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When and how did *Bos indicus* introgress into Mongolian cattle?

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ABSTRACT

The Mongolian cattle are one of the most widespread breeds with strictly *Bos taurus* morphological features in northern China. In our current study, we presented a diversity of mitochondrial DNA (mtDNA) D-loop region and Y chromosome SNP markers in 25 male and 8 female samples of Mongolian cattle from the Xinjiang Uygur autonomous region in Western China, and detected 21 *B. taurus* and four *Bos indicus* (zebu) mtDNA haplotypes. Among four *B. indicus* mtDNA haplotypes, two haplotypes belonged to I1 haplogroup and the remaining two haplotypes belonged to I2 haplogroup. In contrast, all 25 male Mongolian cattle samples revealed *B. taurus* Y chromosome haplotype and no *B. indicus* haplotypes were found. Historical and archeological records indicate that *B. taurus* was introduced to Xinjiang during the second millennium BC and *B. indicus* appeared in this region by the second century AD. The two types of cattle coexisted for many centuries in Xinjiang, as depicted in clay and wooden figurines unearthed in the Astana cemetery in Turfan (3rd–8th century AD). Multiple lines of evidence suggest that the earliest *B. indicus* introgression in the Mongolian cattle may have occurred during the 2nd–7th centuries AD through the Silk Road around the Xinjiang region. This conclusion differs from the previous hypothesis that zebu introgression to Mongolian cattle happened during the Mongol Empire era in the 13th century.

1. Introduction

Cattle is one of the most economically important livestock in China and other parts of the world, and can be classified into two subspecies, *Bos taurus* (BTA) and *Bos indicus* (BIN, or zebu). The Fertile Crescent is considered as the primary center of taurine cattle domestication, whereas evidence for independent domestication events in other locales is currently debated (Edwards et al., 2011). mtDNA sequences revealed that indicine cattle originated from a different wild aurochs population, *Bos primigenius namadicus*, in the Indus Valley approximately 8000 years before present (Chen et al., 2009; Troy et al., 2001). It has turned out that there are five haplogroups (P, E, Q, R, and T) in taurine cattle and two (I1 and I2) in indicine cattle (Achilli et al., 2009). In addition, there are three paternal haplogroups (Y1, Y2 and Y3) detected in cattle based on Y chromosome single nucleotide polymorphism (Y-SNP) markers. Of these, Y1 and Y2 belong to BTA, and Y3 belongs to BIN (Götherström et al., 2005).

As for 28 cattle breeds and many local populations in China, they can be divided into three groups based on their geographic distribution, morphological characteristics and sex chromosome polymorphisms: the northern group in North China, the central group in the middle and lower areas of the Yellow River and the southern group in South China (Chen et al., 1993; Qiu et al., 1998). The studies of sex chromosome and mtDNA polymorphisms revealed a declining south-to-north gradient of female zebu introgression and a geographical hybrid zone in central China of BTA and BIN (Cai et al., 2007; Chen et al., 1993; Lei et al., 2006).

The Mongolian cattle breed, with strictly BTA morphological features, is one of the most commonly distributed breeds among many Chinese indigenous cattle breeds. It is herded mainly in the Inner Mongolian region, and is also widely distributed in the northeast, north and northwest of China (Qiu et al., 1998). It is suggested that the Mongolian cattle originated from BTA based on the Y chromosome karyotype (Chen et al., 1993). However, previous studies detected BIN lineage in Mongolian cattle (Lei et al., 2006; Mannen et al., 2004). Therefore, Mannen et al. (2004) agreed with the hypothesis that the import of zebu and other cattle from Southeast Asia, Southwest Asia and Northern India during the Mongol Empire era in the 13th century with subsequent crossing with native taurine cattle (Rupen, 1979), which could explain the BIN introgression in the Mongolian cattle. Meanwhile, although Cai et al. (2007) did not find any zebu mtDNA introgression in Mongolian cattle, they found lower percentages of zebu mtDNA introgression in Kazakh and Zaosheng cattle, which also belonged to North cattle group. In contrast, other studies speculated that the introgression of zebu cattle in China may originate from North Africa and the hybridization between African zebu and indigenous taurine cattle through the Silk Road of ancient China (Chen and Cao, 2001; Chen et al., 1990).

