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GENETIC DIVERSITY OF THE MONGOLIAN BACTRIAN CAMEL BASED ON MITOCHONDRIAL SEQUENCES

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ABSTRACT

Current genetic diversity of 5 local breeds of Mongolian camels and 2 samples of *Camelus ferus* was studied and compared their levels of variability. DNA was extracted using Qiaamp® DNA kit (Qiagen) and amplified mtDNA D-loop control region from 36 blood samples of Mongolian Bactrian camels and 2 from Mongolian *Camelus ferus*. Three common haplotypes (H1, H2, H6) and 4 unique haplotypes (H3, H4, H5, H8) revealed in the population of domestic camels based on the mitochondrial DNA region. For the further study we carried out best result 26 sequences. We did not find high inbreeding levels in the different breeds. The phylogenetic analysis suggested that domestic and wild camels have two distinct lineages.

Key words: Camel, genetic diversity, mitochondrial DNA, Mongolian camel breeds

The distribution of Mongolian camels is limited and there are relatively few differences between local camels in terms of body size, shape, colour and productivity. These are bred mainly in Tugrug and Tonkhil soums of Gobi-Altai province, Khanbogd soum in Umnugovi province and Mandal-Ovoo soum in Umnugovi province.

Scientists have identified 3 breeds which are based on their productivity, colour and morphological differences. The "Galbyn Gobi Red" and "Khanyn Hetsiin Khuren" were approved as breed while "Tuhum tungalagiin khuren" was approved as breeding group (Baldan *et al,* 2015).

Battsetseg (2018) studied 2 species from genus *Camelus* and domestic camel populations in Mongolia using 18 mitochondrial and nuclear microsatellite markers to detect genetic patterns and evolution and to assess genetic diversity. The genetic characteristics of the camel breeds of the Galbyn Gobi Red, the Khanyn Hetsiin Khuren and the Tuhum tungalagiin khuren were identified, revealing differences in the main genetic parameters of the population, such as allele number, frequency, heterozygosity and genetic distance. Based on neutral markers, little population structure in Mongolian Bactrian camels were observed and the phenotypic differentiation was mainly due to recent anthropogenic selection, which would change allele frequencies in selected genes rather than in neutral markers.

Wild Bactrian camel is similar to Bactrian camel, but morphologically have different characteristic features (Andrei and Bat-Erdene, 1998; Adiya, 2017).

There is a lack of genetic study about the Mongolian Bactrian camel population, however, a comparative study between the genetic diversity of two Mongolian camel populations from China and the area bordering China was conducted (Jianlin *et al,* 2004).

Mongolian camel herders have traditionally selected camels based on morphological and productivity traits for milk, meat and wool (Chuluunbat *et al,* 2014). Considering this morphological characterisation, we investigated the genetic diversity among the described breeds and expected breed-specific population differentiation by analysing nucleotide sequence of the control region in mitochondrial DNA.

Materials and Methods

In total, 36 camels were sampled from 7 different localities of Mongolia (Fig 1). Blood, skin and hair samples of Galbyn Gobi Red camel from Khanbogd soum of Umnugovi province, Khanyn Hetsiin Khuren camel from Mandal-Ovoo soum,

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Tuhum tungalagiin khuren camel from Tugrug soum of Gobi-Altai province and local Mongolian camels populations from Bayantooroi bagh of Tsogt soum of Gobi-Altai province, Aldarkhaan soum of Zavkhan province, Bugat soum of Gobi-Altai province and Baruunturuun soum of Uvs province were collected and stored in the -20°C.

DNA extraction from blood

DNA extraction from EDTA-preserved blood was performed with the Qiaamp® DNA kit (Qiagen). Hair samples were digested with a non-commercial lysis buffer (Pfeiffer *et al,* 2004) and DNA was extracted with the NucleoSpin® Tissue Kit (Macherey-Nagel). PCR amplification using the primer combinations: Pro FCCACCACCAGCACCCAAAGCTG Phe RGGC CAGGTGCCCATCCAGGCAT. The amplification was carried out in a 30 µl reaction volume containing 1 µl of the extracted DNA, 1µl of the 25 mM of dNTPs, 1µl of the each primers (10 pmol), 0.5 units of Dream taq (Thermofisher scientific) and 1xPCR buffer (includes 20 mM $MgCl_2$). The PCR conditions were the following: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 69°C for 1 min and extension at 72°C for 1 min. The final extension was at 72°C for 10 min.

