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ANIMAL GENETICS

Genetic Diversity and Phylogenetic Structures of Four Tibet Yak Populations Using CytB Gene Sequence of Mitochondrial DNA

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Abstract—The yak is one of the important livestock unique to the pastoral areas of the Qinghai–Tibet Plateau. In this study, the genetic diversity and phylogenetic relationships of four yak populations in Tibet Naqu and Ali were assessed using mitochondrial cytochrome genes (CytB). Results showed that the full length of the CytB gene sequences of 129 yak individuals in the four populations was 1140 bp, and the average contents of T, C, A, and G were 26.3, 28.9, 31.7, and 13.1%, respectively. Twenty-one haplotypes were constructed with the full-length region of the CytB gene based on 29 single nucleotide polymorphism sites. The haplotype polymorphisms, nucleotide polymorphisms, and Tajima's D of the four populations ranged from 0.59740 (Gaize Yak) to 0.69970 (Geji Yak), from 0.00214 (Geji Yak) to 0.00273 (Nima Yak), and from –1.4410 (Geji Yak) to –0.3370 (Gaize Yak), respectively. Specifically, the pairwise difference (F_{ST}) ranged from –0.03387 to 0.00383, indicating that the genetic divergence in these yak populations was low. The phylogeny and haplotype network analysis results implied that the four populations were mainly from two matrilineal origin linkages corresponding to two traditionally recognized matrilineal yak populations. Therefore, the genetic diversity in four yak populations with different geographical distributions was evaluated in this study to confirm the rich genetic diversity using CytB sequences. However, genetic differentiation among the populations was small. The result of this study will provide valuable theoretical basis for the diversity evaluation and conversion of domestic Tibet yaks.

Keywords: yak, mitochondrial cytochrome genes, diversity, population structure

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INTRODUCTION

As one of the five major pastoral areas in China, Tibet has large pastures and a rich foundation of nomadic animal husbandry. In recent years, Tibet's animal husbandry has developed rapidly as the basic economic industry. The overall livestock industry is improving with the gradual transformation of people's old backward concepts. The yak occupies a large proportion of the main domestic livestock species in Tibet, and products, such as yak beef, have become important local livestock products. Therefore, the protection and development of the genetic resources of the domestic yak population are greatly beneficial to Tibetan areas.

Domestic yak (*Bos grunniens*) is usually distributed in the Qinghai–Tibet Plateau. The majority of yak populations in the world are mainly distributed in China, and most of them have been domesticated. In recent years, in-depth research and exploration on population genetic diversity have been conducted [1, 2] to understand the conservatory status of the local yak population.

As an effective genetic marker, mitochondrial DNA has a wide range of applications in the study of population genetic diversity in various domestic animals, including pig [3, 4], cattle [5, 6], chicken [7–9], and goat [10, 11].

Mitochondrial cytochrome genes (CytB) are important molecular markers and widely used in the diversity estimation of domestic and wild animals [12–15]. In related studies, the phylogenetic relationship between the yak and the American bison in the Karakorum-Pamir region is closer than that between the yak and the common bison when a full-length CytB sequence is used [16]. Coincidentally, CytB has played an important role in the study of genetic diversity in local yak populations. Tibetan yak populations have a high genetic diversity when full-length CytB sequences are used [17]. The Xinjiang Taxkorgan yak has a high genetic richness, further confirming divi-

Table 1. Geographic location and sample information of four yak populations

Population	Code	Sample size	Altitude, m	East longitude (E) and north latitude (N)	Location
Jiali Yak	JL	33	4501	E 93.23253, N 30.64081	Naqu Jiali County, Tibet
Nima Yak	NM	37	4541	E 87.23677, N 31.78470	Naqu Nima County, Tibet
Geji Yak	GJ	37	5888	E 81.25544, N 32.65881	Ali Geji County, Tibet
Gaize Yak	GZ	22	4700	E 81.98333, N 33.55911	Ali Gize County, Tibet

Table 2. Tajima's D test and genetic diversity of four yak populations using mtDNA CytB gene sequence

Population	Sample size	Haplotype number	Haplotype diversity value	Nucleotide polymorphisms value	Tajima's D	Tajima's D *P*-value
GZ	22	7	0.59740	0.00261	−0.3370	$P > 0.1$
JL	33	8	0.65341	0.00272	−0.5342	$P > 0.1$
GJ	37	12	0.69970	0.00214	−1.4410	$P > 0.1$
NM	37	9	0.68769	0.00273	−0.6116	$P > 0.1$

sion with other yak breeds identified using full CytB sequences [18].

In this study, CytB sequences were used to investigate the genetic diversity and genetic differentiation of 129 yak individuals from four Tibet yak populations. Our result may facilitate the effective conversion and utilization of local yak genetic resources.

