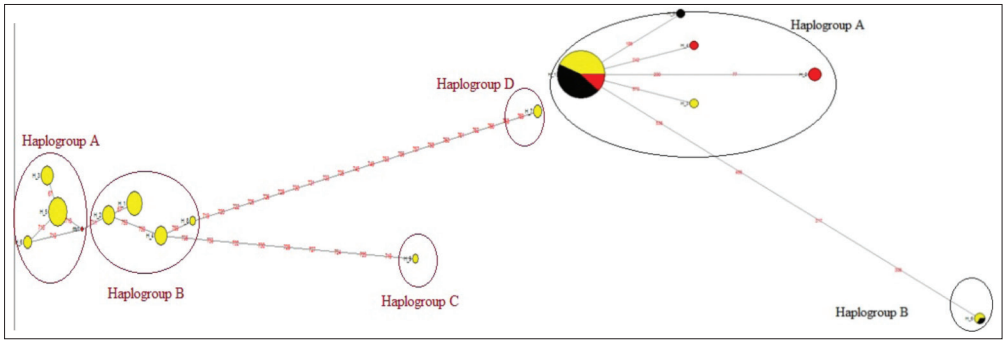

Fig 1. A median-joining haplotype network for *Bactrian camels (Left)* and dromedary camel *(Right:* Yazd Station: Semnan Herd: Trod Station) based on mitochondrial cytochrome b locus. Mv: hypothetical intermediate haplotypes

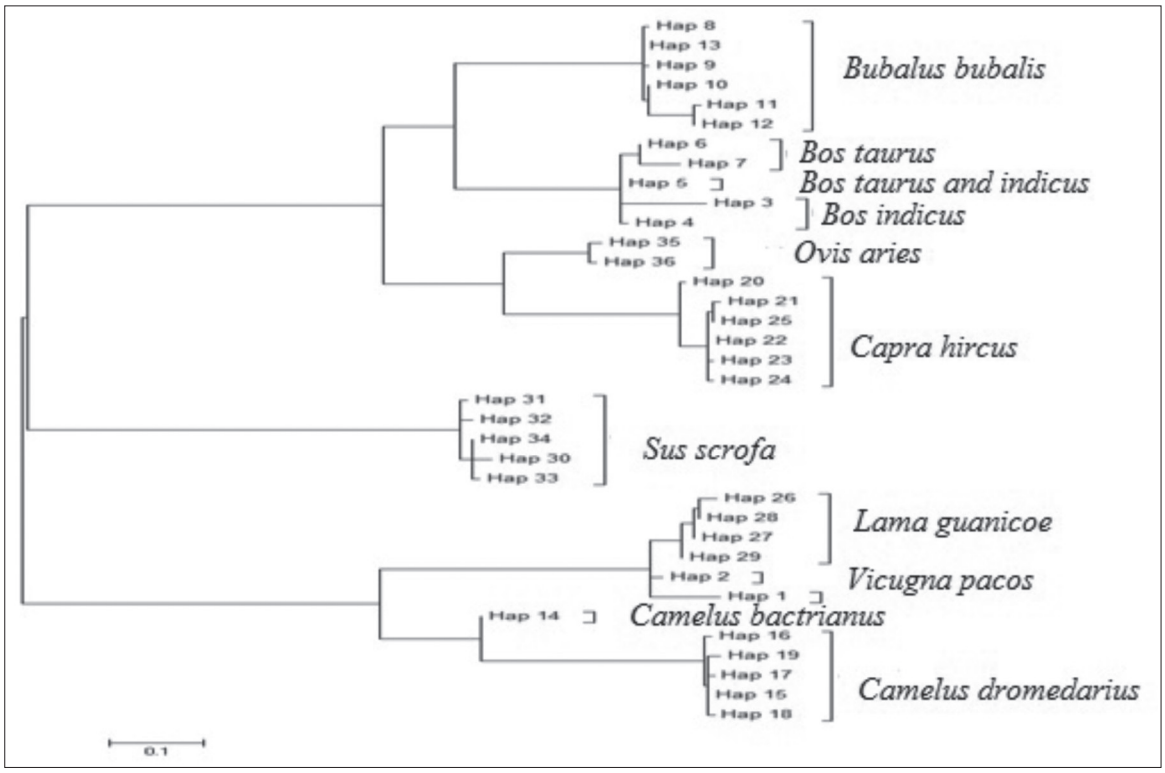

Fig 2. Phylogenetic tree reconstruction of different haplotype between domestic animals

were differed by two and four substitutions, respectively. Nucleotide diversity (π) was similar between dromedary's populations (0.0015, 0.0020, and 0.0010 for Trod station, Semnan Herd, and Yazd station respectively) and it was lower than Bactrian camel with 0.0121. Haplotype diversity in Semnan herd was higher between dromedary populations (0.667). We found high haplotype diversity in Bactrian camel population (0.886) *(Table 1)*.

