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Mitochondrial phylogeography and genetic diversity of Tibetan gazelle (*Procapra picticaudata*): Implications for conservation

Fangfang Zhang, Zhigang Jiang *

Institute of Zoology, Chinese Academy of Sciences, No. 25 Beisihuanxilu, Beijing 100080, China
Graduate School of the Chinese Academy of Sciences, No. 19 (A) Yuquanlu, Beijing 100049, China

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Abstract

The Tibetan gazelle (*Procapra picticaudata*) is a threatened species and distributed on the Qinghai-Tibet Plateau of China (Qinghai Province, Tibet Autonomous Region and the adjacent Gansu Province, Sichuan Province, and Xinjiang Uigur Autonomous Region). Small peripheral populations of Tibetan gazelle were once found in northern Sikkim and Ladakh, but now these are close to extinction. To describe the evolutionary history and to assess the genetic diversity within this monotypic species and population structure among different geographic locations in China, we sequenced mitochondrial DNA from the control region (CR) and cytochrome (cyt) *b* gene for 46 individuals from 12 geographic localities in Qinghai, Tibet, Xinjiang, Gansu, and Sichuan. A total of 25 CR haplotypes and 16 cyt *b* haplotypes were identified from these gazelle samples. CR haplotype diversity (0.98 ± 0.01) and nucleotide diversity (0.08 ± 0.009) were both high. Phylogenetic trees indicate that the Tibetan gazelle in China can be divided into three main clades: Tibet, Sichuan (SCH) and Qinghai-Arjin Shan-Kekexili (QH-ARJ-KKXL). Analysis of molecular variance (AMOVA) and network analysis consistently support this geographic structure in both datasets. Significant differentiation between populations argues for the presence of management units (MUs). Such differentiation may reflect a geographic separation resulting from the uplift of the Qinghai-Tibet Plateau during the Late Pliocene and Pleistocene. Mismatch distribution analysis implies that Tibetan gazelle has undergone complex population changes. We suggest that the present population structure has resulted from habitat fragmentation during the recent glacial period on the Qinghai-Tibet Plateau and population expansion from glacial refugia after the glacial period. It is likely that the present populations of Tibetan gazelle exhibit a pattern reminiscent of several bottlenecks and expansions in the recent past.

Keywords: Ungulates; Mitochondrial DNA; Phylogeography; Qinghai-Tibet Plateau

1. Introduction

In terms of both evolutionary and conservation aspects, the Tibetan gazelle (*Procapra picticaudata*) is a key species living on the Qinghai-Tibet Plateau (28–37°N, 75–103°E). It is one of the representative ungulates on the plateau and its adaptation to this unique geographic environment averaging above 4000 m high is of general interest (Schaller, 1998; Jiang, 2004). The Tibetan gazelle was hunted for meat, hides, and fur before 1980s; over-hunting has led to its disappearance from large areas. Because of habitat destruction and fragmentation by agricultural encroachment and expanding livestock production in its original habitats, as well as roads and railways that impose physical barriers and reinforce the isolation of fragmented populations, the Tibetan gazelle has experienced a sharp population decline in the wild and is becoming a threatened species (Jiang and Wang, 2001; Mallon and Kingswood, 2001). Accordingly, it is now classified as a category II species under the Wild Animal Protection Law in China and is listed as LR in the IUCN Red List of Threatened Species (Mallon, 2003).

Tibetan gazelle is a monotypic species without subspecies based on morphology (Feng et al., 1986). It is now confined to a number of isolated locations, such as Kekexili, Arjin Shan, Chang Tang, Ruoergai, and Mazongshan.

* Corresponding author. Fax: +86 10 62565689.
E-mail address: jiangzg@ioz.ac.cn (Z. Jiang).

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Small populations of several dozen individuals were once found in two adjoining areas (Ladakh and Sikkim) in India, but the populations were probably extirpated (Schaller, 1998). Thus, the Tibetan gazelle has attracted some conservation attention (Jiang and Wang, 2001; Mallon and Kingswood, 2001). However, there has been no attempt to describe the geographical distribution of genetic diversity or the evolutionary history of the species.

Historically, the Qinghai-Tibet Plateau uplifted several times by approximately 3000 m in the Quaternary period (Zhang, 2000). Furthermore, at least four major glaciations have occurred during the Pleistocene and Quaternary period in South-Central Asia (Chen, 1984) and widespread mountain glaciation controlled the Qinghai-Tibetan Plateau in the Lower Pleistocene and Quaternary period (Kwan et al., 1996). The topographic variation of the Qinghai-Tibet Plateau and the climate changes in the Pleistocene are widely regarded as two of the most important factors influencing the current spatial distribution of local species and their genetic diversity (Fort, 1996; Hewitt, 2000). Therefore, phylogeographic analysis is necessary to uncover the evolutionary history of this species and to provide genetic insights for conservation strategies.

Because populations in Ladakh and Sikkim possibly no longer exist (Schaller, 1998) and it is difficult to obtain samples, we excluded the peripheral populations in India from this study. Sampling over a broad geographic scale in China, we explored mitochondrial CR and mitochondrial cytochrome *b* (mtDNA cyt *b*) sequences to investigate the genetic status and evolutionary history of the Tibetan gazelle. We analyzed the phylogeographic patterns of living populations to test the hypothesis that uplifting of the Qinghai-Tibet Plateau in the Pleistocene and glaciations in the Quaternary period may have affected population distribution of the Tibetan gazelle, and to assess implications for conservation through combined analysis of CR and cyt *b* data.

