

COMPARATIVE ANALYSIS OF MITOCHONDRIAL GENOMES SEQUENCES OF BACTRIAN AND DROMEDARIES CAMELS

Nemat Hedayat-Evrigh*, Vahid Vahedi

Department of Animal Science, Faculty of Agricultural and Natural Resources,
University of Mohaghegh Ardabili, Ardabil, Iran

Abstract

Iranian Bactrian camels are in critically endangered status, while dromedary camels with stable population size are economically important in hot desert area regions. This pioneering study was conducted to observe the genetic diversity of Iranian camels using mitochondrial DNA. A fragment of 1657 bp from mitochondrial DNA including Cyt-b (1052bp) and D-loop (605 bp) was amplified. Eleven mtDNA haplotypes and three haplogroups were detected in 120 individuals from four populations. Mitochondrial sequences showed extensive sharing of haplotypes across dromedary populations. For Bactrian camels, we identified 1 and 30 polymorphic sites in D-loop and Cyt-b respectively, while mentioned values were 51 and 58 for dromedaries.

Keywords: Genetic Diversity, Mitochondrial DNA, Dromedary and Bactrian camels, Phylogeny analysis

1. INTRODUCTION

Bactrian and dromedary camel are two extant domesticated species of Camels (*Camelus*, *Camelini*) in the world. These species live in the harsh and desert environments of Africa and Asia (Wu, et al. 2014).

Bactrian camels distributed in northwest area (Ardabil province), their total number is less than 150, While, there are more than 162,000 dromedaries camels, which distributed in all part of the country specially in the eastern and central desert regions (Animal breeding center of Iran, 2015).

Because of climate changes, there is a challenge for conservation the livestock which are resistant to harsh weather conditions. This is particularly true for native animals because they carry specific genes and gene combinations that might be useful to manage the challenge of global climate change and food quality in the future.

Evaluation of genetic variability is an essential factor in working out keep genetic resources, support future breeding options, to design rational breeding strategies for optimal use and conservation of available genetic variability (Sharma, et al. 2015). Cyt-b gene and D-loop region are two parts of mtDNA that play an important role as markers for the characterization of different genetic resources (Goldstein and Pollock, 1997). These markers have been widely applied to the analysis of phylogenetic relationships among mammals at species and breed level.

The purpose of this study was to evaluate the genetic relationships between Iranian Bactrian and dromedary camel populations using mitochondrial markers polymorphisms.

2. MATERIALS AND METHODS

2.1. Materials

Blood samples in 4 ml were collected from Jugular vein of 120 camels including 85 dromedary camels (*Camelus dromedaries*) (40, 28 and 17 from Yazd station, Trod station and Semnan herd respectively) and 35 Bactrian camels (*Camelus bactrianus*) from Bactrian camel research station in Ardabil province (As a gene bank for Bactrian camels in Iran). DNA was extracted by RBC mini kit for mammalian blood (Real Biotech Corporation, RBC, South Korea).

2.2. Methods

Specific primers for amplification of the mitochondrial Cyt-b gene and D-Loop were designed by Primer 3 software (version 4.0). The PCR product lengths were 1052 and 605 bp for Cyt-b and D-loop respectively and were sequenced using sanger sequencing technique (KX622076-KX622082).

MEGA 6.0 software (based on the maximum likelihood (ML) algorithm) was used for reconstructing a phylogenetic tree and Tajima's D (Tamura, et al. 2011) was calculated as a neutrality test. Alignment of Cyt-b region sequence fragments were performed using bioEdit software V.7.0.1 (Hall, 2004). Nucleotide diversity (π) and haplotype variability (h) were calculated by DnaSP 5.10 (Rozas, et al. 2009).

Genetic differentiation between all pairs of populations assessed by pairwise F_{ST} values using mitochondrial markers. A hierarchical analysis of molecular variance applied to assess the between and within population differences. A Median Joining Network analysis (Bandelt, et al. 1999) generated by Network V.4.6.1.1 (fluxus-engineering.com) was also applied.

3. RESULTS

All of 120 samples were analyzed for mitochondrial markers. A fragment of 1657 bp including Cyt-b and D-loop regions amplified. This fragment represents 351 Amino acids, corresponding to 1052 nucleotides of Cyt-b gene in the camel's mitochondrial genome. 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 58 mutations for dromedary camels comprising 49 singletons and 9 parsimony informative sites. We identified 1 and 51 mutations for D-loop region in Bactrian and dromedary camel respectively that all sites were parsimony. The most variability occurred in the coding region (Cyt-b) (53%,; 97%, for dromedaries and Bactrian camels respectively). These results indicated that Bactrian camels are more variable in Cyt-b gene in comparing with none-coding regions, but dromedary camels were variants in both coding and none coding regions.