Results and Discussion

The study included a total of 36 samples, which consisted of 6 samples from each population such as Besreg, Khanyn Khetsiin Khuren, local Mongolian camel, wild Bactrian camel, Galbyn Gobi Red and Tuhum tungalagiin khuren. The 1100 base pairs of the mitochondrial DNA control regions were amplified and analysed (Fig 2). For the further study we carried out best result 26 sequences.

The 657bp sequences of the control region in 26 samples were aligned with the reference sequences (NC 009628.2 *Camelus bactrianus*, NC 009629.2 *Camelus ferus*) in MEGA v.10.0.4. A total of 9 haplotypes were identified using DnaSP v.5.10 software (Rozas *et al,* 2017) to determine the haplotype of the DNA control region, but no common haplotypes were found between a wild camel and domestic camel populations. Two samples of wild camels were included in one haplotype (H7), while the domestic camel population was divided into 8 haplotypes.

Burger (2012) and Silbermayr *et al* (2009) have sequenced the cyt b gene and D-loop in the mitochondrial genome of Bactrian camels and identified 6 haplotypes (D1-D6) from 41 domestic

Fig 1. Geographic distribution of the domestic Bactrian camel samples analysed in the present study. 1. Umnugovi province, Han-bogd: Galbyn Gobi Red camel; 2. Umnugovi province, Manal- ovoo: Khanyn Hetsiin Khuren camel; 3. Gobi-Altai province, Tugrug: Tuhum tungalagiin khuren camel; 4. Gobi-Altai province, Tsogt: Mongolian camels; 5. Zavkhan province, Aldarkhaan: Mongolian camels; 6. Gobi-Altai, Bugat: Mongolian camels; 7. Uvs province, Baruunturuun: Mongolian camels.

camels and 2 haplotypes from 27 wild camels (W1, W2) were detected. Based on this, domestic and wild camels have many common haplotypes. Previous studies have also shown that the mitochondrial cyt b gene has a slower evolution rate than the D-loop. In other words, because camel species are less susceptible to evolution, some genes in the mitochondrial structure, such as the mitochondrial cyt b gene, are relatively conservative, while a relatively large number of mutations have accumulated in the D-loop, hence is more appropriate for detecting genetic differences in camel species.

A total of 9 haplotypes were detected in the nucleotide sequences of the mtDNA control regions in the 26 samples. In order to avoid duplication of nucleotide sequences in the mtDNA control region, one sample was selected as representative from the samples with a common haplotype in each haplotype and a total of 9 newly discovered haplotypes were registered with the gene bank (NCBI) (Table 1).

A total of 16 nucleotide substitutions were observed in domestic camels and wild camels when compared with the reference sequence from the gene bank (NC 009629.2 *Camelus ferus*, NC 009628.2 *Camelus bactrianus*) (Tables 1 and 2). In them, 12 nucleotides were replaced in wild camels and 5 nucleotides were replaced in domestic camels. The absence of a

common haplotype between domestic camels and wild camels (H9) indicates that there is a significant genetic difference. In the domestic Bactrian camel, all 3 main haplotypes were identified - H1, H2 and H3 (which is often found in Chinese, Russian and Mongolian Bactrian camels). Haplotypes specific to a particular geographical region (H6, H4, H5, H8) were also found.

A phylogenetic tree from all the detected haplotypes was constructed by neighbour joining method based on the Tamura-Nei model using 1000 bootstrap replications in MEGA v.10.0.4. The haplotype of the wild camel was in the same cluster with the reference sequence of wild camels from the gene bank (NC 009629.2 *Camelus ferus*) and the other domestic camels were clustered together with *Camelus bactrianus* from the gene bank (NC 009628.2 *Camelus bactrianus*) (Fig 3).

Genetic diversity was averaged over nucleotide diversity, haplotype diversity and nucleotide differences (Table 3). Domestic camel populations had high genetic diversity according to an mtDNA control region.