2. Materials and methods

2.1. Sample collection

Muscle, hair, and skin samples ($n = 46$, Table 1) were collected throughout the species' distribution in China, including locations in Tibet, Kekexili (KKXL), Arjin Shan (ARJ), Qinghai and Sichuan (SCH) (Fig. 1). Five hair samples were plucked from captive animals in Beijing Zoo and Xining Zoo in December 2004 and another four hair samples were obtained from the museum collection of the Administrative Bureau of Kekexili Nature Reserve and the Forestry Bureau of Tianjun County, Qinghai Province. Nineteen muscle and skin samples were obtained from carcasses of Tibetan gazelle that died from poaching, hunting, natural enemy predation and disease in the Kekexili Nature Reserve, Qinghai Province, Arjin Shan Nature Reserve, Xinjiang Uigur Autonomous Region, Ruoergai Nature Reserve, Sichuan Province, Dulan International Hunting Park, Qinghai Province, and Harshihar International Hunting Park, Gansu Province from February 2004 to September 2005; another 18 muscle and skin samples were collected from the museum collection of the Institute of Zoology, Chinese Academy of Sciences, the Forestry Bureau of Tibet Autonomous Region, and the Forestry Bureau of Tianjun County, Qinghai Province.

2.2. DNA extraction and sequencing of mtDNA cyt b and control region

All samples in the present study were stored dry. Genomic DNA was isolated from muscle, hair, and skin using the standard proteinase K digestion and phenol/chloroform extraction procedures (Sambrook et al., 1989), after washing with excess NTE (0.05 M Tris–HCl, 0.01 M NaCl, 0.02 M EDTA, pH 9.0) to remove possible protease or PCR inhibitors (Hall et al., 1997). Polymerase chain reaction (PCR) was used to amplify approximately 800 bp of the CR and 425 bp of cyt *b*. CR sequences were amplified using the universal primer pair L15926 (5′-ATATACTGGTCTTG TAAACC-3′) and H16498 (5′-CCTGAAGTAGGAACC AGATG-3′) (Zhang and Ryder, 1993). Cyt *b* was amplified using the primer pair L14724 (5′-CGAAGCTTGATATG AAAAACCATCGTTG-3′; Irwin et al., 1991) and H15149 (5′-CCTCAGAAAGATATTTGTCCTC-3′; Kocher et al., 1989). Amplification was performed in a total volume of 50 µl containing 50 mM KCl, 10 mM Tris–HCl, 1.5 mM Mg^{2+}, 200 µmol of each dNTP, 0.2 µmol of each primer, 2 U of TaKaRa *Taq* DNA Polymerase (TaKaRa, China) and

Table 1
Sample names, sources, and types

Sample	*n*	Source	Type
Tibet01-06	6	Museum collecting[a]	Skin
Tibet07, 08	2	The Forestry Bureau of Tibet	Skin
Tibet09, 10	2	Beijing Zoo	Hair
KKXL01-08	8	Kekexili Nature Reserve	Hair and skin
ARJ01-05	5	Arjin Shan Nature Reserve	Skin
QH01-12	12	The Forestry Bureau of Tianjun County	Muscle and hair
QH13, 14	2	Dulan International Hunting Park	Muscle
QH15-17	3	Xining Zoo	Hair
SCH01-05	5	Ruoergai Nature Reserve	Skin
GS01	1	Harshihar International Hunting Park	Muscle

[a] Samples came from the Institute of Zoology, Chinese Academy of Sciences.

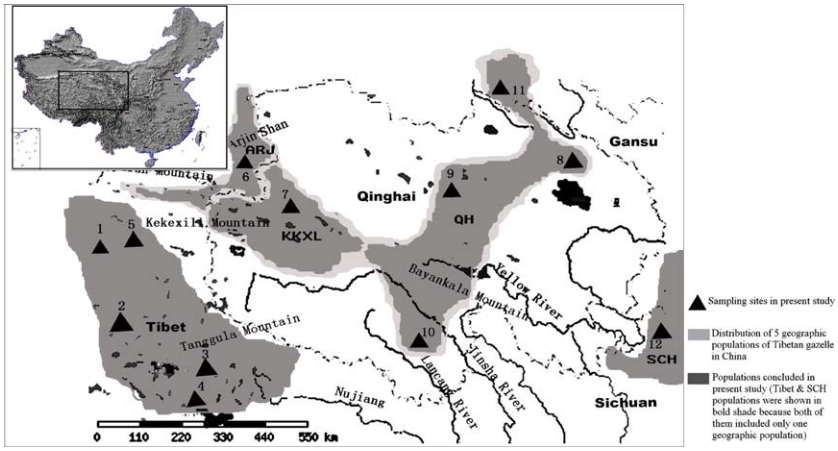

Fig. 1. Map of the distribution and sampling locations in the present study. The sampling locations are indicated by black triangles (▲). The Arabic numerals indicate sampling locations (sample sizes in parentheses): 1, Geji (2); 2, Bange (2); 3, Mangkang (1); 4, Shengzha (1); 5, Qiangtang (4); 6, Arjin Shan (5); 7, Kekexili (8); 8, Tianjun (13); 9, Doulan (2); 10, Yushu (2); 11, Harshihar (1); and 12, Ruoergai (5).

approximately 10 ng of genomic DNA. A total of 35–40 amplification cycles were carried out on a Perkin-Elmer Cetus 9700 DNA thermocycler with denaturation at 94 °C for 30 s, annealing at 56 and 50 °C for 30 s for cyt *b* and the D-loop, respectively, extension at 72 °C for 45 s, and a final extension at 72 °C for 10 min, then held at 4 °C. PCR products were purified using a Qiagen PCR purification kit and subsequently sequenced using ABI BigDye chemistry on an ABI 377 Genetic Analyzer according to the manufacturer's instructions.

2.3. Data analysis

2.3.1. mtDNA sequence analysis and phylogeny

Consensus sequences for all individuals were aligned using the CLUSTAL X program (Thompson et al., 1997) and checked by visual inspection. Genetic diversity among all geographic locations was estimated using haplotype (*h*) and nucleotide diversities (π) as implemented in DnaSP version 4.0 (Rozas et al., 2003). Sequence divergence within and among geographic regions was estimated using DNAdist in PHYLIP (Felsenstein, 1989). Phylogenetic relationships among haplotypes of cyt *b* and CR sequences were estimated using maximum-likelihood (ML), neighbor-joining (NJ; Saitou and Nei, 1987) and maximum-parsimony (MP) by heuristic search methods carried out by PAUP*4.0b8 (Swofford, 2001) separately. The robustness of these analyses was assessed using bootstrap replications, with 1000 replications for MP and NJ and 100 replications for ML. The settings for the DNA substitution model that best fitted the data were selected by a hierarchical likelihood ratio test using the programs MODELTEST 3.06 (Posada and Crandall, 1998) and PAUP*. We also conducted a median-joining network (MJN) approach (Bandelt et al., 1999) to depict the relationships among the Tibetan gazelle haplotypes. This approach has proved to yield the best genealogies among other rooting and network procedures (Cassens et al., 2003). The MJN was estimated using NETWORK4107 software (Bandelt et al., 1999; http://www.fluxus-engineering.com).

