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Molecular phylogeny of the Bactrian camel based on mitochondrial *Cytochrome b* gene sequences

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ABSTRACT. The Bactrian camel is an important domesticated animal providing milk, meat, and other products in desert countries. In this study, 111 individuals representing 11 domestic Bactrian camel breeds from China, Mongolia, Russia, and one wild Bactrian camel group from Mongolia were selected for the preparation of mitochondrial DNA. The 1140-bp fragments of the *cytochrome b* gene (*Cytb*) were amplified by polymerase chain reaction and sequenced directly. Sequences of the 92 domestic and 19 wild Bactrian camel samples were analyzed with DNASTAR, and a phylogenic tree was constructed using MEGA. The analysis revealed sixteen haplotypes among the samples that were divided into two haplogroups: a domestic haplogroup (H1-H13, H15,

and H16) and a wild haplogroup (H14). Haplotype diversity values were from 0.356 in the HosZogdort, to 0.889 in the Sunit Bactrian camel breed. The Sunit breed displayed the highest nucleotide diversity value (0.00115), and the HosZogdort breed had the lowest value (0.00031). All domestic Bactrian camels formed a single monophyletic lineage that is the sister group to wild Bactrian camels, a finding consistent with a single domestication event and independent maternal inheritance since domestication. In addition, the most common mitochondrial haplotypes (H1, H3, and H4) were shared between Chinese, Mongolian, and Russian domestic Bactrian camels, which indicated that there was no distinguishing geographic structure among the domestic breeds from these three regions. These findings provide important insights into patterns of relatedness among Bactrian camels from the Chinese, Mongolian, and Russian regions.

Key words: *Cytochrome b* gene (*Cytb*); Domestic Bactrian camel; Wild Bactrian camel; Bactrian camel breed; Haplotype; Phylogenetic tree

INTRODUCTION

The Bactrian camel, also known as the two-humped camel, is uniquely adapted to hot and arid environments (Schwartz, 1992). Bactrian camels can provide a range of products and services, including milk, meat, wool, and blood, to the people who inhabit cold-arid and semi-arid desert regions (Groeneveld et al., 2010), which makes the Bactrian camel one of the most useful animals that humans have ever domesticated. In addition, scholars believe that the Bactrian camel made a great contribution to transportation on the Silk Road and could be portrayed as a bridge between the Eastern and Western cultures (Potts, 2005). This has enhanced the cultural and economic development of human societies (Ji et al., 2009a).

Bactrian camels include the domestic Bactrian camel (*Camelus bactrianus*) and the wild Bactrian camel (*Camelus ferus*). The domestic Bactrian camel is mainly distributed in central Asia and surrounding cooler areas (Ji et al., 2009a). Extant populations of the wild Bactrian camel are mainly distributed in the regions of the Gobi and Taklamakan Deserts of Mongolia and Xinjiang.

Mitochondrial DNA (mtDNA) has advantages for phylogenetic analysis, including its simple structure, small molecular weight, maternal inheritance, rare recombination, and high mutation rate compared with many nuclear markers. As a molecular marker, these characteristics of mtDNA sequences have been very important for studies of molecular evolution over past decades (Quan et al., 2000; He et al., 2009; Chuluunbat, et al., 2014). The *cytochrome b* gene (*Cytb*) in the mtDNA genome is an important protein-encoding gene for studies of phylogenetic evolution and species classification (Johns and Avise, 1998; Li et al., 2005; Zhong et al., 2014). Thus far, the genetic diversity of different breeds of Bactrian camels from China, Mongolia, and Russia have never been characterized based on the *Cytb* gene, and only a partial survey of the breeds in China have been reported (Quan et al., 2000). Therefore, in this study, we collected samples from seven representative Bactrian camel breeds (Alxa,

Gobi Red, Qinghai, Mulei, Zhungeer, Tarim, and Sunit Bactrian camel) from China, three representative breeds (Galbiin Gobiin Ulaan camel, HosZogdort camel and Haniin Hetsiin Huren camel) from Mongolia, and one representative breed (Kalmyk Bactrian camel) from Russia; wild Bactrian camel samples were also collected from Mongolia. We analyzed sequence divergence and phylogenetic relationships based on the *Cytb* gene to discuss the genetic diversity and evolutionary relationships among these domestic Bactrian camel breeds and one wild Bactrian camel group.