MATERIALS AND METHODS

First, 10 mL of venous blood sample was collected per individual with EDTA tubes. The populations were from the two ecotype regions (Naqu and Ali) of Tibet (Table 1). Then, the blood samples were frozen and stored in a refrigerator at −40°C.

The full-length sequence of the CytB gene was amplified using primers YAK mtDNA CytB-F: 5'-GTTCCGTAGCCATAGCCG-3' and YAK mtDNA CytB-R: 5'-TTGAGTCTTAGGGAGGTT-3'. The PCR reaction conditions were as follows: 95°C pre denaturation for 5 min; 94°C denaturation for 30 s, 51.5°C annealing for 30 s, 72°C extension for 80 s, 40 cycles; and 72°C extension for 10 min. The amplified PCR products were detected through 1.0% agarose gel electrophoresis and sequenced using a 3130XL genetic analyzer (AB Applied Biosystems, USA) with double PCR primers' direction by Wuhan TianyiHuiyuan Biological Co., Ltd.

Nucleotide polymorphism, haplotype number, average number of nucleotide differences (Kxy), mutation sites of CytB sequence, and Tajima's D neutral test were estimated using DNAsp 6.12.03 [19]. The alignment of sequences with CLUSTAL W [20] and phylogenetic tree of CytB haplotype sequence was performed using MEGA 7.0.26 [21]. The best fitting model was obtained using jModelTest V. 0.1.1 [22]. Pairwise difference (F_{ST}) was calculated using Arlequin 3.5.2.2 [23]. Finally, the neighbor-joining network of CytB haplotype sequences and the frequency distribution were constructed using Network 5.0.1.1 software package [24].

RESULTS

The CytB gene sequences of all the yak individuals were obtained, and the complete mitochondrial gene sequence of the yak was aligned (GenBank: GQ464270.1). The results revealed that the full lengths of the CytB sequences of 129 individuals were 1140 bp. The average contents of T, C, A, and G were 26.3, 28.9, 31.7, 13.1%, respectively, the average content of A + T was 58.0%, and the average content of G + C was 42.0%. Twenty-one haplotypes were identified in the CytB genes of 129 individuals when 29 single nucleotide polymorphism sites were used (29, 2.5%). In the CytB gene haplotype distribution of four yak populations, the GJ population carried the most number of haplotypes (12), and the GZ populations carried the least haplotypes (7). Haplotype diversity in the four populations ranged from 0.59740 (GZ) to 0.69970 (GJ), and the nucleotide polymorphisms ranged from 0.00214 (GJ) to 0.00273 (NM). The result of Tajima's D showed that the lowest and highest values were GJ (−1.4410) and GZ (−0.3370), respectively. However, the *P* value of Tajima's D was nonsignificant ($P > 0.1$) in the four populations (Table 2).

A phylogenetic tree was constructed from 21 mtDNA CytB haplotype sequences with the HKY model in MEGA 7.0.26 (Fig. 1a). The 21 haplotypes were divided into two main branches, that is, H_2 and H_3 were included the first branch, and the 19 remaining haplotypes were included in the second

Table 3. Results of the best fitting model estimation for the phylogenetic tree of 21 mtDNA CytB haplotypes

Model	#Param	BIC	AICc	lnL	Invariant	Gamma
HKY	43.00	3963.93	3616.50	−1765.17	n/a	n/a
HKY + G	44.00	3973.28	3617.78	−1764.80	n/a	0.38
HKY + I	44.00	3973.36	3617.86	−1764.85	0.49	n/a
TN93	44.00	3973.48	3617.98	−1764.91	n/a	n/a
TN93 + G	45.00	3982.82	3619.25	−1764.54	n/a	0.38
TN93 + I	45.00	3982.91	3619.34	−1764.58	0.49	n/a
HKY + G + I	45.00	3983.35	3619.77	−1764.80	0.63	200.00
TN93 + G + I	46.00	3992.90	3621.25	−1764.53	0.63	200.00
GTR	47.00	4003.15	3623.42	−1764.61	n/a	n/a
GTR + G	48.00	4012.45	3624.65	−1764.23	n/a	0.39
GTR + I	48.00	4012.58	3624.78	−1764.29	0.49	n/a
T92	41.00	4020.87	3689.60	−1803.73	n/a	n/a
GTR + G + I	49.00	4022.48	3626.60	−1764.20	0.62	200.00
T92 + G	42.00	4030.11	3690.76	−1803.30	n/a	0.32
T92 + I	42.00	4030.25	3690.90	−1803.37	0.49	n/a
K2	40.00	4039.89	3716.70	−1818.28	n/a	n/a
T92 + G + I	43.00	4040.18	3692.76	−1803.30	0.66	200.00
K2 + G	41.00	4049.18	3717.91	−1817.88	n/a	0.35
K2 + I	41.00	4049.30	3718.02	−1817.94	0.49	n/a
K2 + G + I	42.00	4059.25	3719.91	−1817.88	0.64	200.00
JC	39.00	4066.61	3751.49	−1836.68	n/a	n/a
JC + G	40.00	4075.89	3752.69	−1836.28	n/a	0.34
JC + I	40.00	4076.01	3752.82	−1836.34	0.49	n/a
JC + G + I	41.00	4085.97	3754.69	−1836.28	0.65	200.00