Abbreviations: mtDNA, mitochondrial DNA; BTA, *Bos taurus*; BIN, *Bos indicus*.

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In order to clarify the above debates regarding the BIN introgression in the Mongolian cattle, we analyzed the mtDNA D-loop and Y-SNP polymorphisms of 33 Mongolian cattle samples from Bayanbulak steppe in Xinjiang, China. Furthermore, we present the DNA results incorporating relevant historical and archeological information about the first appearances of cattle in Xinjiang, with an attempt to determine when and how the earliest BIN may have introgressed into the Mongolian cattle. Particular attention is given to the context of the ancient southern Silk Road, which connected northwestern China with India.

2. Materials and methods

2.1. Samples collection, DNA extraction and GenBank sequence mining

Blood samples of 33 Mongolian cattle (25 males, 8 females) were collected from Bayanbulak steppe in Hejing County, Xinjiang Uygur autonomous region, China (Fig. 1). The sample collection was permitted by the owner of cattle, and the owners were interviewed in detail to ensure unrelatedness among the sampled individuals. The present study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Northwest A&F University. Genomic DNA was extracted using standard phenol–chloroform protocol. Fifteen mtDNA D-loop sequences, of which 6 sequences have been assigned to BIN I1 haplogroup (GenBank accession nos: AB268563, EF417976, EF417979–EF417980, DQ166127–DQ166128) (Chen et al., 2009; Jia et al., 2010), 8 sequences to I2 haplogroup (GenBank accession nos: AB268560–AB268562, AB268568–AB268570, AB268574–AB268575) (Chen et al., 2009), and one reference mitochondrial DNA genome sequence (GenBank accession no: NC_006853) of *B. taurus*, were collected to use as phylogenetic analysis. A complete mtDNA sequence (GenBank accession no: EU780708) of buffalo was used as an outgroup in the phylogenetic tree construction.

2.2. PCR amplification and sequencing

The PCR protocol was as follows: each 25 μl reaction contained 20 ng of genomic DNA, 20 pmol of each primer, 0.2 mM of dNTPs, 1× PCR buffer (including 2.5 mM Mg^{2+}), and 1.0 U Taq DNA polymerase (Tiangen Biotech, Beijing, China). Thermocycling consisted of 4 min of denaturation at 95 °C, 35 cycles of 30 s at 94 °C, 60 s at 54 °C (condition for Y-markers see Table S1) and 90 s at 72 °C, and a final extension for 10 min at 72 °C. PCR products were purified with Watson PCR Purification Kit (Watson Biotechnologies, Shanghai) and sequenced using an ABI model 3730 automated sequencer (Applied Biosystems).

2.3. Analysis of bovine mtDNA sequences and bovine Y-SNP diversity

The complete mtDNA D-loop was amplified using a pair of primers: 5-CTGCAGTCTCACCATCAACC-3 and 5-GATTATAGAACAGGCTCCTC-3 (Loftus et al., 1994). mtDNA sequences were edited using DNASTAR 5.0 (DNASTAR, Madison, WI) and aligned using ClustalX (Thompson et al., 1997). All positions containing gaps were eliminated from the analysis. The polymorphisms in the analyzed segments were exported using MEGA 5.0 (Tamura et al., 2011). A Maximum likelihood (ML) tree, using Hasegawa–Kishino–Yano model with an additional parameter of 1000 bootstrapping replicates, Gamma distribution (+G) was constructed in MEGA 5.0 (Tamura et al., 2011). The Bayesian phylogenetic tree was also constructed using TOPALi 2.5 (Milne et al., 2004). The haplotype diversity and nucleotide diversity for Mongolian cattle breed were estimated using Arlequin 2.0 package (Schneider et al., 2000). The pairwise mismatch distribution between mtDNA sequences was generated using DnaSP 5.0 program. The divergence time and most recent common ancestor were calculated using MEGA 5.0. The mutation rate was assumed to be 32%/million years (Troy et al., 2001).