MATERIAL AND METHODS

Sample collection and DNA extraction

Whole blood samples from 92 domestic Bactrian camels (*C. bactrianus*) representing 11 domestic breeds were collected from Bactrian camel breeding villages belonging to three Chinese provinces (Inner Mongolia, Xinjiang, and Qinghai), three regions in Mongolia (Hanbogd soum, Mandal-Ovoo soum, and Tugrug soum), and one region in Russia (Astrakhan City) (Figure 1). Ear tissue samples from 19 wild Bactrian camels (*C. ferus*) were collected from Gobi Altai in Mongolia (Figure 1). All blood samples were stored at -70°C. Total genomic DNA was extracted from blood or ear tissue using the standard phenol-chloroform extraction method described by Chengjia (Cheng et al., 2009). Detailed information of each Bactrian camel breed's name, geographic distribution, and sample size is provided in Table 1.

Figure 1. Geographic distribution of the 11 domestic Bactrian camel breeds and 1 wild Bactrian camel group analyzed in this study.

Table 1. Breed name, geographic distribution, collection of sample size, number of haplotypes, haplotype diversity, and nucleotide diversity for each different breed of Bactrian camel used in our study.

Bactrian camel breed	Geographic distribution	Number of individuals	Number of haplotypes	Haplotype diversity	Nucleotide diversity/10^{-2}
Alxa	China, Inner Mongolia	10	4	0.644	0.066
Gobi Red	China, Inner Mongolia	10	4	0.644	0.080
Sunit	China, Inner Mongolia	10	6	0.889	0.115
QingHai	China, Qinghai	10	4	0.533	0.053
Zhungeer	China, Xinjiang	5	2	0.400	0.035
Tarim	China, Xinjiang	5	2	0.600	0.105
MuLei	China, Xinjiang	5	3	0.800	0.105
Galbiin Gobiin Ulaan	Mongolia, Hanbogd soum	8	4	0.643	0.066
Haniin Hetsiin Huren	Mongolia, Mandal-Ovoo soum	10	6	0.778	0.088
HosZogdort	Mongolia, Tugrug soum	10	2	0.356	0.031
Kalmyk	Russia, Astrakhan	9	3	0.667	0.068
Wild	Mongolia, Gobi Altai	19	1	-	-

PCR amplification and product sequencing.

Polymerase chain reaction (PCR) primers for the amplification of the *Cytb* gene were designed by Zhang et al. (2008): forward 5'-ATG ACA AAC ATC CGA AAT CAC ACC-3' and reverse 5'-TCT TCA TTT TAG GAT ACG GTT TTC A-3'. The primers were designed using Primer 5.0 software and synthesized by Shanghai Sangon Biological Engineering Technology and Service Company (Shanghai, China). PCR amplification was implemented in a 50 μL reaction mixture comprising 5 μL 10X reaction buffer, 1 μL 10 mM dNTPs, 1 μL of each primer (10 μM), 1.25 U Taq DNA polymerase (TaKaRa Biosystems, Tokyo, Japan), and approximately 200 ng template (the genomic DNA of each sample that was used as a template for PCR). The PCR mixture was subjected to the following conditions: denaturation for 5 min at 95°C, followed by 32 cycles of 60 s at 94°C, 60 s at 52°C, 90 s at 72°C, and finally annealing for 10 min at 72°C. The PCR products were identified by agarose-gel electrophoresis, and DNA sequencing was performed on an ABI 3730 xl DNA sequencer according to the manufacturer instructions.