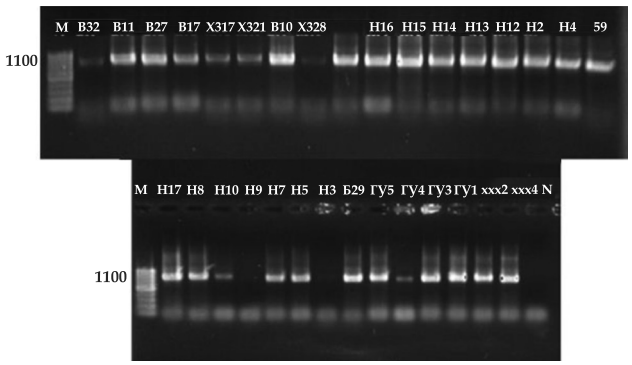

Fig 2. PCR product of control region mtDNA in 1% agarose gel electrophores. M- 100bp marker, N-negative control, Б-Besreg, Х3- Tuhum tungalagiin khuren, Н- local Mongolian camel, ГУ- Galbyn Gobi red camel, XXX- Khanyn khetsiin khuren.

Although, the genetic diversity of the Galbyn Gobi red and local Mongolian camel populations is greater than that of other populations, it may have depended on the number of samples. However, the nucleotide diversity of the Bearded camel is lesser than that of the Khoz Zogdort populations where sample numbers are the same. Due to the small number of wild camel samples, genetic diversity could not be determined.

At the end of *cyt b* gene and the beginning of the control region of mitochondrial DNA in Mongolian camel breeds and local Mongolian camel populations, the molecular diversity index showed high haplotype diversity, averaging H_d = 0.725 (± 0.044), nucleotide diversity was π= 0.00195 and the average number of genetic differences was k = 1,566 (Battsetseg, 2018).

In present study, the haplotype diversity was H_d = 0.6-0.93, the nucleotide diversity was π= 0.0009-0.002 and the average number of nucleotide differences was k = 0.6-1.6.

When calculating the genetic distance between two species and populations of camels, there was a significant genetic difference between wild camel populations and domestic camels. However, there were not many genetic differences between domestic camel populations (Table 6).

When comparing to the nucleotide sequences of 15120-15923 site from mitochondrial DNA, Mongolian camel Khanyn Khetsiin Khuren, Tuhum tungalagiin khuren, Galbyn Gobi Red, local Mongolian camel populations and wild camel were compared based on Tamura-Nei model in MEGA v6 and the genetic distance between Tuhum tungalagiin khuren - Khanyn Khetsiin Khuren was 0.0063, Galbyn Gobi Red-Khanyn Khetsiin Khuren was 0.0025, Galbyn Gobi red- Tuhum tungalagiin khuren was 0.0037, local Mongolian camels- domestic camel breed was

Table 1. The haplotype of domestic and wild camels and the gene bank accession number.

Populations	Numbers of Samples	Haplotype	Sample	NCBI GenBank Accession number
Domestic camels	7	H1	Б11, Б9, Б10, Х315, Х36, ГУ1, ГУ33	MT582437
	9	H2	Б27, Б17 Х310, Х326, НУ14, НУ2, ГУ5, ГУ34, ХХХ2	MT582438
	1	H3	НУ13	MT582439
	1	H4	НУ15	MT582440
	1	H5	НУ16	MT582441
	3	H6	НУ12, ХХХ4, ХХХ13	MT582442
	1	H7	ГУ3	MT582443
	1	H8	ГУ50	MT582444
Wild camel	2	H9	ХАВТГАЙ 9, ХАВТГАЙ 8	MT582445

Table 2. Nucleotide substitution in the control region of mtDNA.