Eleven mtDNA haplotypes detected from 120 individual samples across 4 populations. Most haplotypes were different within single or two substitutions from other haplotypes except for hap1, hap2, hap3 and hap4 that differed by four, twenty-one, thirty-two and eight substitution respectively. Four hypothetical intermediate haplotypes detected that not found in the current study. The ratios of transitions per transversions pairs were 3.14 and 4.0 for dromedaries and Bactrian camels respectively. Two haplotypes (hap2 and hap 4) were for Bactrian camel populations and nine haplotypes belonged to dromedary camels (hap1, hap3, hap5, hap6, hap7, hap8, hap9, hap10 and hap11) that were sampled from three populations. Hap3 was the dominant haplotype comprising 60 individual that shared between three populations and hap1 shared between Trod station and Semnan herds. Three specific haplotypes detected for the Semnan herd (hap6, hap7, and hap8) and Yazd station (hap5, hap10 and hap11) and also one specific haplotype detected for Trod station (hap9).

Nucleotide diversity (π) was similar between dromedaries camel populations (0.01648, 0.02382, and 0.0128 for Trod station, Semnan Herd, and Yazd station respectively) and it was approximately higher than Bactrian camel with 0.00908. Haplotype diversity in Semnan herd was similar to Bactrian camels (0.822 ± 0.097 and 0.83 ± 0.046 , respectively) but higher than other dromedaries camel populations (Trod station and Yazd station with 0.36 ± 0.145 and 0.363 ± 0.138 respectively). Tajima's D was calculated 1.04 for Bactrian camels as well as 0.71 for dromedary camels that provided no proof of deviations from selective neutrality.

Based on Bayesian and Akaike parameter, the best substitution model for D-loop and Cyt-b sequences were a General Time Reversible model with Gamma distribution and invariable site (GTR+G+I) (INL= -5156.079; G=0.51; I=0.49)

Phylogenetic analyses for haplotypes (Figure 1) performed base on Maximum likelihood using GTR+G+I model as substitution model.

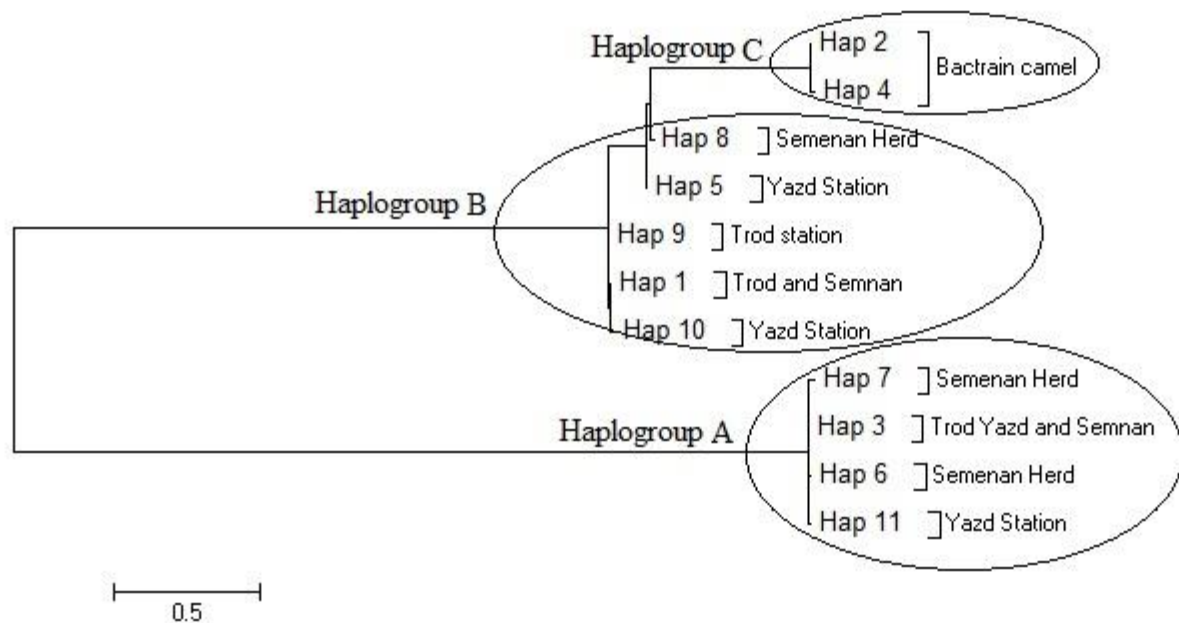


Figure 1. Phylogenetic tree reconstruction of a different haplotype in Iranian camel Population.

The genetic differentiation value (F_{ST}) was calculated using mtDNA markers. As expected, high genetic diversity was observed among Bactrian and dromedaries. Results indicate that highest diversity was between Bactrian camels and dromedaries of Yazd station ($F_{ST} = 0.90756$) and lowest between dromedaries of Trod station and SemnanHerd ($F_{ST} = -0.05918$).

In the current study, Cyt-b gene and D-loop region sequence data were used to investigate genetic structure and dispersal among dromedaries and Bactrian camels in Iran. We found a relatively low distance and high gene flow between dromedary camels populations.

Iranian camels fragmented by land that shows differentiation between Bactrian and dromedary camels which inhabited to cold and hot area respectively. We observed a relatively large number of haplotypes in four populations under study, some of which were unique to each population.