2.3.2. Phylogeographic analysis

Genetic differentiation between populations was assessed by comparing the average number of pairwise differences between populations (PiXY), the average number of pairwise differences within populations (PiX and PiY) and the corrected average pairwise difference (PiXY − (PiX + PiY)/2) using the program Arlequin 2.000 (Schneider et al., 2000). Differentiation between pairs of populations was tested by exact test (Raymond and Rousset, 1995) using 10,000 Markov chain steps as implemented in Arlequin 2.000 (Schneider et al., 2000). The same program was used for analysis of molecular variance (AMOVA; Excoffier et al., 1992) to test for differentiation between geographical units. In AMOVA the correlation of haplotype frequency is used as an F-statistic analog at various hierarchical levels. Φ_{ST} estimates the proportion of genetic variation within populations relative to the genetic variation for the whole sample, Φ_{CT} estimates the proportion of genetic variation among groups of populations relative to the whole species, and Φ_{SC} estimates the variation among populations relative to a regional grouping of populations. The significance of Φ-statistics was tested by random permutations of sequences among populations. The groupings that maximize values of Φ_{CT} and are statistically significant indicate the most parsimonious geographical subdivisions.

2.3.3. Analysis of demographic history

Signatures of population demographic changes (e.g., bottlenecks or expansions) in the Tibetan gazelle were examined using two different approaches. First, we investigated the demographic history by comparing mismatch distributions in each geographic sample with those expected in constant populations using DnaSP. The shape of the mismatch distribution of pairwise differences is usually

multimodal in samples drawn from populations at demographic equilibrium, whereas a unimodal distribution is generally found in populations having passed through a recent demographic expansion (Rogers and Harpending, 1992; Harpending et al., 1998). It is important to note, however, that such a wave in the distribution could also be generated by a bottleneck, and that distinguishing between both demographic events remains difficult (Rogers and Harpending, 1992). The overall validity of the estimated demographic model is tested by obtaining the distribution of a tested statistic SSD (the sum of squared differences) between the observed and estimated mismatch distributions. A significant SSD value is taken as evidence of departure from the estimated demographic model of a sudden population expansion. In addition, the Tajima's (Tajima, 1989) *D* statistic and the Fu's (Fu, 1997) *Fs* statistic were used to test whether CR and cyt *b* data conformed to expectations of neutrality, considering that departures from neutrality could also be due to factors other than selective effects, such as a population bottleneck, a population expansion, or heterogeneity of the mutation rate. *Fs* differences were tested for significance with a coalescent simulation program (1000 simulations), as implemented in Arlequin 2.000 (Schneider et al., 2000).

3. Results

3.1. Mitochondrial CR and cyt b variability

A fragment from approximately 570–720 bp of mtDNA control region was sequenced in 46 Tibetan gazelle individuals from five geographic populations (Tibet, QH, KKXL, ARJ and SCH). Two deletions of approximately 40 and 80 bp were found at approximate positions 121 and 221 in all individuals of the SCH population and some individuals of the Tibet population (Fig. 2a), but these haplotypes were restricted to the two geographic populations only. After exclusion of gaps, 25 haplotypes (GenBank Accession Nos. DQ017352–DQ017355 and DQ423488–DQ423508) were defined by 193 polymorphic sites, of which 130 were parsimony-informative. As a result, the overall haplotype diversity (0.98 ± 0.01) and the nucleotide diversity (0.08 ± 0.009) of CR were both high; as some sequences differed by more than 50 mutations, the mean sequence divergence was high overall (0.15), with a maximum of 0.18 (among individuals within the Tibet geographic population). The 425 bp of cyt *b* sequences were generated from the same 46 individuals; a total of 113 variable nucleotide sites defined 16 haplotypes (Fig. 2b; GenBank Accession Nos. DQ001163–DQ001166 and DQ423513–DQ423524. As expected, generally lower haplotype diversity (0.88 ± 0.039) and nucleotide diversity (0.06 ± 0.019) were observed in cyt *b* sequences.

3.2. Phylogenetic relationships

Hierarchical likelihood ratio tests indicated that Hasegawa et al.'s (1985) HKY model of substitution and γ distribution was the best for the CR fragment data (HKY + G, $-\ln L = 2841.40$; Ti/Tv ratio = 4.64, γ distribution shape parameter = 0.41). Equal numbers of transition (Ti) and transversion (Tv) were observed amongst cyt *b* sequences. The Tamura-Nei's TrN model (Tamura and Nei, 1993) was