Sample of camels	Name of population	Haplotype	Nucleotide of substitution																		
NC 009629.2 *Camelus ferus*	Reference suence		G	T	T	.	T	G	C	.	T	G	C	.	T	A	T	A	T	T
NC 009628.2 *Camelus bactrianus*			.	.	.	.	.	.	.	C	.	.	.	.	.	.		.	C	C
b11	Besreg	H1	A	C	.	A	C	A	A	T	C	A	T	A	C	C	C	C	-	-
b27		H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	-	-
b17		H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	-	-
b9		H1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-
b10		H1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-
hos zogdort 15	Khos zogdort	H1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-
hos zogdort 10		H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	-	-
hos zogdort 26		H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	-	-
hos zogdort 6		H1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-
Nutgiin temee 13	Domestic camels	H5	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.	.	-	-
Nutgiin temee 15		H4	.	.	.	G	.	.	.	.	.	.	.	.	.	.	T	A	-	-
Nutgiin temee 14		H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	-	-
Nutgiin temee 16		H3	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	A	-	-
Nutgiin temee 12		H6	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	-
Nutgiin temee 2		H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	-	-
Hawtgai 9	Wild camels	H9	G	T	T	.	T	G	C	.	T	G	C	.	T	A	T	-	-	-
Hawtgai 8		H9	G	T	T	.	T	G	C	.	T	G	C	.	T	A	T	-	-	-
Galbiin ulaan 5	Galbyn gobi red camel	H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	-	-
Galbiin ulaan 3		H7	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	-	-
Galbiin ulaan 1		H1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-
Galbiin ulaan 50		H8	.	.	.	.	T	.	.	.	.	.	.	C	.	.	T	A	-	-
Galbiin ulaan 33		H1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-
Galbiin ulaan 34		H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	-	-
Haniin hets 2	Khanyn khets	H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	-	-
Haniin hets 4		H6	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	-
Haniin hets 13		H6	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	-

low (0.0025-0.0050) and wild Bactrian camel-Bactrian camel was high (0.3369-0.3432) (Battsetseg, 2018). The nucleotide sequence of the mtDNA control region in the study, the genetic distance between Galbyn Gobi Red-Khanyn Khetsiin Khuren was 0.00297, local Mongolian camels-domestic camel breed was low (0.00153-0.00297) and wild Bactrian camel-Bactrian camel was high (0.01584-0.01652).

From the mid-90s to 2014, the population of Mongolian Bactrian camels declined from 700,000 to less than half of their previous population, according to the census. The consequences of such a severe reduction could have implications for the future sustainable use and conservation of this important livestock species. To assess the impact of this recent demographic event on genetic variability, a comprehensive study of mitochondrial and nuclear genetic diversity was conducted in three phenotypically different Mongolian domestic breeds compared to ordinary local Mongolian camels (Battsetseg *et al,* 2014). They concluded that Mongolian Bactrian camels have a genetic diversity comparable to Chinese Bactrian camels and dromedaries. They found neither a high level of inbreeding in various Mongolian breeds nor signs of a bottleneck. In recent years, due to economic needs (wool), over the past 10 years, the actual number of Mongolian Bactrian camels has increased by 68%.

