Maternal genetic diversity, differentiation and phylogeny of wild yak and four domestic yak breeds in Qinghai, China inferred from mitochondrial Cytb variations

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Abstract

Yak (Bos grunniens) is a unique livestock animal originating from the Qinghai-Tibet Plateau in China. In the current study, we investigated the maternal genetic diversity, differentiation and phylogeny of wild yak population and four domestic yak breeds (Qinghai-Gaoyuan, Huanhu, Xueduo, and Yushu) in Qinghai, China by analyzing 166 mitochondrial cytochrome b (Cytb) gene sequence variations. Our results indicated that the haplotype and nucleotide diversities of wild yak were 0.883±0.044 and 0.004±0.002, while the total haplotype and nucleotide diversities of four Qinghai domestic yak breeds were 0.646±0.040 and 0.003±0.001, respectively. Among the four Qinghai domestic yak breeds, the haplotype diversity was found to be highest in Yushu yak breed (Hd = 0.770±0.053), while the lowest was recorded in Huanhu yak breed (Hd = 0.501±0.088). Estimates of FST values showed a moderate genetic differentiation between wild yak and Huanhu yak (FST = 0.058) as well as that between Huanhu yak and Yushu yak breeds (FST = 0.052), but a weak genetic differentiation was observed between the other yak breeds/populations (-0.021¡FST¡0.037). Additionally, the clustering analysis based on RST values showed that Xueduo yak and Huanhu yak were clustered into one group, and each of the other three yak breeds/populations was separated into one group, respectively. Overall, the clustering relationship between wild yak and Yushu yak was closer. Maternal phylogenetic analysis showed that wild yak and four local yak breeds/populations in Qinghai represented in three maternal lineages (Mt-I, Mt-II, and Mt-III), indicating three maternal origins in yak. Our study would provide valuable information for the conservation and utilization of wild yak and Qinghai domestic yak breeds.

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China by analyzing 166 mitochondrial cytochrome b (*Cytb*) gene sequence variations. Our results indicated that the haplotype and nucleotide diversities of wild yak were 0.883±0.044 and 0.004±0.002, while the total haplotype and nucleotide diversities of four Qinghai domestic yak breeds were 0.646±0.040 and 0.003±0.001, respectively. Among the four Qinghai domestic yak breeds, the haplotype diversity was found to be highest in Yushu yak breed (Hd = 0.770±0.053), while the lowest was recorded in Huanhu yak breed (Hd = 0.501±0.088). Estimates ofF_{ST} values showed a moderate genetic differentiation between wild yak and Huanhu yak ($F_{\mathrm{ST}} = 0.058$) as well as that between Huanhu yak and Yushu yak breeds ($F_{\mathrm{ST}} = 0.052$), but a weak genetic differentiation was observed between the other yak breeds/populations (-0.021¡F_{ST} ¡0.037). Additionally, the clustering analysis based on R_{ST} values showed that Xueduo yak and Huanhu yak were clustered into one group, and each of the other three yak breeds/populations was separated into one group, respectively. Overall, the clustering relationship between wild yak and Yushu yak was closer. Maternal phylogenetic analysis showed that wild yak and four local yak breeds/populations in Qinghai represented in three maternal lineages (Mt-I, Mt-II, and Mt-III), indicating three maternal origins in yak. Our study would provide valuable information for the conservation and utilization of wild yak and Qinghai domestic yak breeds.

Keywords

Bos grunniens , *Cytb* , Genetic diversity, Differentiation, Phylogenetic relationship