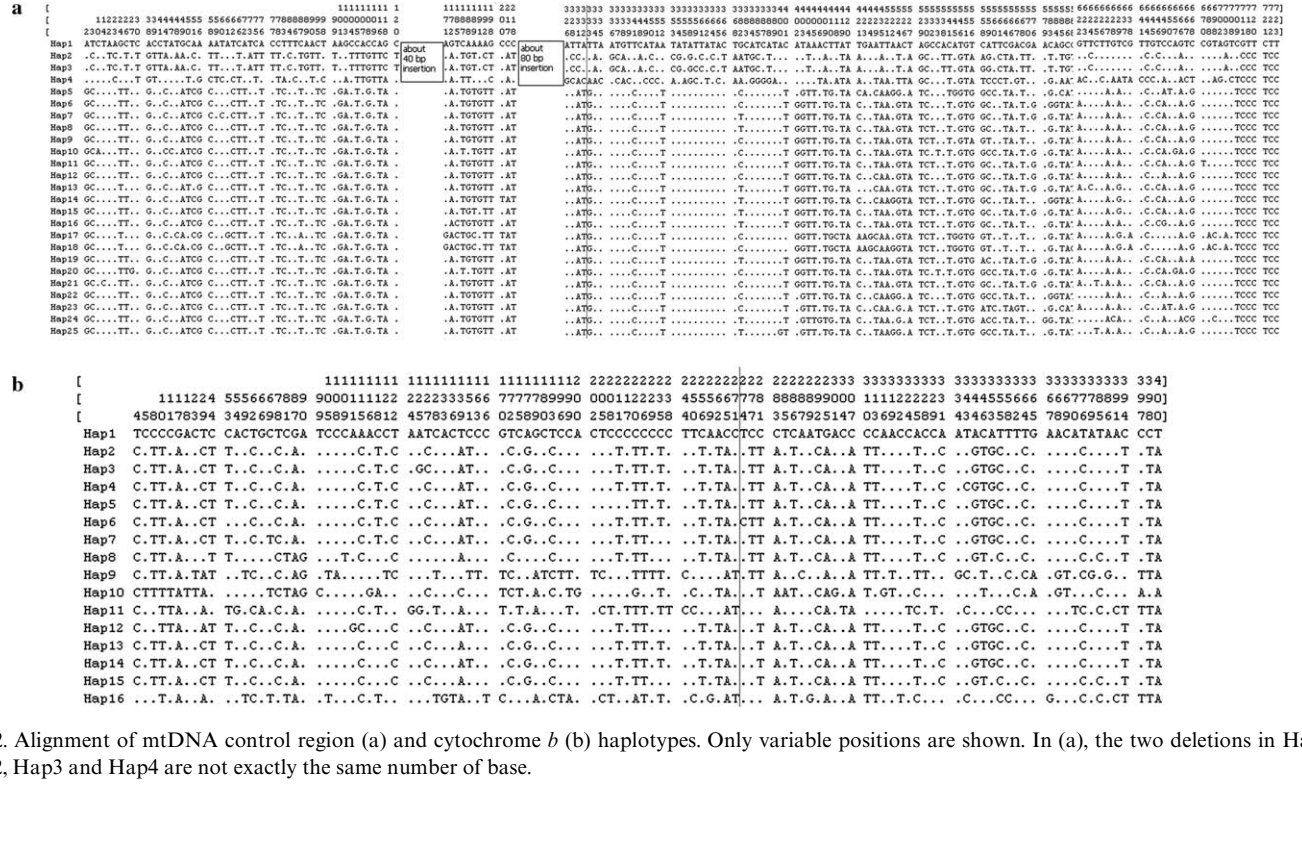

Fig. 2. Alignment of mtDNA control region (a) and cytochrome *b* (b) haplotypes. Only variable positions are shown. In (a), the two deletions in Hap1, Hap2, Hap3 and Hap4 are not exactly the same number of base.

selected as the best for the data ($-\ln L = 1360.49$; Ti/Tv ratio = 1). The MP trees for CR and cyt *b* haplotypes separately (Fig. 3A) and jointly (Fig. 3B) indicated a relatively high resolution and high bootstrap supported phylogenetic structure. By excluding haplotypes Hap1, Hap5, Hap17 and Hap18, the 21 haplotypes of CR showed three major clusters: Tibet, SCH and QH-ARJ-KKXL. The Tibet and SCH cluster were formed with high bootstrap support (BP = 96% and 91%, respectively), but the QH-ARJ-KKXL cluster showed moderate bootstrap support (64%). Haplotypes Hap1 and Hap5 were composed of sequences from the Tibet and Arjin Shan localities, with dispersion into the SCH and Tibet clusters, respectively, suggesting that they possibly represent the most ancestral haplotypes. The geographical structure of the Tibetan gazelle is also shown in the medium-joining network (Fig. 4), which is star-like from the core haplotype H8 (QH), but divergent from other haplotypes.

3.3. Population structure and gene flow

The genetic differentiation was detected and significant between SCH versus each of other four geographic populations and Tibet versus the other three geographic populations excluding ARJ both in pairwise Φ_{ST} and $(PiXY - (PiX + PiY)/2)$ values (Table 3). Analysis of AMOVA based both on genetic distance and haplotype frequency indicated a single significant Φ_{CT} value in one of the possible population grouping patterns: Tibet, SCH and QH-ARJ-KKXL. Significant difference ($p < 0.01$; Table 4, 3000 permutations) among the three groups was observed. In addition, this grouping pattern gave the highest Φ_{CT}