Data analysis

Multiple alignments of nucleotide sequences were performed using Clustal W (Thompson et al., 1994), with variation sites deleted from the alignments. Indices of population diversity contained nucleotide diversity, haplotype diversity, and the number of haplotypes to be calculated using the DnaSP 5.0 software (Rozas et al., 2003). The phylogenetic tree was constructed from aligned sequences using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) (Sokal and Michener, 1958; Atteson, 1999) in the MEGA 5.1 software (Kumar et al., 2001). The Dromedary camel (*Camelus dromedarius*) was used as the outgroup.

RESULTS

Analysis of nucleotide sequences

After sequencing of the PCR products, 1140 nucleotides were determined in *Cytb* gene sequences of all 111 samples. The average contents of T, A, G, and C were 27.77, 28.96, 15.07, and 28.20%, respectively, which shows that the GC content (43.27%) was less than the AT content (56.73%). In this study, we detected only nucleotide substitutions, and no deletions

or insertion mutations were observed. Among the 16 different haplotypes identified from the 111 *Cytb* sequences, we identified 17 variable sites (16 transitions and 1 transversion) (Table 2).

Table 2. Variation sites of the *Cytb* gene of wild and domestic Bactrian camels from Mongolia, China, and Russia.

Haplotype	Number of individuals	128	214	463	472	492	525	553	570	618	709	711	714	723	730	819	823	834
H1	52	T	G	T	A	T	C	C	G	T	T	A	T	T	C	T	C	C
H2	1	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H3	14	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H4	9	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-
H5	1	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	T
H6	1	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-
H7	2	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-
H8	1	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-
H9	3	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-
H10	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-
H11	1	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-
H12	1	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
H13	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-
H14	19	-	-	-	-	-	-	T	A	-	C	-	-	-	-	C	-	-
H15	2	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-
H16	2	-	-	C	-	-	T	-	-	-	-	-	-	-	-	-	-	-

We found that the number of haplotypes from different breeds varied from 1 to 6, and haplotype diversity values from 0.356 in the HosZogdort to 0.889 in the Sunit Bactrian camel breed (Table 2). The Sunit Bactrian camel displayed the highest nucleotide diversity value (0.00115), whereas the HosZogdort breed had the lowest value (0.00031). When comparing *Cytb* sequence variation in all Chinese domestic Bactrian camels with those of Mongolian and Russian domestic Bactrian camels, the level of diversity within China (nucleotide diversity: 0.00084) was higher than that of Russia (0.00068) and Mongolia (0.00060), whereas the level of haplotype diversity of China (0.679) was also higher than that of Russia (0.667) and Mongolia (0.582).

Haplotype analysis of *cytb* sequences from domestic and wild Bactrian camels

We found that the 16 different haplotypes were arranged into two haplogroups: the domestic Bactrian camel haplogroup and the wild Bactrian camel haplogroup (Figure 2). The domestic Bactrian camel haplogroup included 15 haplotypes, and the largest haplotype (H1) consisted of 52 individuals. There were three domesticated haplotype groups (H1, H3, and H4) that included 9 or more individuals, and the other domesticated haplotypes contained fewer individuals. The main mitochondrial haplotypes (H1, H3, and H4) were shared between Chinese, Mongolian, and Russian domestic Bactrian camels, which indicated that there was little geographical structuring and apparent genetic admixture among the domestic breeds from the three regions. Moreover haplotypes specific to a geographic region were also discovered. For example, haplotypes H2, H5, H10-13, H15, and H16 were only found in Chinese domestic Bactrian camels; haplotypes H6, H7, and H8 were only found in Mongolian domestic Bactrian camels; but there were no specific haplotypes in Russian domestic Bactrian camels. In this study, all wild Bactrian camels consisted of only one haplotype (H14), in contrast to three haplotypes reported by a previous study (Ji et al., 2009b). Our results also suggest an extremely small effective population size of wild Bactrian camels, which are considered critically endangered by the International Union for Conservation of Nature.