Four Y-SNP markers (DDX3Y-7, ZFY-9, ZFY-10 and UTY-19) were selected (Ginja et al., 2009; Götherström et al., 2005) to distinguish BTA and BIN Y chromosome haplotypes in 25 male Mongolian cattle samples. Y chromosome regions, primer sequences, fragment sizes, and annealing temperatures of the above four markers are shown in Table S1 (Ginja et al., 2009; Götherström et al., 2005). The PCR products of the 25 male samples were sequenced in both directions and the sequences were analyzed with SEQMAN TM II v6.1 (DNASTAR Inc.). Y chromosome haplogroup assignment was performed following Ginja et al. (2009).

3. Results

3.1. Variation of mtDNA D-loop sequences in Mongolian cattle

Within the 33 complete mtDNA D-loop sequences analyzed in this study, 25 haplotypes (Fig. S1) were defined by the polymorphisms at 79 sites: 57 transitions, 20 transversions, and 2 coexistence sites of transition and transversion. Mongolian cattle showed high haplotype diversity of 0.978 and nucleotide diversity of 0.017. The haplotypes H1, H4, H6, H13 and H19 represented 2, 3, 2, 4 and 2 individuals, respectively, whereas the remaining 20 haplotypes occurred only once, respectively (Fig. S1).

3.2. Phylogenetic tree of mtDNA in Mongolian cattle

The ML (Fig. 2) and Bayesian (Fig. 3) trees were constructed based on 25 mtDNA haplotypes in Mongolian cattle and combined with 15 published BIN (14) and BTA (1) mtDNA sequences as controls, and buffalo mtDNA sequence as an outgroup. Both of the ML and Bayesian trees

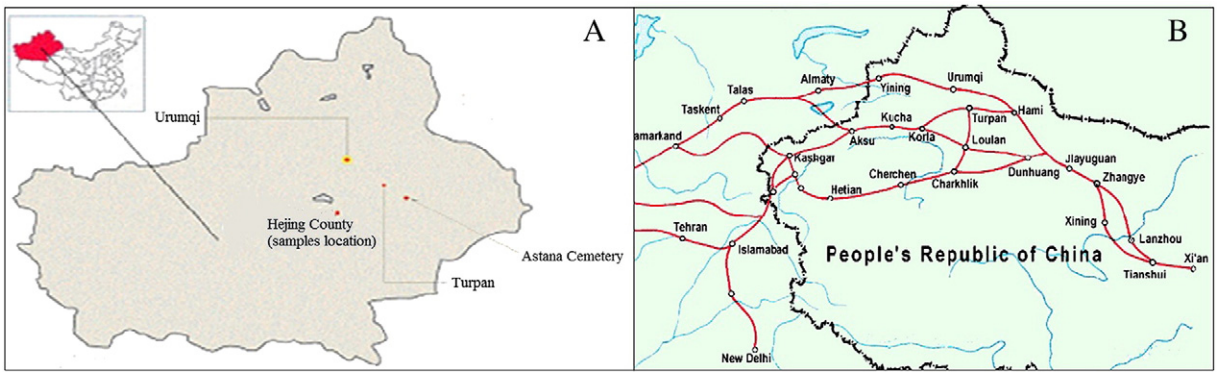

Fig. 1. The location of samples and Astana cemetery and the map of partial Silk Road route. (A) The map of Xinjiang Uygur autonomous region, it lists the sample location, and the location of Ancient Astana Cemetery. (B) The map of the partial Silk Road route.