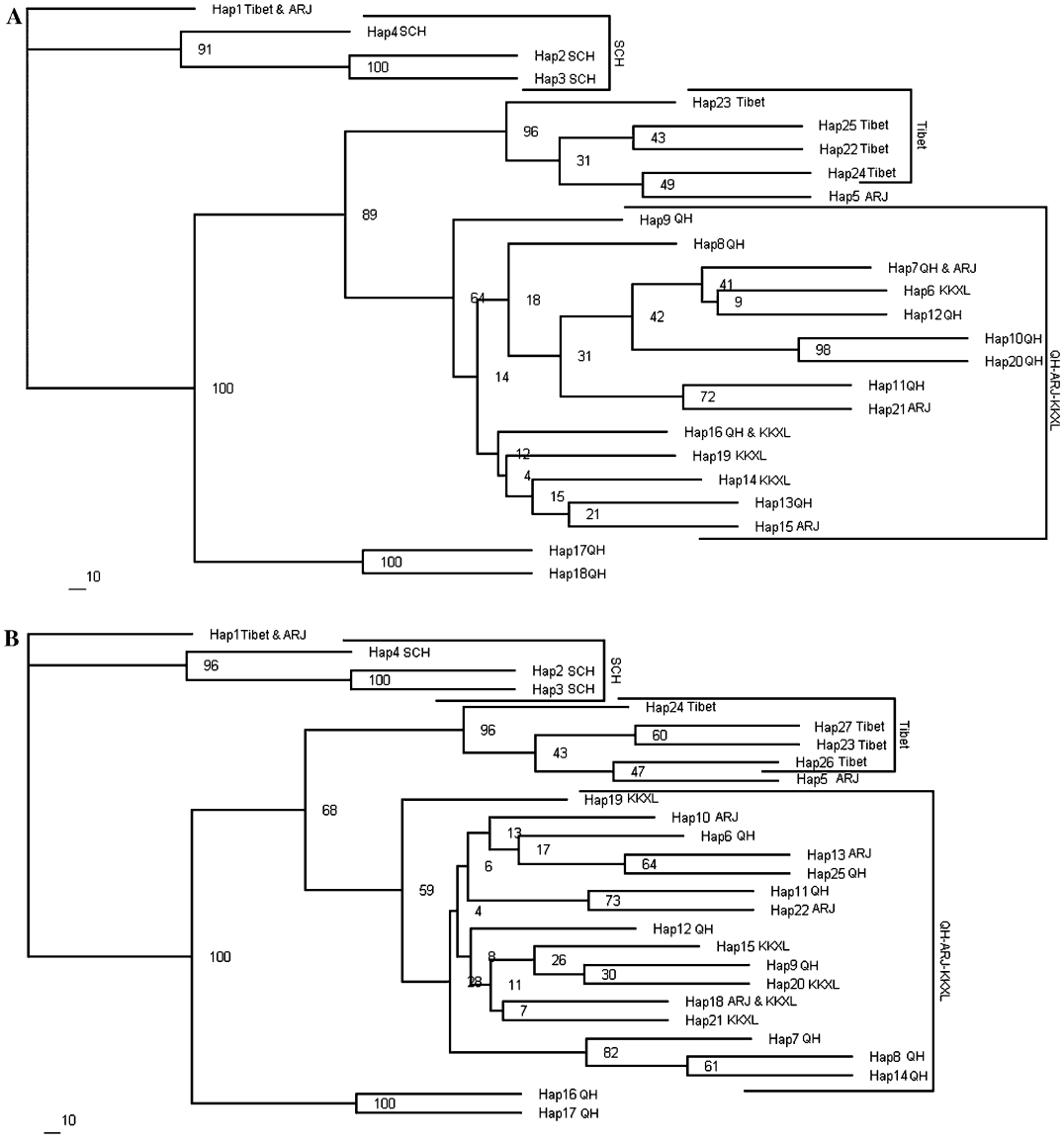

Fig. 3. Maximum parsimony (MP) trees computed by PAUP based on HKY distance among the mitochondrial control region and CR with cyt *b* haplotypes of the Tibetan gazelle. Bootstrap percentage values are indicated next to nodes of 1000 bootstrap simulations.

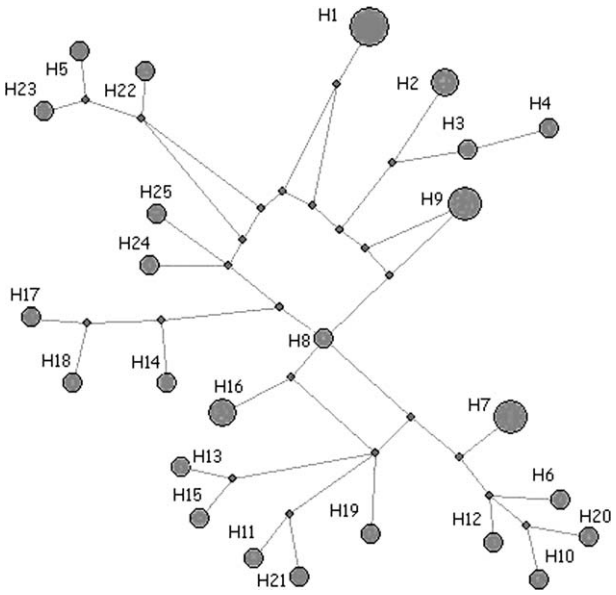

Fig. 4. Median-joining network of the 25 control region haplotypes of the Tibetan gazelle (*Procapra picticaudata*).

value (0.0676). These results indicate a relatively strong genetic structure for the Tibetan gazelle.

3.4. Demographic analysis

Mismatch distribution analysis of the overall and each population separately indicated different patterns (Fig. 5). The shapes of the functions for overall, Tibet and SCH populations were ragged and multimodal, which suggests that they may include populations or lineages that were previously separated but now have merged together. Fu's F_s test for neutrality indicated no significant population expansion ($p > 0.1$) for the total nor for any of the geographic populations (Table 2), but a significance ($p = 0.017$) for Tajima's D test suggested an expansion in the Tibet population. In addition, both Tajima's D value and Fu's Fs value support the results of mismatch distribution. The results shed lights on the early history of the once separated Tibetan gazelle populations before mixing.

4. Discussion

4.1. Genetic diversity and phylogeography

The Presence of 25 CR and 16 cyt *b* haplotypes from 46 samples indicated high haplotype diversity in the Tibetan gazelle populations (Table 2). Significant genetic differentiation was detected between the SCH and each of other four geographic populations and the Tibet and the other three geographic populations, excluding ARJ (Table 3). Presumably, the Tibetan gazelle in Arjin Shan, Xinjiang Province, and the populations in Kekexili and other regions in Qinghai Province once formed a single large population with continuous distribution on the plateau. Measures of genetic diversity in Tibetan gazelle are comparable to that found in other bovid species of similar endangered status (Arctander et al., 1996). High genetic diversity suggests that these species probably had large effective population sizes in their recent history (Christopher et al., 2003). On the other hand, the high mitochondrial DNA diversity in the Tibetan gazelle may be not only a species-associated feature, but also the result of its recent evolutionary history and some contemporary events. AMOVA analysis detected a significant Φ_{CT} value for the population grouping pattern: Tibet, SCH and QH-ARJ-KKXL. Patterns of molecular differentiation observed in the Tibetan gazelle indicate recent subsequent isolation processes caused by uplifting of the Qinghai-Tibet Plateau. Thus, much of the current haplotype diversity is probably a consequence of strong phylogeographic structure. For example, although each of the

