Journal of Animal and Veterinary Advances 8 (7): 1312-1315, 2009
ISSN: 1680-5593

Mitochondrial DNA Diversity and Origin of Chinese Leiqiong Cattle

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Abstract: To determine the origin and genetic diversity of Chinese Leiqiong cattle, the complete mtDNA cyt b gene sequences were analyzed. There haplotypes of 18 individuals were identified from 2 polymorphic sites with 1140 bp in lengh. The average haplotype diversity and nucleotide diversity were 0.0741 and 0.0012 indicating rare genetic diversity in Leiqiong cattle. The nucleotide comparison also demonstrated a strong bias towards transition. A neighbor-joining tree was constructed and revealed that Leiqiong cattle only originated from *Bos indicus* and had no direct relationship with *Bos taurus*, *Bos grunniens* and *Bos javanicus* (banteng). The network relationships of haplotypes from *Bos indicus* supported the hypothesis that Chinese Leiqiong cattle and other Asian zebus possessed the independent domestication event. It was inferred an independent domestication centre of somewhere in south China.

Key words: mtDNA, origin, cyt b gene, Leiqiong cattle, haplotypes, China

INTRODUCTION

Cattle are widespread throughout all provinces of China. Chinese cattle breeds are roughly divided into 3 groups according to different ecological characteristics and sex chromosome polymorphisms: the southern group, the central group and the northern group (Qiu *et al*., 1988; Lei *et al*., 2000). Leiqiong cattle is one of the most typical breeds of the southern group and is mainly distributed on Leizhou peninsula and Heinan island in China. It is also, the dominant cattle breed in these 2 regions.

Whether, there exist pure *Bos indicus* type cattle breeds is always a focusing question in China. Few people think that pure *Bos indicus* type cattle breed exists and regard Leiqiong cattle as the only 1 in China. But there are no enough powerful evidences had been put forword to support the speculation till now. In order to provide genetic evidence for the origin of Leiqiong cattle, we examined the sequence of mtDNA cyt b gene in the full length and constructed phylogenetic tree with other bovine species.

MATERIALS AND METHODS

Sampling and sequencing: Fresh blood samples of 18 individuals from indigenous Leiqiong cattle of in Leizhou city of China. Genomic DNA was extracted by standard phenol-chloroform method.

The complete cyt b gene was amplified by using forward primer 5'-CCATAAATAGGTGAAGGTTTGG-3' and reverse primer 5'-TTGATGGTGAGACTGCAGTT-3'. The PCR were done with about 100 ng genomic DNA as a template in a volume of 50 μL of 1×reaction buffer, 1.5 mM $Mgcl_2$, 25 μM dNTPs, 0.4 μM of each primer and 2U Taq polymerase. Amplification was performed using a stand PCR program with 2 min denaturation at 95°C, 30 cycles for 40 sec at 94°C, 40 sec annealing at 51°C, 90 sec extension at 72°C and final extension for 8 min at 72°C.

PCR products were purified on spin columns (Watson Biotechnologies, Shanghai, China) and were directly sequenced by stand double-strand DNA cycle sequencing on an ABI 3730 automated sequencer.

Data collection and analysis: For comparison with data, additional mtDNA cyt b sequences were obtained from GenBank using a Blast program. The sequences came from 4 bovine species including *Bos taurus*, *Bos indicus*, *Bos grunniens* and *Bos javanicus*.

The cited sequences of *Bos taurus* were obtained from Japanese Black cattle (GenBank Accession No. AB074962 and AB074967), Korean cattle (GenBank Accession No. AY526085, DQ124371, DQ124372, DQ124374 and DQ124389), American cattle (GenBank Accession No. AF490528, AY676860, AY676861 and

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AY676866), England cattle (GenBank Accession No. V00654), Holland Holstein-Friesian cattle (GenBank Accession No. DQ124413) and Germany cattle (GenBank Accession No. DQ124413).

The cited sequences of *Bos indicus* were obtained from Asian zebus (GenBank Accession No. AF492350, AF531473, AY126697 and AY689190).