Models with the lowest Bayesian Information Criterion scores were considered to describe the best substitution pattern. GTR: general time reversible, HKY: Hasegawa–Kishino–Yano, TN93: Tamura–Nei, T92: Tamura three-parameter, K2: Kimura two-parameter, and JC: Jukes–Cantor.

branch. The phylogenetic network and frequency distribution of 21 haplotypes (Fig. 1b) showed that H_1 had the highest frequency and was shared by the four populations. H_2 and H_5 were shared by the four populations, H_7 (GZ and GJ) and H_18 (NM and GJ) were shared by two populations, and H_3 (GZ, JL, and NM) and H_11 (JL, GJ, and NM) were shared by three populations. The 17 remaining haplotypes were unique to each population, accounting for 68% of all the haplotypes.

The pairwise difference of populations (F_{ST}) was calculated, and the genetic distance between the four yak populations in Tibet was determined (Table 4, Fig. 2a). The genetic distance (F_{ST}) between populations ranged from –0.03387 to 0.00383, that between the GJ and JL populations was the farthest (0.00383), and that between the GZ and JL populations was the smallest (–0.03387), indicating that the genetic relationship between the GZ and JL populations was the closest. In addition, the average nucleotide difference coefficient (Kxy) ranged from 2.705 to 3.075, and the maximum value was obtained between JL and NM (3.075). The average nucleotide difference between GJ and GZ was the smallest (2.705).

DISCUSSION

The population genetic diversity and phylogeny of various domestic and wild animals were estimated using mtDNA polymorphisms [25, 26]. The mtDNA

Table 4. Genetic differences between different populations using Kxy and F_{ST}

Code	GZ	JL	GJ	NM
GZ	\	2.939	2.705	3.025
JL	−0.03387	\	2.776	3.075
GJ	0.00127	0.00383	\	2.743
NM	−0.00754	−0.01007	−0.01163	\

Above the diagonal is Kxy (average number of nucleotide differences), and below the diagonal is F_{ST}.

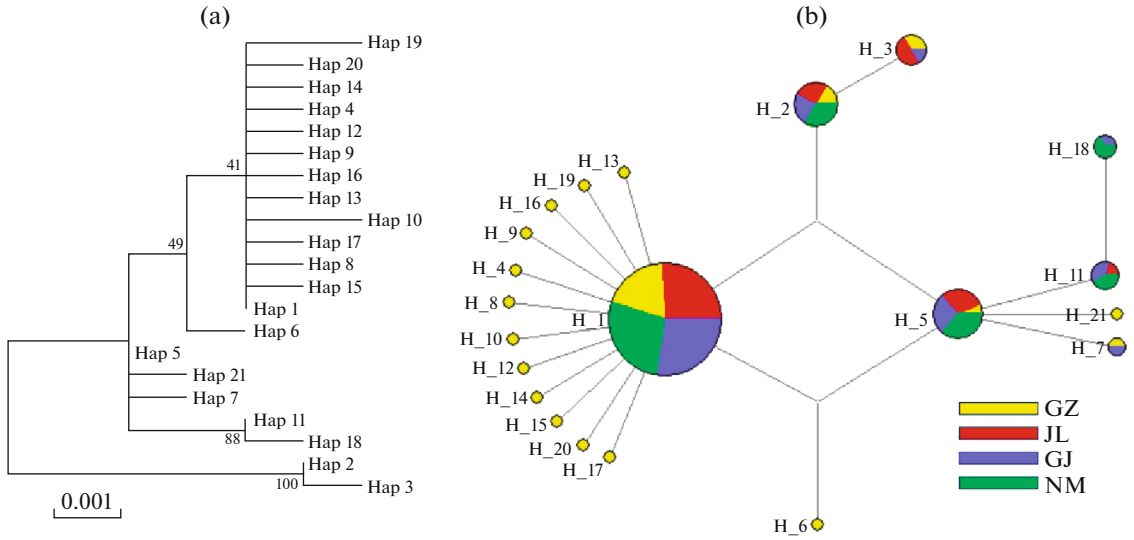

Fig. 1. Phylogenetic tree and haploid frequency diagram of CytB sequence. (a) Molecular system analysis of 21 yak mtDNA CytB haplotypes by maximum likelihood method. (b) Network and frequency distribution of 21 yak mtDNA CytB haplotypes.