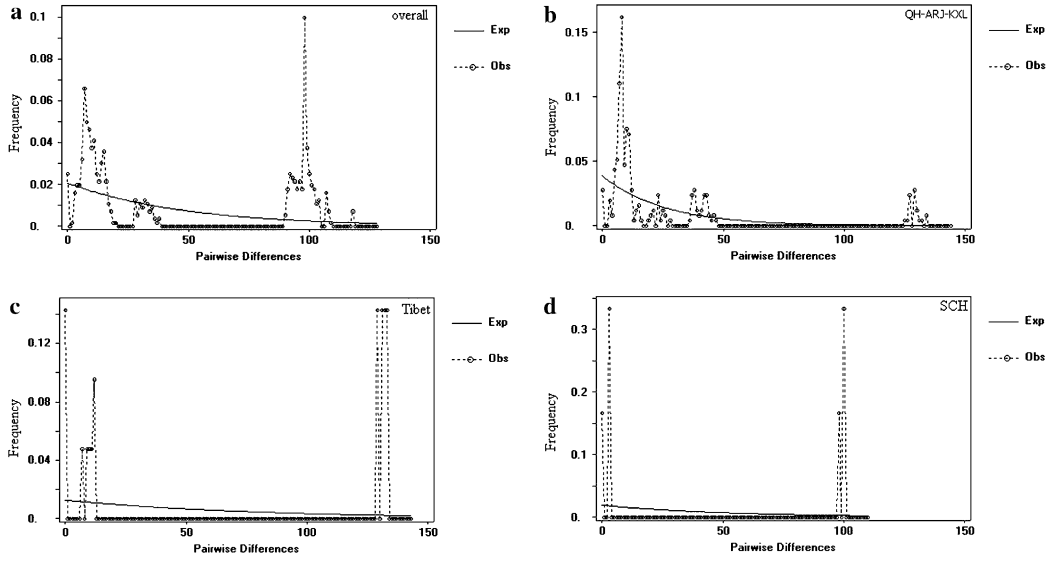

Fig. 5. Mismatch distributions of the Tibetan gazelle mtDNA control region in the overall (a), QH-ARJ-KKXL (b), Tibet (c) and SCH (d) populations.

Table 2
Gene diversity (h), nucleotide diversity (π), sequence divergence (D_{xy}), Tajima's (D), Fu's (Fs), and mismatch distribution tests (SSD) for CR sequences

Geographic origin	n	h	π	Mean D_{xy}	SSD (p value)	D (p value)	Fs (p value)
Overall	46	0.975(0.012)	0.081(0.009)	0.154	0.0239(0.26)	0.043(0.506)	0.971(0.712)
ARJ	5	1.000(0.177)	0.156(0.087)	0.151	0.2600(0.07)	−0.304(0.487)	2.213(0.550)
KKXL	8	1.000(0.126)	0.015(0.008)	0.012	0.1603(0.06)	−0.267(0.529)	−0.182(0.453)
Tibet[a]	10	0.857(0.137)	0.160(0.030)	0.183	0.2119(0.02)	1.788(0.973)	6.040(0.987)
SCH[a]	5	0.833(0.222)	0.119(0.069)	0.112	0.2469(0.21)	−0.741(0.190)	−0.752(0.188)
QH-ARJ-KKXL	31	0.972(0.022)	0.052(0.020)	0.062	0.0152(0.09)	−1.906(0.017)	−1.027(0.337)

Standard deviations for h and π are in brackets.
[a] The group includes samples from a single geographic origin.

clades includes multiple haplotypes, no common haplotype was shared among the three populations. This suggests some degree of spatial genetic structure within *P. picticaudata* and limited dispersal of individuals among local populations.

Our results also suggest that recent history may have shaped the genetic patterns observed in the Tibetan gazelle. The phylogenetic tree revealed a relatively deep structure among the haplotypes, suggesting divergence between the geographic groups and high polymorphism. A rate of sequence divergence for bovid cyt *b* was reported as 0.7% per million years (Hassanin and Emmanuel, 1999) and the substitution rate for the CR was estimated to be 5–10 times faster than for the cyt *b* block in other bovids (Birungi and Arctander, 2000); thus, the estimated rate for the CR is 3.5–7% per million years. Based on the estimated divergence rate of the CR, a recent coalescence time of approximately 2.2–4.4 million years was predicted among the Tibetan gazelle samples (average sequence divergence 15.4%, shown in Table 2). This estimate matches the conclusion by An et al. (2001) from their geographical study that enhanced uplift of the Qinghai-Tibet Plateau along the northern and eastern margins occurred 3.6–2.6 million years ago. In addition, it is consistent with the historical glaciation in the Pleistocene (Li, 1998), which was an important event for the phylogeographic pattern of ungulate distribution over the Qinghai-Tibet Plateau. On the other hand, the tree with some haplotypes lying on old branches outside all of the three clades is evidence of old co-ancestral haplotypes that have been maintained over a long-term separation. During the Mid-Pleistocene and Quaternary period, much of the Qinghai-Tibetan Plateau was covered by the largest glacier ever known in the region (Zhang, 1999), and the population of Tibetan gazelle may have been restricted to areas of low altitude during glaciations. This hypothesis, however, needs to be tested further for other ungulates in the same distribution region.

However, because the Qinghai-Tibet Plateau is a unique geographic area, studies on other species of similar range are not available for comparison. Intraspecies mtDNA CR studies of terrestrial mammals, for example, the African buffalo (*Syncerus caffer*, Simonsen et al., 1998) and impala (*Aepyceros melampus*, Arctander et al., 1996), found variation values of $\leqslant 5.5\%$ within a single population. However, large sequence divergences have been reported, for example, among divergent mtDNA genotypes separated by geographical barriers or distance (Morin et al., 1994) or within hybrid zones (Ferris et al., 1993; Avise et al., 1984). The phylogeographic divergence between the Tibet and QH-ARJ-KKXL populations, the Tibet and SCH populations, and the QH-ARJ-KKXL and SCH populations may possibly be related to geographic barriers. For example, the Kunlun Mountains, Tanggula Mountains, Lanchang River, Nujiang River, Jinshajiang River, Qionglai Mountains, and Daxueshan Mountains are all natural barriers that separate the Tibet, Qinghai, and Sichuan populations.