The transitions/transversion ratios were 5.902 and 7.66 for Bactrian and dromedary camels, respectively. Obtained results revealed that Bactrian camel population divided

Table 2. *F_{ST} (lower) D_{XY} (upper) between populations using and mitochondrial markers (the bold number is significant in P<0.05)*

Population	DC-Yazd	DC-Trod	DC-Semnan Herd	*Camelus Bactrianus*
DC-Yazd	-	0.001	0.003	**0.089**
DC-Trod	-0.009	-	0.003	**0.089**
DC-Semnan Herd	0.206	0.202	-	**0.089**
Camelus Bactrianus	**0.995**	**0.996**	**0.981**	-

into four distinct mtDNA haplogroups. A median-joining network constructed of haplotypes for Bactrian and dromedary camels is shown in *Fig. 1*. None significant Tajima's D was calculated-1.299 for Bactrian camels as well as-1.564 for dromedary camels that provided no proof of deviations from selective neutrality.

Analysis of cyt-b gene showed differentiation between domestic animals. It is worth to mentioned that phylogenetic tree constructed with haplotypes between Iranian camels and other species was shown in *Fig.* 2. Bovidae family was more similar to each other, and it diverged from Sus scrofa and camelid in the past. Based on cyt-b gene the sus scrofa was more similar to camelid than bovidea. Our data further suggested that camelid family differentiated several years ago, and it might be having some genes that would be important to manage the challenge of global climate changing in future.

Gentic Differentiation Between Populations

As expected, high genetic diversity was observed among Bactrian and dromedaries populations. Results indicate that the highest diversity was between Bactrian camels and dromedaries of Trod station (F_{ST}=0.996) and the lowest was between dromedaries of Trod and Yazd station (F_{ST}=-0.009) *(Table 2)*.

DISCUSSION

Despite specific features of camels and their importance as a food source for many people in Africa and Asia, researchers have paid less attention to the breeding of this species compared to other livestock. Understanding of genetic variation in camel populations would be useful for planning the breeding schemes, maintaining of genetic diversity and preventing loss of beneficial alleles. In the current study, cyt-b gene sequence data were used to investigate genetic structure among Iranian dromedaries and Bactrian camels. We found a relatively low distance values (D_{XY}) between dromedary camel populations *(Table 2)*.

Iranian camels fragmented by land that shows differentiation between Bactrian and dromedary camels which inhabited to cold and hot area respectively. Iran is one of the few countries where to have both species of camels (Bactrian and dromedary). Evidence for structuring within camel groups was found to show there are subpopulations within each population. We observed a relatively adequate number of haplotypes in four populations under study, some of which were unique to each population *(Table 1)*.

Based on assessment of mitochondrial sequences in present study, broad sharing of haplotypes (six haplotypes) was observed across dromedary camel populations. This is a likely legacy of extensive crossbreeding occurring between populations of dromedary camels in Iran. Iranian Bactrian camels were categorized into nine haplotypes. Our analysis indicated that four haplogroups of Bactrian camels are consistently different from each other. Despite the low number of Bactrian camels in Iran, (about 150 camels; Animal breeding center of Iran 2015), our results revealed that there is high genetic diversity among Bactrian camels in Iran. It might be useful for making specific breeding strategies and their genetic conservation scheme.

We categorize six haplotypes of Iranian dromedaries into two haplogroups (A and B) that was supported by results of Almathen et al.[12]. Haplogroup A including 68 individuals was the largest group that comprising 5 haplotypes and haplogroup B was diverse by four mutations. For dromedaries, all populations were shared in both haplogroups A, but haplogroup B was observed in two population, this could be due to high levels of migration from one area to another. Similar to other research, phylogeographic pattern was not detectable, because the major haplotypes were observed across the global range of the populations.

Haplotype and nucleotide diversity for Bactrian camels in present study (0.886, 0.0012, respectively) were higher than reported values for Mongolian Bactrian camels (0.725, 0.0019, respectively) [13] and Kalmyk breed of Russia (0.722 and 0.0017), while the mentioned parameters were lower than Zhungeer breed in China (0.900 and 0.0032) [13]. Average haplotype diversity of Pakistanis camel breeds (0.894 and 0.588 [12,14] respectively) and also Australian (0.814), Iranian (0.717), Indian (0.632) and South Asian (0.711) [12] camels was higher than Iranian dromedaries camels (0.362) in this study.

We found six haplotypes in three studied dromedaries populations that demonstrated a moderate mtDNA genetic diversity in the Iranian camel populations. Hap1 was predominant and central haplotype for dromedary camels. The phylogenetically central and most frequent

be assumed to be the most ancient node in the network as the oldest ancestral allele is the root of the phylogeny, whereas the haplotypes at the tip or periphery of the network regarded as most recently arisen [15]. The negative results of neutrality test (Tajima's D) might explain purifying selection in both dromedary and Bactrian camel populations but the result is not statistically significant ($P<0.05$). Our findings showed the presence of at least four genetically differentiated common ancestors in Bactrian camels based on haplotype analysis.

Analysis of Cyt-b gene showed differentiation between domestic animals. Phylogenetic tree constructed with haplotypes between species was shown in *Fig. 2*. Bovidae family was more similar to each other, and it diverged from Sus scrofa and camelid in the past. Based on Cyt-b gene the sus scrofa was more similar to camelid than bovidea. Our data further suggested that camelid family differentiated several years ago, and it might be having some genes that would be important to manage the challenge of global climate changing in future.

The current study, which to our knowledge is the first detailed analysis of genetic diversity and population structure of Iranian camels, has shown a weak genetic structure and a high level of genetic migration in the dromedary populations. Also, high genetic variability was observed in Bactrian population that is listed critically endangered in Iran. We suggest that results of this research could be employed in designing of effective conservation programs for this species.

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