1 INTRODUCTION

Yak (*Bos grunniens*), known as the "boat of the plateau", is a vulnerable bovine species endemic to the Qinghai-Tibet Plateau (QTP) with an altitude of 2,000˜6,000m above sea level (Qiu et al., 2012; Wang et al., 2011). At present, there are more than 15 million wild and domestic individuals worldwide, but about 95% of which are lived in China (Wiener et al., 2003). Qinghai Province of China owns abundant yak genetic resources. It possessed four officially recognized indigenous breeds (Qinghai-Gaoyuan, Huanhu, Xueduo, Yushu) and two improved breeds (Datong and Ashdan). Mitochondrial DNA (mtDNA) owns the characteristics of maternal inheritance and high variation rate, that making it as an important molecular marker to explore the evolution history, origin and genetic diversity of mammals (Bruford et al., 2003; Lunkina et al., 2004; Srivastava et al., 2015; Maltsev et al., 2015). The mammalian cytochrome b gene (*Cytb*), located in the functional region of mitogenome, is a mitochondrial oxidative phosphorylation complex protein encoding 379 amino acids (Hatefi, 2018). Compared with the mtDNA D-Loop region, *Cytb* is more conserved and also has been widely used in the studies on animal's population genetics in recent years. Previously, some studies analyzed mtDNA D-loop region to explore the maternal genetic diversity of wild yak and Qinghai domestic yak populations (Ma et al., 2010; Wang et al., 2010), but relatively few studies are done based on *Cytb*variations. Again, to date, most *Cytb* studies conducted have focused explicitly on a few Chinese yak populations (e.g. Bazhou, Jiali, Sangsang, Sangri, Gongbujiangda, Sibu, Pali, Kangbu, Jiangda, Leiwuqi, Dingqing, Baqing, Zhongdian, Taxkorgan, Karakorum-Pamir, Shenzha, and wild yak) (Yang et al., 2009; Chang et al., 2010; Ji et al., 2012; Wang et al., 2013; Tu et al., 2016; Hu et al., 2018; Liu et al., 2019; Li et al., 2019; Ji et al., 2019; Wang et al., 2021), but which were limited to four domestic yak breeds in Qinghai Province of China (Wang et al., 2021).

In this study, we comprehensively analyzed maternal genetic diversity and differentiation of six wild and domestic yak breeds/populations (wild, Qinghai-Gaoyuan, Huanhu, Xueduo, and Yushu yak) in Qinghai, and explored their clustering relationship and phylogeny based on*Cytb* sequence variations so as to provide baseline information for the conservation and utilization of these valuable yak genetic resource.

2 MATERIALS AND METHODS

2.1 Sample Collection and genomic DNA extraction

Muscle tissue samples of two wild yak was collected from the Hoh Xil nature reserve of Qinghai Province and Yanchiwan nature reserve of Gansu Province in China, respectively. Moreover, we collected jugular vein blood samples from three Qinghai-Gaoyuan yak in Golmud City, China (N: 36deg 24' 8.64", E: 94deg 54' 11.88")

(Table S1). The genomic DNA was extracted using Blood DNA Extraction kit (Aidlab Biotechnologies Co., Ltd, Beijing, China) according to the manufacturer recommendations, diluted to 10 to 100 ng/μL and kept it at -80 until to use.

2.2 PCR amplification and Sequencing

According to the previously reported methods (Yang et al., 2009), the complete *Cytb* sequences were amplified using a pair of primers: PF 5'-gttccgtagccatagccg-3'; PR 5'- ttgagtcttagggaggtt-3'. PCR amplification was conducted in a 25-μL reaction mixture containing 12.5 μL of 2×PreMix, 1 μL of each primer (10 pmol/μL), 1 μL of genomic DNA (10-20ng/μL), and 9.5 μL of ddH_2O. The PCR was carried out using a standard program with 4 min predenaturation , then consisted of 35 thermal cycles with a denaturation step (45 sec at 95), a hybridization step (50 sec at 53.2) and an elongation step (120 sec at 72) for each cycle, and final extension for 5 min at 72, followed by cooling to 4 . PCR products with appropriate band size were then sent to Beijing Tsingke Biotechnology Co., Ltd (Beijing, China) for sequencing.