Table 3
Pairwise population differences and Φ_{ST} values for control region sequences

	Tibet	ARJ	SCH	KKXL	QH
Tibet	61.29	−0.144	0.5384**	0.262**	0.422**
ARJ	−6.33	56.67	0.546**	0.057	0.233
SCH	46.35**	46.83**	49.67	0.742**	0.830**
KKXL	16.91*	0.22	66.67**	7.40	−0.027
QH	16.09**	0.02	65.09**	0.03	10.84

Above diagonal: pairwise Φ_{ST} values between populations. Diagonal elements: average number of pairwise differences within population (PiX). Below diagonal: corrected average pairwise difference (PiXY − (PiX + PiY)/2). Pairwise Φ_{ST} values and corrected average pairwise differences that are statistically different are indicated.
* $p < 0.05$.
** $p < 0.01$.

Table 4
AMOVA for grouping of populations estimated using Φ-statistics based on control region sequence

Groups	Among pops within groups (Φ_{ST})	Within pops (Φ_{ST})	Among groups (Φ_{CT})	(%) Among groups	p (Among groups)
[Tibet] [QH, KKXL, ARJ & SCH]	0.0494	0.0812	0.0335	3.35	0.1906
[Tibet] [QH, KKXL, ARJ] [SCH]	0.0190	0.0854	0.0676**	6.76	<0.01
[Tibet, QH, KKXL, ARJ] [SCH]	0.0475	0.1027	0.0579	5.79	0.1945
[Tibet, KKXL, ARJ] [QH] [SCH]	0.0139	0.0750	0.0620	6.2	0.0968
[Tibet, KKXL] [ARJ, QH] [SCH]	0.0503	0.0673	0.0179	1.79	0.0626
[Tibet, SCH] [ARJ, KKXL, QH]	0.0538	0.0704	0.0176	1.76	0.1046

** Significant at 0.01 level.

4.2. Demographic history

High haplotype and sequence diversity, relatively deep phylogenetic tree, and Tajima's *D* and Fu's *Fs* values (Table 2), all indicate there was no single expansion or contraction in all populations of Tibetan gazelle, but suggest that the SCH and Tibet populations were once fragmented in history but have since merged. Furthermore, the mismatch distribution analysis showed atypical distribution shapes, revealing a complicated demographic history for the populations; in particular, with zero frequency in the moderate pairwise difference interval, but high frequency in the low and high intervals, the overall, Tibet and SCH populations showed distribution patterns that deviated from the simulation. Results for the Tibet and SCH populations imply that they consist of lineages that were previously isolated but are now merged. This may be related to the effects of climate oscillations during glaciations in the Pleistocene and Quaternary period and to postglacial population expansions. The topographical diversity of the Qinghai-Tibetan Plateau might have created both networks for refugia during glaciations and complex barriers to subsequent expansion (Hewitt, 2004). When the top of the plateau was covered by glaciers (Flint, 1965), the animals withdrew to the fringe areas of the plateau for refuge and some gazelle individuals returned to the heartland of plateau after the glaciations, but this presumption need more samples to validate by uncovering the phylogenetic relationship within populations. The mismatch distribution deviation of the overall population could be the result of high sequence diversity of the individuals from different geographic locations. As the topographic features changed, climate oscillated and vegetation varied during uplifting of the Qinghai-Tibet Plateau and during glaciations in the Quaternary Period, the species may have suffered comprehensive population fluctuations, whereas the main populations remain genetically healthy.

4.3. Implications for conservation

Tibetan gazelles are now facing extirpation in India (Schaller, 1998). Phylogeographic analysis of intraspecies genetic variation provides valuable information on how genetic variation is partitioned within species and identifies evolutionarily significant units (ESUs) and management units (MUs) for conservation (Moritz, 1994). According to the model proposed by Moritz (1994), ESUs are designated on the basis of reciprocal monophyly at mitochondrial markers, while MUs are identified by significant differences in allele frequency distributions and significant divergence in mitochondrial or nuclear loci. Considering these criteria, populations with genotypes that are closely related to but not shared with other populations would be described as MUs; thus, the Tibet, SCH and QH-ARJ-KKXL populations of *P. picticaudata* would be described as MUs. Our data imply that the Tibetan gazelle shows a relatively healthy genetic status both for the overall population and each of the populations in China. Moreover, the SCH population is probably special, with unique haplotypes but small population size. Therefore, as a conservative approach, we recommend protection of Indian populations if they still exist, and to enhance efforts to protect the overall population of Tibetan gazelle in China. Furthermore, special attention should be allocated to preserving the SCH population; on the other hand, we would advise avoiding the introduction of genes to any of the populations unless genetic erosion occurs.

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References

An, Z., John, E.K., Warren, L.P., Stephen, C.P., 2001. Evolution of Asian monsoons and phased uplift of the Himalaya-Tibetan plateau since Late Miocene times. Nature 411, 62–66.

Arctander, P., Kat, P.W., Simonsen, B.T., Siegesmund, H.R., 1996. Population Genetics of Kenyan Impalas-Consequences for Conservation, Molecular Genetic Approaches in Conservation. Oxford University Press, Oxford.

Arctander, P., Kat, P.W., Aman, A.R., Siegismund, H.R., 1996. Extreme genetic differences among populations of Grant's gazelle *Gazelle granti* in Kenya. Heredity 76, 465–475.

Avise, J.C., Bermingham, E., Kessler, L.G., Saunders, N.C., 1984. Characterization of mitochondrial DNA variability in a hybrid swarm between subspecies of bluegill sunfish (*Lepomis macrochirus*). Evolution 38, 931–941.

Bandelt, H.J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48.

Birungi, J., Arctander, P., 2000. Large sequence divergence of mitochondrial DNA genotypes of the control region within populations of the African antelope, kob (*Kobus kob*). Mol. Ecol. 9, 1997–2008.

Cassens, I., Waerebeek, K., Best, P., Crespo, E., Reyes, J., Milinkovitch, M., 2003. The phylogeography of dusky dolphins (*Lagenorhynchus obscurus*): a critical examination of network methods and rooting procedure. Mol. Ecol. 12, 1781–1792.

