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RESEARCH ARTICLE

High occurrence of length heteroplasmy in domestic Bactrian camel (*Camelus bactrianus*)

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ABSTRACT

Heteroplasmy is the presence of more than one mitochondrial DNA (mtDNA) variant within a cell, tissue, or individual. In this study, sequence variation was investigated in the control region (CR) of mitochondrial DNA (mtDNA) from 135 individuals belonging to five primary domestic Bactrian camel breeds in China and Mongolia. Due to variation of the repeat unit G(T/C)(AC)n, length heteroplasmy was detected within each camel by direct sequencing and fragment analysis. A high occurrence of mtDNA heteroplasmy, up to 100 percentages was observed in five camel populations. Our study provides the first evidence of extensive length heteroplasmy in Bactrian camels.

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Bactrian camel; control region; length heteroplasmy; mitochondrial DNA

Introduction

Length heteroplasmy in the mtDNA control region (CR) has been reported in a wide range of animals, such as Mytilus (Hoeh et al. 1991), eastern spadefoot toad (Munwes et al. 2011), crested ibis (He et al. 2013), cetaceans (Vollmer et al. 2011), dog (Savolainen et al. 2000), rabbit (Mignotte et al. 1990), Nepalese sheep (Gorkhali et al. 2016) and evening bat (Wilkinson & Chapman 1991). The Bactrian camel (*Camelus bactrianus)* is a two-humped camel native to the steppes of Central Asia. The complete mtDNA sequence of the wild Bactrian camel has revealed that length variation mainly occurs in a tandem repeat (ACGTAC)*n* between the conserved sequence blocks (CSB) I and II in the CR (Cui et al. 2007). Heteroplasmy has also been observed in a similar position in South American camelids (Maté et al. 2007). Thus, we hypothesize that domestic Bactrian camels carry length heteroplasmy. In this study, our aim was to investigate the existence, frequency and pattern of heteroplasmy in five primary Bactrian camel breeds from China and Mongolia by direct sequencing and fragment analysis.

Material and methods

Ear tissue samples from 135 domestic Bactrian camels were collected from five breeds from China and Mongolia (Table 1). The sample set did not include any sets of parents and offspring for representing heteroplasmy profile on the population level. Total genomic DNA was extracted using a conventional phenol/chloroform protocol (Sambrook & Russell 2001). Two sequencing primers of MF (5′-TCGCAGGACATAA CTACAACAC-3′) and MR (5′-TGGCAGGACTGTCTGGTGTAT-3′) were designed for the region including the heteroplasmy fragment (Figure 1). The primer MF was labelled by a 6-FAM florescent dye at its 5′-end to detect the size variations in the heteroplasmy fragment analysis. PCR amplicons were directly sequenced using primers MF and MR.

In the analyses, NUMTs were detected through blasting the Bactrian camel mitochondrial genome (NC_009628.2) against the Bactrian camel nuclear genome (JARL00000000.1) sequence using BLASTN program with a threshold of 10E − 4. Eastern spadefoot toad was used for the selection of raw data and analysis method in the study (Munwes et al. 2011).

Results and discussion

mtDNA sequence variation in domestic Bactrian camels

Sequencing chromatograms from the 135 camels showed remarkable sequence variation in the repeat region between CSB I and II. This region comprised tandem repeats with a consensus sequence 5′-GT/C(AC)n-3′ (Figure 1). These tandem repeats were similar in both position and structure to those found in wild Bactrian camels (Cui et al. 2007). The six base

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Supplemental data for this article can be accessed here.

Table 1. Sample size and distribution of five domestic Bactrian camel breeds.