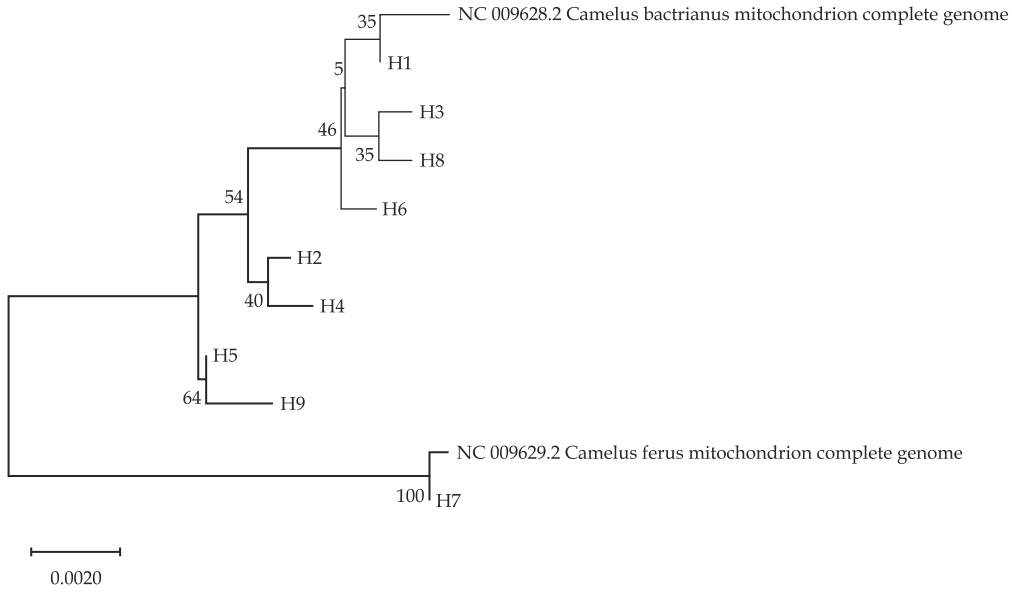

Fig 3. Phylogenetic tree established using haplotype of mtDNA control region. The evolutionary history was inferred using the Neighbour-Joining method.

Table 3. Genetic diversity comparison of mtDNA control regionNote: N-sample number; H-haplotype number; H d - haplotype diversity; Number of S-variable sites; π-nucleotide diversity; κ- Average number of nucleotide difference, SE-standard error.

Population	N	H	S	Hd±SE	π±SE	K
Wild camel	2	1	-	-	-	-
Galbyn Gobi red	6	4	4	0.86	0.002	1.6
Besreg camel	5	2	1	0.6	0.0009	0.6
Khanyn Khetsiin brown	3	2	2	0.66	0.002	1.3
Tuhum tungalagiin khuren	4	2	1	0.66	0.001	0.6
Local Mongolian camel	6	5	3	0.93	0.002	1.6

Table 6. Domestic camel populations and wild camels genetic distance of control region of mtDNA.

	Besreg	**Tuhum tungalagiin khuren**	**Local Mongolian camel**	**Wild camel**	**Galbyn gobi red**
Besreg					
Tuhum tungalagiin khuren	0.00153				
Local Mongolian camel	0.00290	0.00280			
Wild camel	0.01652	0.01636	0.01584		
Galbyn Gobi Red	0.00229	0.00229	0.00314	0.01663	
Khanyn Khetsiin khuren	0.00244	0.00254	0.00297	0.01558	0.00297

Mitochondrial DNA analysis has proven to be a useful tool for rapid screening of wild populations for maternal hybridisation and is also suitable for noninvasively collected samples, such as hair flakes from bushes (Lucas Lipp, 2013).

Based on the mtDNA D loop region, a small population structure in the Mongolian Bactrian camel could be seen. Three common haplotypes (H1, H2, H6) and 4 unique haplotypes (H3, H4, H5, H8) were found in the population of domestic camels. This suggests that the presence of unique haplotypes in the local populations of the Galbyn Gobi red indicates greater genetic diversity than in other populations. We assume that this diversity of haplogroups is related to the history of Bactrian camels (along trade routes between Asia and Europe). When compared with the populations of Galbyn Gobi Red and Khanyn Khetsiin Khuren, the value of the genetic distance shows an increase (from 0.0025 to 0.00297), indicating greater genetic diversity than in other populations.

As for local Mongolian camels and domestic camel breeds, the value of the genetic distance shows a decrease (from 0.0025-0.0050 to 0.00153-0.00297), which means the low genetic diversity of populations.

Wild Bactrian camels showed lower levels of nucleotide diversity and haplotype diversity, which may be due to the extremely small effective population size of the wild Bactrian camel (Ming, 2016). In our result, the value of the genetic distance between Wild and Bactrian camels was higher (0.3369-0.3432 to 0.01584-0.01652).

Conclusion

In present study, 9 haplotypes were identified in the Mongolian camel population from 16 haplotypes genus of Camelus, such a variety of haplogroups is probably related to the history of Bactrian camels (along trade routes between Asia and Europe). The Galbyn Gobi red and Khanyn Khesin Khuren has a the greater genetic diversity than in other populations. In contrary, low genetic diversity of populations has been shown for local Mongolian camel breeds.

Camelus bactrianus and *Camelus ferus* evolved as 2 distinct lineages and it is assumed that these do not have the same maternal origin. *Camelus ferus* showed lower levels of nucleotide diversity and haplotype diversity, which might be due to the extremely small effective population size of wild camels. On the other hand, our results and other research emphasises the importance of preserving the current variability of wild camel populations as a very valuable species in the desert.

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