Chen, Y., 1984. Quaternary climates. In: Eastern China Normal University, Hebei Normal University, Central China Normal University and Southern China Normal University (Eds.), Quaternary Geology. Hebei Normal University Press, Shijiazhuang, China, pp. 40–72 [in Chinese].

Christopher, N., Balakrishaan, S.L., Minfort, A.G., Lalji, S., Michael, D.S., 2003. Phyogeography and conservation genetics of Eld's deer (*Cervus eldi*). Mol. Ecol. 12, 1–10.

Excoffier, L., Smouse, P.D., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 476–494.

Felsenstein, J., 1989. PHYLIP-Phylogeny Inference Package (Version 3.2). Cladistics 5, 164–166.

Feng, Z., Cai, G., Zhen, C., 1986. The Mammals of Tibet. A Comprehensive Scientific Expedition to the Qinghai-Tibetan Plateau. Science, Press, Beijing [in Chinese].

Ferris, S.D., Sage, R.D., Huang, C.M., Nielsen, J.T., Ritter, U., Wilson, A.C., 1993. Flow of mitochondrial DNA across a species boundary. Proc. Natl. Acad. Sci. USA 80, 2290–2294.

Flint, R.F., 1965. The Pliocene-Pleistocene, international studies on the Quaternary. Geol. Soc. Am. 84, 497–533.

Fort, M., 1996. Late Cenozoic environmental changes and uplift on the northern side of the central Himalaya: a reappraisal from field data. Palaeogeography, Palaeoclimatology, Palaeoecology 120, 123–145.

Fu, Y., 1997. Statistical test of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147, 915–925.

Hall, L., Willcox, M., Jones, D., 1997. Association of enzyme inhibition with methods of museum skin preparation. Biotechniques 22, 928–930.

Harpending, H., Batzer, M., Gurven, M., Jorde, L., Rogers, A., Sherry, S., 1998. Genetic traces of ancient demography. Proc. Natl. Acad. Sci. USA 95, 1691–1697.

Hasegawa, M., Kishino, K., Yano, T., 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Ecol. 22, 160–174.

Hassanin, A., Emmanuel, J.P.D., 1999. The tribal radiation of the family bovidae (*Artiodactyla*) and the evolution of the mitochondrial cytochrome *b* gene. Mol. Phylogenet. Evol. 13, 227–243.

Hewitt, G., 2000. The genetic legacy of the Quaternary ice age. Nature 405, 907–913.

Hewitt, G., 2004. Genetic consequences of climatic oscillations in the Quaternary. Phi. Trans. R. Soc. Lond. B 359, 183–195.

Irwin, D.M., Kocher, T.D., Wilson, A.C., 1991. Evolution of the cytochrome *b* gene of mammals. J. Mol. Evol. 32, 128–144.

Jiang, Z., 2004. Taxonomy of gazelles. In: Jiang, Z. (Ed.), Przewalski's Gazelle. China Forestry Publishing House, Beijing, China, pp. 26–39 [in Chinese].

Jiang, Z., Wang, S., 2001. IUCN Antelope Survey and Action Plan Part 4: North America, the Middle East and Asia, Chapter 33 China, pp. 168–177. In: Mallon, D.P., Kingswood, S.C. (Eds.), Global Survey and Regional Action Plans on Antelope. Gland and Cambridge: IUCN.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86, 6196–6200.

Kwan, H.K., Choung, M.L., Liu, T., Ding, M., Derbyshire, E., 1996. Gravel deposits on the margins of the Qinghai-Xizang (Tibetan) Plateau, and their environment significance. Palaeogeography, Palaeoclimatology, Palaeoecology 120, 159–170.

Li, W., 1998. Quaternary Vegetation and Environment in China. Science Press, Beijing [in Chinese].

Mallon, D.P., Kingswood, S.C. (Eds.), 2001. Global Survey and Regional Action Plans on Antelope. Gland and Cambridge, IUCN.

Mallon, D.P., 2003. *Procapra picticaudata*. In: IUCN 2004. 2004 IUCN Red List of Threatened Species. <www.iucnredlist.org>.

Morin, P.A., Moore, J.J., Chakraorty, R., Jin, L., Goodall, J., Woodruff, D.S., 1994. Kin selection, social structure, geneflow, and the evolution of chimpanzees. Science 265, 1193–1201.

Moritz, C., 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. Mol. Ecol. 3, 401–411.

Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Raymond, M., Rousset, F., 1995. An exact test for population differentiation. Evolution 49, 1280–1283.

Rogers, A., Harpending, H.M., 1992. Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. 9, 552–569.

Rozas, J., Sánchez-DelBarrio, J.C., Messeguer, X., Rozas, R., 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19, 2496–2497.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Sambrook, J., Fritsh, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, New York.

Schaller, G.B., 1998. Wildlife of the Tibetan Steppe. The University of Chicago Press, Chicago USA.

Schneider, S., Kueffer, J.M., Roessli, D., Excoffier, L., 2000. Arlequin ver. 2.000: A Software for Population Genetics Data Analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.

Simonsen, B.T., Siegismund, H.R., Arctander, P., 1998. Population structure of African buffalo inferred from mtDNA sequences and microsatellite loci: high variation but low differentiation. Mol. Ecol. 7, 225–237.

Swofford, D.L., 2001. PAUP*: phylogenetic analysis using parsimony (* and other methods), Version 4.0b8, Sinauer Associates, Sunderland, MA.

Tajima, F., 1989. Statistical methods for testing the neutral mutation hypothesis for DNA polymorphism. Genetics 123, 585–595.

Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10, 512–526.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.

Zhang, D., 2000. Eco-environmental effects of the Qinghai-Tibet Plateau uplift during the Quaternary in China. Environ. Geol. 39, 1352–1358.

Zhang, R., 1999. Zoogeography of China. Science Press, Beijing [in Chinese].

Zhang, Y., Ryder, O.A., 1993. Mitochondrial DNA evolution in the Artoidea. Proc. Natl. Acad. Sci. USA 90, 9557–9961.