CytB sequences of mammalian animals have become important mtDNA genetic markers for studying the relationship between population genetic diversity and phylogeny because of their relatively moderate evolution speed and rich genetic information [27–29].

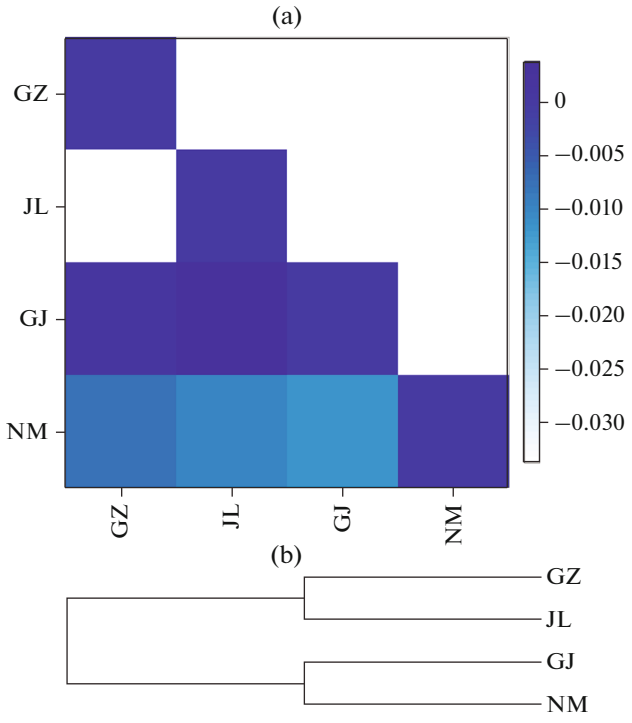

Fig. 2. Phylogenetic genetic divergence of four yak populations using F_{ST} and Kxy. (a) Paired F_{ST} matrix of four ecotype yak populations. (b) Phylogenetic relationship of four ecotype yak populations based on the average number of nucleotide differences (Kxy).

In recent years, the conversion and development of local yak genetic resources have attracted attention. Animal husbandry in Tibet has undergone continuous development. The conversion of local yak populations has become one of the important means for effectively utilizing yak resources [30, 31]. Thus far, numerous assessments on the genetic diversity level of local yak populations and related research have been published [32].

In this study, 129 individual mtDNA CytB region sequences were obtained from the four yak populations. Our analysis results showed that the complete length of the CytB gene region sequence was 1140 bp, and the base content of A + T (58.0%) was much greater than that of G + C (42.0%) showing a certain bias. These results were consistent with those of previous studies [33, 34]. Nucleotide polymorphism within the population of this study was lower than that determined by Ji et al. [35], indicating that the yak populations may have been affected to a certain extent. For example, the artificial intervention in reproduction and the small populations of the yak's ancestors have affected the genetic diversity of the yak. Second, haplotype diversity in the yak population in Tibet was much greater than nucleotide diversity, suggesting a new maternal breach in the yak population and corresponding to the research results in Banan yak and Sino–Burmese yak populations [36, 37].

The haplotype N-J network and frequency distribution among the four yak populations showed that most of the high-frequency haplotypes were shared by different populations as well as each yak population had unique haplotype types, indicating that a wide gene flew among the four yak populations. Furthermore, according to F_{ST}, no significant difference was found among these populations, although their genetic divergence was consistent with the geographi-

cal distance of habitats between them. This finding can also be attributed to the yak's feeding habit in the Qinghai–Tibet Plateau. Extensive nomadic grazing and grazing management were implemented. Specifically, the yak populations were accompanied by genetic material exchanges during the nomadic process [38]. Therefore, the degree of genetic differentiation among yak populations in this area was greatly reduced.

Furthermore, the Tajima's D analysis of CytB gene found that the D values of each population was negative but nonsignificant, indicating that no historical expansion occurred in the four yak populations, and the Tibet yaks were not affected by large-scale artificial selection during domestication.

Finally, according to the phylogenetic tree and haplotype network analysis of mtDNA CytB, yaks had two main maternal branches, which corresponded to the two generally considered maternal branches of the domestic Chinese yaks in previous studies [38, 39]. The Naqu and Ali areas of the Tibet autonomous region are the important habitats for the Tibet yak. Therefore, this study will help understand the status of their genetic resource conversion and domestication history.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflicts of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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