The cited sequences of *Bos grunniens* were obtained from China and other regions (GenBank Accession No. AF091631, AY374124, AY684273, AY955225, AY955226, EF494177, EF494178, EF494179 and NC_006380).

The cited sequences of *Bos javanicus* were obtained from Asian (GenBank Accession No. AY689188, EF197952, D34636, D82889, DQ459558 and DQ459559).

Alignment of sequences was achieved using the CLUSTAL W package (Thompson *et al*., 1994). Nucleotide diversity and haplotype diversity were performed in DnaSP package (Rozas and Rodzs, 1995). The Neighbor Joining (NJ) tree based on the cyt b gene sequences was reconstructed using Kimura 2-parameter model in the MEGA package (Kumar *et al*., 2004). The statistical confidence of each node in the tree was also estimated by 1000 random bootstrap resamplings. Network relationship of haplotypes of *Bos indicus* was analyzed by TCS1.18 package (Clement *et al*., 2004).

RESULTS

Sequence variation and haplotype: We have examined 18 complete mtDNA cyt b gene sequences of Chinese Leiqiong cattle which are all 1140 bp in lengh. Comparison of the 18 sequences revealed 3 different haplotypes with 2 polymorphic sites. The 3 haplotypes were named as LQ1, LQ2 and LQ3 (GenBank Accesion No EU096517, EU096518 and EU096519), respectively. The mtDNA sequences comparison of the complete alignment revealed 360 A<->G and 870 A<->G transitions, but only 2 transitions, demonstrating the strong bias towards transition.

The cyt b gene of Leiqiong cattle is rich in A/T nucleotides and shows a slightly higher A/T continent (56.8%) than C/G continent (43.2%). The average haplotype diversity and nucleotide diversity were 0.0741 and 0.0012, respectively, indicating rare genetic diversity in Leiqiong cattle.

Phylogenetic tree of the haplotypes and network map of the *Bos indicus* lineage: The different haplotypes found in this study and cited sequences from other 4 bovine

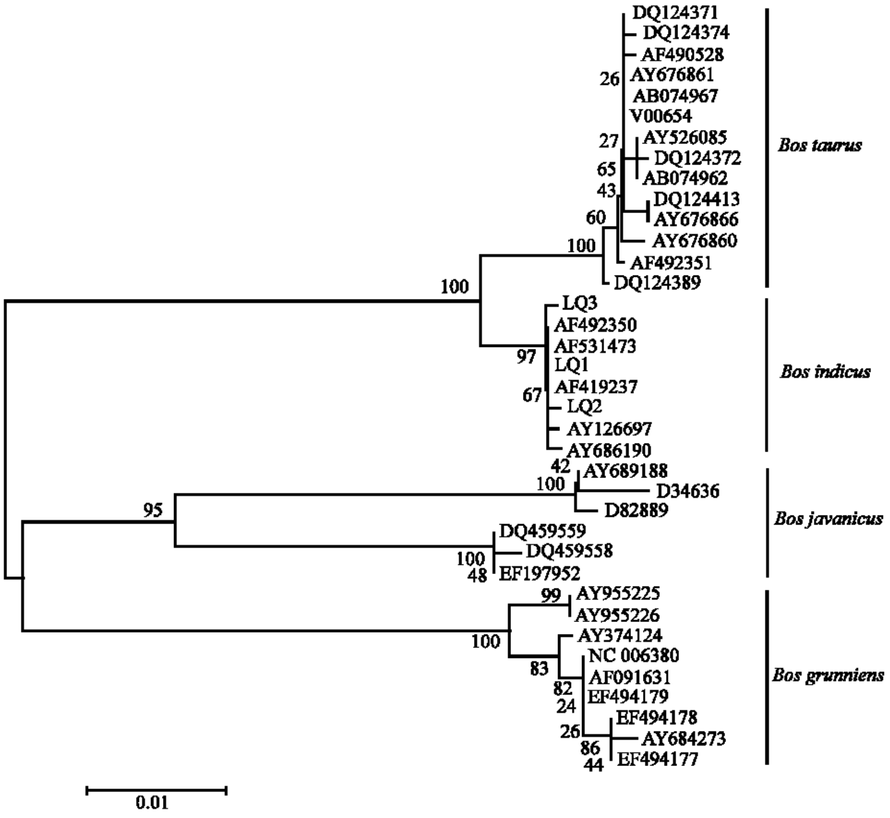

Fig. 1: NJ tree of cyt b gene sequences of 4 bovine species (The bootstrap values of the branches)