Country	Breed	Code	Type	Sample size	Distribution
China (CH)	Alxa Bactrian camel	AX	Desert type (AXD)	17	Alashan Prefecture, Inner Mongolian Autonomous Region, China
			Gobi type (AXG)	22	
	Sonid Bactrian camel	SN		19	West Sunite Banner, Silinghol League, Inner Mongolian Autonomous Region, China
	Hexi Bactrian camel	HX		21	Beidangchengwan Town, Jiuquan City, Gansu Province, China
	Qinghai Bactrian camel	QH		40	Haixi Mongolian & Tibetan Autonomous Prefecture, Qinghai Province, China
Mongolia (MG)	Mongolian Bactrian camel	MG		16	Bayandale County, South Gobi Province, Mongolia
	Total			135	

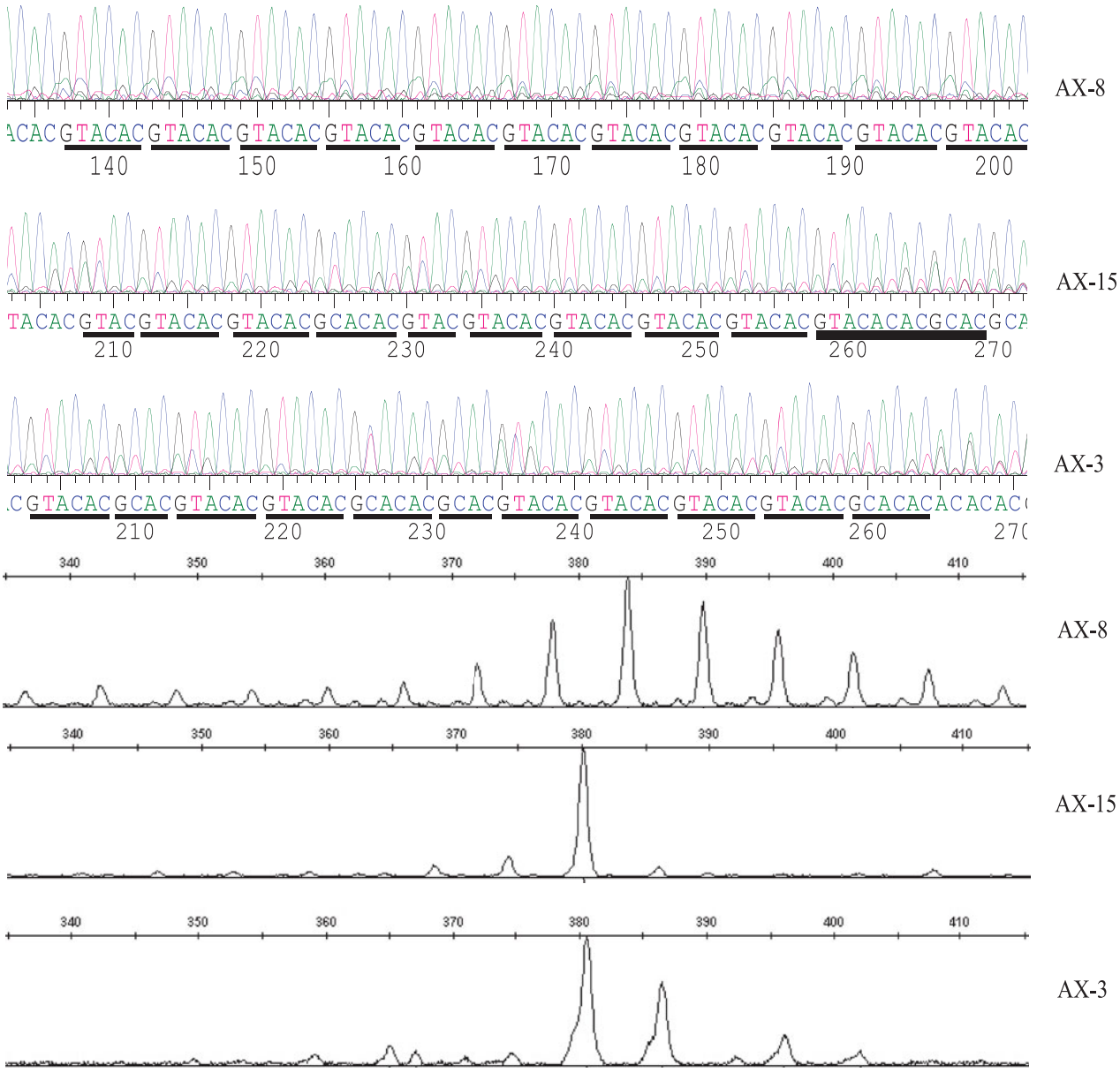

Figure 1. Partial sequencing chromatograms and the peak distributions of genotyping profiles of the representative camel samples showing the length polymorphism and heteroplasmy. Individual AX-8 had the repeat unit GT(AC)2. Individual AX-15 had a combination of three tandem repeats: GTAC, GT(AC)2, and GC(AC)2. Individual AX-3 had a similar combination of three tandem repeats: GT(AC)2, GCAC, and GC(AC)2. All three individuals had one predominant peak and additional minor peaks. Most repeat regions ended with a sequence of GT(AC)3GCAC, as in individual AX-15.

pair GTACAC repeat is also extensively distributed in the mtCR of many mammals (Wei et al. 2011), which indicates a widespread occurrence of the repeat unit across many animals. The sequence variation of the tandem repeat region was mainly caused by three factors: the T/C replacement; the number of "AC" units; and the number of repeats. The repeat region usually ended with 5′-GT(AC)3-GCAC-3′ (Figure 1).

The intra-individual multi-fragments were not caused by NUMTs

The PCR products for all individuals contained more than two fragment sizes, suggesting the presence of intra-individual variation in the mitochondrial DNA (mtDNA). At least two factors could cause this pattern: nuclear-encoded mitochondrial pseudogenes (NUMTs) and heteroplasmy. The NUMTs were detected by blasting a mitochondrial DNA genome sequence to nuclear genome data. Our study revealed the presence of NUMTs and heteroplasmy in different mtDNA regions, and showed that intra-individual multi-fragments were caused by length heteroplasmy and not by NUMTs (Table S1).

High occurrence of length heteroplasmy in camel populations