2.3. Sequences download and Data Analysis

161 previously reported *Cytb* sequences from wild yak and four Qinghai domestic yak breeds/populations were downloaded in Genbank (Table S1). Combined with five new *Cytb* sequences from two wild yak and three Qinghai-Gaoyuan yak, 166 mtDNA *Cytb* sequences were analyzed totally in this study (Table S1). Raw *Cytb* sequences were checked and aligned with the Clustal W multiple alignment algorithm of BioEdit v7.2.5 software (Hall, 1999). The number of variable sites, haplotype diversity (Hd) and nucleotide diversity (Pi) were estimated using Dnasp 5.10 (Librado& Rozas, 2009) and Arlequin 3.11 (Excoffier et al., 2007). Fixation index (F_{ST}) was also calculated using the Arlequin 3.11 software. TheR_{ST} values were calculated by the linearizedF_{ST} value, namely, $R_{ST} = F_{ST}/(1\text{-}F_{ST})$. Multidimensional scaling (MDS) analysis was performed based onR_{ST} values using SPSS 18.0 software. Taking the American bison (*Bison bison*) counterpart as an out-group (Genbank accession No.: EU177871), the phylogenetic tree was constructed by using the Neighbor-joining (NJ) method in Mega 6.0 (Tamura et al., 2013). A median-joining (MJ) network was generated using the Network 10.2.0 software (Bandelt et al., 1999).

3 RESULTS AND DISCUSSION

3.1 Maternal genetic diversity of five yak breeds/populations

The sequences of two wild yak and three Qinghai-Gaoyuan yak were deposited in GenBank under the accession numbers ON077034-ON077035 and OP389990-OP389992, respectively. A set of the 166 mtDNA *Cytb*sequences from one wild yak population and four Qinghai domestic yak breeds was analyzed, which revealed 34 polymorphic sites, including 16 singleton variable sites and 18 parsimonious informative sites. A total of 11 variable sites were detected, and 11, 11, 11,12, and 25 haplotypes were identified in Qinghai-Gaoyuan, Huanhu, Xueduo, Yushu, and wild yak, respectively (Table S2). Totally, 29 haplotypes were defined in 166 yak samples (Figure 2,Table S2). Of these, H1, H4, and H10 were the most frequent, being represented in 86 (51.81%), 26 (15.66%), and 20 (12.05%) individuals of all samples respectively, followed by H14 identified in three yak (1.81%). H2, H5, H19, H21, H22, and H28 were each found in two yak individuals and the remaining 19 haplotypes were found in single individual (Figure 2.). At the same time, a total of 7 haplotypes were found in Qinghai-Gaoyuan yak (4 specific haplotypes), 5 in Huanhu yak (2 specific haplotypes), 8 in Xueduo yak (3 specific haplotypes), 9 in Yushu yak (4 specific haplotypes), and 13 in wild yak (8 specific haplotypes), respectively, indicating that both wild yak and four Qinghai domestic yak breeds/populations own unique maternal genetic information. The analysis of genetic diversity of five yak breeds/populations showed that the haplotype and nucleotide diversities of wild yak (0.883±0.044 and 0.004±0.002) were higher than that of the total haplotype diversity (0.646±0.040) and nucleotide diversity (0.003±0.001) of four Qinghai domestic yak breeds, respectively (Table S2), indicating a rich genetic diversity in five yak breeeds/populations, but the wild yak population have highest maternal genetic diversity. Of the four domestic yak breeds, the haplotype diversity reached a maximum in the Yushu yak breed (0.770±0.053) and a minimum in the Huanhu yak breed (0.501±0.088). Notably, the result was broadly consistent with the previous studies on domestic and wild yak breeds/populations in Qinghai (Li et al., 2022).

3.2 Genetic differentiation and clustering relationships among five yak breeds/populations

F_{ST} (Fixation Index) is the index of genetic differentiation among populations, which can be used to evaluate the degree of differentiation among populations. The genetic differentiation index of 0 to 0.05 shows a very weak population differentiation, that of 0.05-0.15 shows moderately differentiated populations, whereas, 0.15-0.25 indicates significant population difference. The degree of differentiation is considered extremely significant when the index reaches above 0.25 (Curnow & Wright, 1978). Here, our analysis revealed that the F_{ST} values between wild yak and three Qinghai local yak breeds (Qinghai-Gaoyuan, Xueduo, and Yushu) exhibited a very weak genetic differentiation (-0.021¡F_{ST} ¡0.037), but a moderate differentiation was observed between wild yak population and Huanhu yak breed ($F_{ST} = 0.058$) (Table S3). Additionally, of four domestic yak breeds, the results suggested a moderate differentiation was found between Yushu yak breed and Huanhu yak breed ($F_{ST} = 0.052$). In contrast, a very weak genetic differentiation was observed between the other domestic yak breeds (Table S3). Overall, the genetic differentiation among the wild yak population and four Qinghai domestic yak breeds showed a weak level, which was largely consisted with the previous results based on paternal genetic differentiation (Li et al., 2022)