Mitochondrial sequences revealed extensive sharing of haplotypes (nine haplotypes) across dromedary camel populations in this study. This is a likely legacy of extensive crossing occurring between populations of dromedary camels. Iranian Bactrian camels were categorized into two haplotypes (Hap2, Hap4). Low genetic variation of Bactrian camels in Iran, caused by limited population size. Captive population through inbreeding and genetic drift leads to loss of genetic variation. Our analysis indicated that two groups of Bactrian camels are consistently much different from each other because the Bactrian population comprise from individuals of different region and might have been an isolated area from each other, in the past. This result showed that there were two different populations of Bactrian camels in Iran, and it might be useful for making specific breeding strategies and their genetic conservation programs.

Based on our results, Iranian camels are divided into three haplogroups (A,B,C) (Figure 2). Dromedary individuals belonged to group A and B, while Bactrian owned by group C. Haplogroup A including 68 individual was the largest group and haplogroup B was the most diverse, comprising 5 haplotypes. For dromedaries, all populations were shared in both haplogroups A and B; this could be due to high levels of migration from one area to another.

Haplotype diversity and nucleotide diversity in Iranian Bactrian camels (0.83, 0.0091, respectively) were higher than reported those of Mongolian Bactrian camels (0.725, 0.0019, respectively)

(Chuluunbat, et al. 2014). Average haplotype diversity of Pakistanis camel breeds (0.894) (Babar, et al. 2015) was similar to Semnan herd (0.822), approximately, but higher than Trod and Yazd station (0.36 and 0.363, respectively).

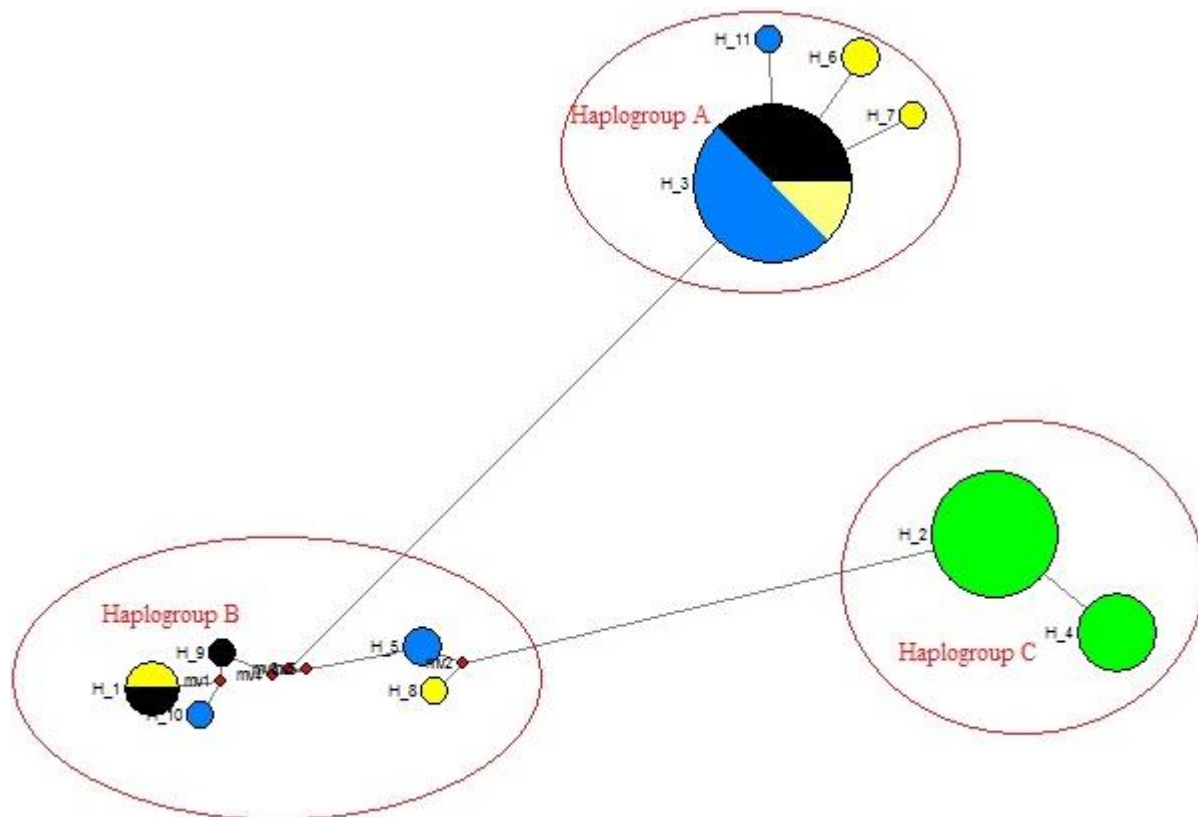


Figure 2. A median-joining haplotype network for Iranian camels based on 1657 bp mitochondrial control region and cytochrome b loci.

■:Yazd Station; ■:Semnan Herd; ■:Bactrian Camel; ■:Trod Station.

Mv= hypothetical intermediate haplotypes not found in the current study.

Detected Eleven haplotypes in four studied populations demonstrated that a high mtDNA genetic diversity has existed in Iranian camel populations. Hap2 and hap3 were predominant and central haplotypes for dromedaries and Bactrian camels, respectively.

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