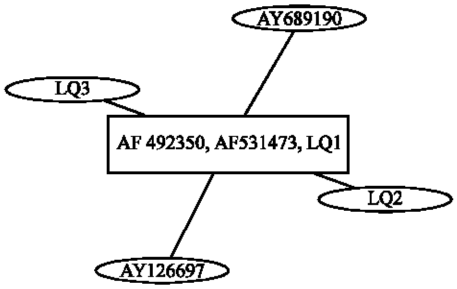

Fig. 2: Network relationships of haplotypes from *Bos indicus*

species were used to construct a NJ tree to reveal the origin of Chinese Leiqiong cattle (Fig. 1). The NJ tree showed 4 different mtDNA lineages: *Bos taurus*, *Bos indicus*, *Bos grunniens* and *Bos javanicus*. The 4 distinct lineages could be interpreted as evidence for 4 separate maternal origin. The 3 haplotypes of Chinese Leiqiong cattle were all clustered into the *Bos indicus* lineage with a high bootstrap probability (>50%).

To identify the relationship between Chinese Leiqiong cattle and Asian zebus, We compared haplotypes of the *Bos indicus* branch. The network showed a star-like phylogenetic pattern with a high frequency (43%) haplotype located in the centre (Fig. 2). The most predominant haplotype represented by sequences (GenBank Accession No. AF419237) was shared by Chinese Leiqiong cattle and Asian zebus. The predominant haplotype had direct mutational connections to other haplotypes. This kind of haplotype, which is related by mutational steps to >1 haplotype and uaually displaying a high frequency, is referred to as interior or ancestral haplotype (Posada and Crandall, 2001).

DISCUSSION

There were different opinions about the origin of Chinese Leiqiong cattle and have been disputed for a long time. The origin of Leiqiong cattle was much more complicated, with the speculation that it might originate from *Bos indicus*, *Bos indicus* and even *Bos javanicus* (banteng) (Chen *et al*., 1990; Payne, 1997; Yu *et al*., 1999).

In this study, we determined the complete mtDNA cyt b gene sequences of Leiqiong cattle, with the representative samples from its central colony. The overall haplotypes fell into the *Bos indicus* lineage revealing a single maternal origin. It was firmly believed that Leiqiong cattle only originated from *Bos indicus* and had no direct relationship with *Bos taurus*, *Bos grunniens* and *Bos javanicus* (banteng).

The network relationships of haplotypes from *Bos indicus*, Chinese Leiqiong cattle shared an ancestral haplotype with Asian zebus. Geographical isolation lied between Leiqiong cattle and Asian zebus. But no sufficient data could certificate that Asian zebus in other regions of Asian once had enter into Leizhou peninsula in history.

We could eliminate the probability of gene flow between them primarily and support the hypothesis that they possessed the independent domestication event. They maybe both origin from wild *Bos namadicus* and domesticated in different region. Futher more, we could infer an independent domestication centre of somewhere in south China. This speculation was also, supported by historical data, archaeological data and modern molecular research results (Chang *et al*., 1991; Cai *et al*., 2006).

ACKNOWLEDGEMENTS

This study was supported by the Project of the Basic Natural Science Foundation for Colleges and Universities in Jiangsu Province (Grant No. 08 KJB230002), the Qing Lan Project for Colleges and Universities in Jiangsu Province and the National Natural Science Foundation of P.R. China (Grant No. 30571323).

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