According to the analysis of multi-dimensional scale (MDS) among five yak breeds/populations in this study, the five yak breeds/populations were divided into four groups/categories. In dimension one, wild yak and Yushu yak were separated into one group, Qinghai-Gaoyuan, Xueduo, and Huanhu yak were separated into another one group. In dimension two, Qinghai-Gaoyuan yak was separated from Xueduo and Huanhu yak, wild yak and Yushu yak were separated again (Figure 3). Based on the results of genetic differentiation and cluster analysis, the cluster relationship between Yushu yak and wild yak was closer, while the cluster of Xueduo, Huanhu, and Qinghai-Gaoyuan yak was closer.

3.3 Phylogenetic analysis of five yak breeds/populations

Phylogenetic analysis revealed that 29 haplotypes was separated into eight haplogroups (A-H), among which four haplogroups (A, B, C, and G) were found in wild yak population, four haplogroups (A, B, C, and F) in Qinghai-Gaoyuan yak breed, three haplogroups (A, B, and C) in Huanhu yak breed, six haplogroups (A, B, C, D, E, and G) in Xueduo yak breed, and six haplogroups (A, B, C, D, F, and H) in Yushu yak breed. Furthermore, only three haplogroups (A, B, and C) were shared by wild yak population and four domestic yak breeds (Figure 1, Table S2).

In our present study, a neighbor-joining tree and a network diagram of 166 yak individuals was constructed to explore the yak phylogeny (Figure 4 and Figure 5). Our results showed that the diagram comprised three branches, eight haplogroups were separated into three distinct maternal lineages (Mt-I, Mt-II, and Mt-III), indicating three maternal origins in yak. Here, Mt-I showed the highest frequency (77.71%) of the total yak individuals, followed by Mt-II (21.08%), and Mt-III (1.21%). Of these, Mt-I, Mt-II, and Mt-III including 4 haplogroups (A, B, E, and F), 3 haplogroups (C, D, and H), and 1 haplogroup (G), respectively. It was worth noting that Mt-III included only one Xueduo yak and one wild yak (Figure 2 and Figure 4). Compared with the previous findings of wild yak and Qinghai domestic yak breeds/populations based on the whole-mito genome variants (Ma et al., 2021; Wang et al., 2010; Wang et al., 2021; Li et al., 2022), this experiment reconfirmed that yak consisted of three maternal lineages, dominated by Mt-I, and presumably has three maternal origins.

As one of the five major pastoral areas in China, Qinghai has extremely abundant yak resources. In this study, five yak breeds/populations have high genetic diversity and each yak breed/population owns unique haplotypes. Both domestic and wild yak were clustered into three lineages, probably with three maternal origins. Based on the above results, we provide baseline information for the wild yak and Qinghai local yak breed and the genetic relationship among each other, which is conducive to the preservation of these valuable yak genetic resources. Overall, further exploration of Qinghai domestic yak and wild yak based on the whole-genome level in the future is of critical importance.

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CONFLICTS OF INTEREST

No potential conflict of interest was reported by the authors.

AUTHOR CONTRIBUTIONS

Zhijie Ma conceived and designed the project. Ruizhe Li, Yuhui Xu, Weixing Guo,Shengmei Chen, Wenhao Li, Weihua Huang, and Zhijie Ma carried out sampling. Donghui Xu and Zhijie Ma performed the experiment and data analyses. Donghui Xu wrote the original manuscript. Zhijie Ma and Chuzhao Lei revised the manuscript. All authors reviewed and approved the final manuscript.

DATA AVAILABILITY STATEMENT

All relevant data for this study are included in and accessible through this manuscript.

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