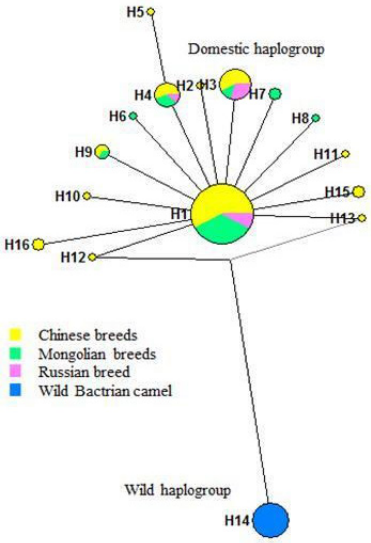

Figure 2. Median-joining network of 16 *Cytb* haplotypes of domestic and wild Bactrian camels. The circled area is proportional to the frequency of the haplotype.

Phylogenetic tree construction

In this study, the UPGMA tree was constructed using 16 haplotypes of all Chinese, Mongolian, Russian domestic Bactrian camels and wild Bactrian camels, with the Dromedary camel (*D. camelus*) as the outgroup (GenBank accession No. X56281) (Figure 3). The phylogenetic tree clearly showed two major groups. The haplotype of the wild Bactrian camel (H14) formed one branch and is a sister group to a monophyletic lineage made up of all haplotypes of the domestic Bactrian camel. The results show that the extant wild Bactrian camel and domestic Bactrian camel form distinct mitochondrial lineages with independent maternal inheritance.

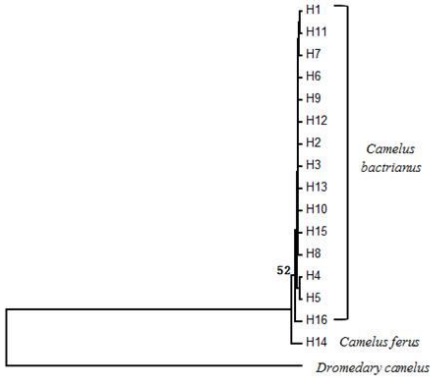

Figure 3. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) phylogenetic tree of complete mitochondrial Bactrian camel cytb sequences showing the phylogenetic relationship of haplotypes (H1 to H16).

DISCUSSION

Molecular genetic analysis of mitochondrial (Jianlin et al., 2004; Ji et al., 2009b; Silbermmayr et al., 2010) and nuclear markers (Silbermmayr and Burger, 2012) provides estimates of the time of separation between wild and domestic Bactrian camels: 0.7-1.5 million years ago in the Pleistocene, which is much longer than the history of domestication. Archaeological research suggests that Bactrian camels migrated from North America through the Bering Strait, and then arrived in Asia about 3 million years ago (Harrison, 1985). Later, during the Pleistocene Epoch (1.8 million to 10 000 years ago), Bactrian camel populations may have again been subdivided geographically, leading to a split of the two lineages (wild and domestic lineages) (Ji et al., 2009b). In this study, we found that 16 different haplotypes were divided into a domestic haplogroup and a wild haplogroup, and the phylogeographic analysis showed two major groups (wild and domestic Bactrian camel groups). This finding provides evidence that the extant wild Bactrian camel and domestic Bactrian camel carry distinct, maternally inherited mitochondrial lineages (Ji et al., 2009b).

In addition, there was no distinguishing geographic structuring of *Cytb* haplotypes among Mongolian, Chinese, and Russian domestic Bactrian camel breeds. This result is consistent with prior research, implying that domesticated Bactrian camels originated from a single maternal lineage (Ji et al., 2009b). In addition, strong gene flow existed among domestic Bactrian camel populations throughout history. For example, on the Silk Road, the merchants, pilgrims, soldiers, and nomads used a large population of domestic Bactrian camels to transport their goods and military supplies between different geographic areas in China, Russia, and Mongolia. Thus, it can be predicted domestic Bactrian camels frequently travelled between different geographic regions. Thus, the Silk Road could have led to the frequent hybridization and admixture of domestic Bactrian camels from different geographic regions.

A possible caveat of our study was that *Cytb* sequence variation alone might not be sufficient to recover population genetic divergence in different geographic regions. Recently, the whole-genome sequence of the Bactrian camel was published (Ji et al., 2012). Future studies comparing whole-genome variation could be adopted for a more sophisticated investigation on the evolution and domestication of the Bactrian camel.

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