Both the sequencing results and genotyping profiles of each sample confirmed 100% incidence of length heteroplasmy within all camels. The incidence of mtDNA heteroplasmy in the five populations is as high as that found in the Crested Ibis (100%) (He et al. 2013), and much higher than that of animals such as the Atlantic spotted dolphin (58.9%) (Vollmer et al. 2011), Nepalese sheep (45%) (Gorkhali et al. 2016) and evening bat (29%) (Wilkinson & Chapman 1991). The high occurrence of length heteroplasmy in camels may be explained by sequence features of the tandem repeats. The core tandem repeat unit G(T/C)AC or G(T/C)ACAC in this study is an imperfect repeat unit, which could increase the stability of the slipped strand or could block polymerase action, thus enhancing the slipped mispairing of repeat units (Savolainen et al. 2000). The slipped-strand mispairing is considered the most plausible explanation for length heteroplasmy (Hoelzel 1991; Hoelzel et al. 1993; Savolainen et al. 2000).

We thus confirmed that both the number of the tandem repeats and the number of "AC" units contributed to the inter-individual length polymorphism as well as the intra-individual length heteroplasmy in the domestic Bactrian camels. Additionally, the number of heteroplasmic fragments varied from 3 to 21 in all the Bactrian camels sampled, further indicating a high level of diversity in the repeat region.

Conclusion

Based on this study, we conclude that domestic Bactrian camel populations have high proportions of length heteroplasmy (100%) generated mainly through DNA slippage. However, the complete mechanism and the functional importance of this high rate of heteroplasmy in domestic Bactrian camels are still not clear. Therefore, a comprehensive investigation is warranted, with samples taken at different ages and from different tissue types.

MtDNA length polymorphism among domestic Bactrian camels

Length variation was further genotyped by fragment analysis in all 135 camels. Each peak represented one DNA sequence in the fragment analysis map. From a total of 135 individuals, 120 (89%) individuals carried one highest peak (called the predominant fragment) and 15 (11%) individuals carried two highest peaks that were identical in height. The longest predominant fragment contained 41 tandem repeat units (425 bp), while the smallest one contained 36 tandem repeat units (337 bp). Most of the fragments ranged in length from 340 bp to 410 bp, suggesting abundant length polymorphisms in the tandem repeat region among domestic Bactrian camels.

Disclosure statement

The authors declare that they have no competing interests.

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