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RESEARCH ARTICLE

Phylogeography and Population Structure of the Golden Monkeys (*Rhinopithecus roxellana*): Inferred from Mitochondrial DNA Sequences

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The golden monkey (*Rhinopithecus roxellana*) is one of the most endangered primate species due to its dramatically shrinking distribution during the past 400 years. Its populations are restricted to three isolated regions, Qinglin (QL), Sichuan/Gansu (SG), and Shennongjia (SNJ) in China. As with other snub-nosed monkeys in China and Vietnam, the biology and evolution of this species is still poorly known. To assess genetic differentiation and explore the relationships among populations of golden monkeys from different geographic locations, 379 bp of mitochondrial DNA control region (CR) hypervariable segment I (HVI) was studied from 60 individuals. Twelve haplotypes were identified from seven populations within the three regions. Haplotype diversity was high (0.845), whereas nucleotide diversity among all haplotypes was low (0.0331). The most recent common ancestor (TMRCA) among mtDNA haplotypes was estimated to have lived approximately 0.48–0.32 million years ago. None of the haplotypes is shared among any of the three regions. Phylogenetic analysis and AMOVA revealed clear and significant phylogeographic structure between the three regions. However, only SG contained haplotypes of the two main clades, indicating either incomplete random sorting of haplotypes or a complex history with phases of population subdivisions and merging of populations. The phylogeographic structure implies that *R. roxellana* should be regarded as separate management units (MUs) for each of the three regions. It is likely that recent phylogeographic history has shaped the pattern of genetic

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differentiation observed in the golden monkey and that its populations have suffered significant demographic fluctuation. Am. J. Primatol. 69:1195–1209, 2007. © 2007 Wiley-Liss, Inc.

Key words: *Rhinopithecus roxellana*; mitochondrial DNA; phylogeography; population structure; management unit; conservation

INTRODUCTION

The genus of snub-nosed monkeys (*Rhinopithecus*, Colobinae, Cercopithecidae) comprises four species, three of which are endemic to China (the golden or Sichuan snub-nosed monkey, *R. roxellana*; the black or Yunnan snub-nosed monkey, *R. bieti*; and the Gray or Guizhou snub-nosed monkey, *R. brelichi*). The fourth is distributed in northern Vietnam (Tonkin's snub-nosed monkey, *R. avunculus*). The systematics and phylogenetic relationships within the genus and the relationships between the genus and other odd-nosed colobines in Asia, in particular the doc langurs (*Pygathrix*), are still controversial [Jablonski, 1998a; Pan et al., 2004; Rowe, 1996]. A recent study [Li et al., 2004] based on mitochondrial cytochrome b and 12S rRNA sequences implied that *Rhinopithecus* could be regarded as a sister genus of *Pygathrix* and confirmed the status of the four independent species in the genus. The divergence time among those four species were estimated to be 2.08–2.84 million years ago (Mya) based on analysis of the mitochondrial cytochrome b gene [Li et al., 2004]. Fossil records indicate that snub-nosed monkeys were widely distributed in China and Vietnam during the Pleistocene [Jablonski, 1998a,b; Quan and Xie, 2002]. Now the habitats of the extant species are separated from each other and they are confined to very limited areas in isolated mountainous regions in Sichuan, Yunnan, Guizhou, Hubei, Shanxi, Gansu, Tibet and northern Vietnam [Jablonski, 1998a,b; Quan and Xie, 2002]. Current census data suggests a very gloomy picture for these species, with only about 20,000 individuals of *R. roxellana*, 1,500 *R. bieti*, 800 *R. brelichi*, and 130–350 *R. avunculus* remaining in the wild [Jablonski, 1998a,b; Li et al., 2002; Long et al., 1994, 1996; Quan and Xie, 2002; Ren et al., 1998].

R. roxellana, widely known as golden monkey or snub-nosed monkey for its shining golden coat and funny unturned nose, has suffered dramatic distribution reduction and decrease of population size during the past 400 years [Li et al., 2002]. Because of its distinct appearance and rarity, the golden monkey is regarded as one of the favorite national animal icons, next to the giant panda (*Ailuropoda melanoleuca*). Golden monkeys used to be more broadly distributed than any other snub-nosed species in China. Studies of the fossil record showed that this species was widely dispersed in south and central China during the late Pleistocene and early Holocene [Gu & Jablonski, 1989; Gu & Hu, 1991; Han, 1982; Jablonski & Pan, 1988; Jablonski, 1998a,b; Pan, 1995]. But now, its populations are only found at the east edge of the Qinghai-Tibet Plateau and the mountains in central China, restricted to three isolated regions: Sichuan/Gansu (SG), Qinglin Mountain (QL), and Shennongjia (SNJ) [Li et al., 2002] (Fig. 1). Because of its small numbers, major habitat reduction and serious fragmentation, *R. roxellana* was recognized as a ''vulnerable'' animal species in the 2006 IUCN List category (http://www.redlist.org/), and has been categorized as Class I of the Protected Status by the Chinese government.

Research on intraspecific variation of the golden monkey has been limited and does not provide much relevant information about the species. One approach based on external morphological and anatomical variation postulated that three

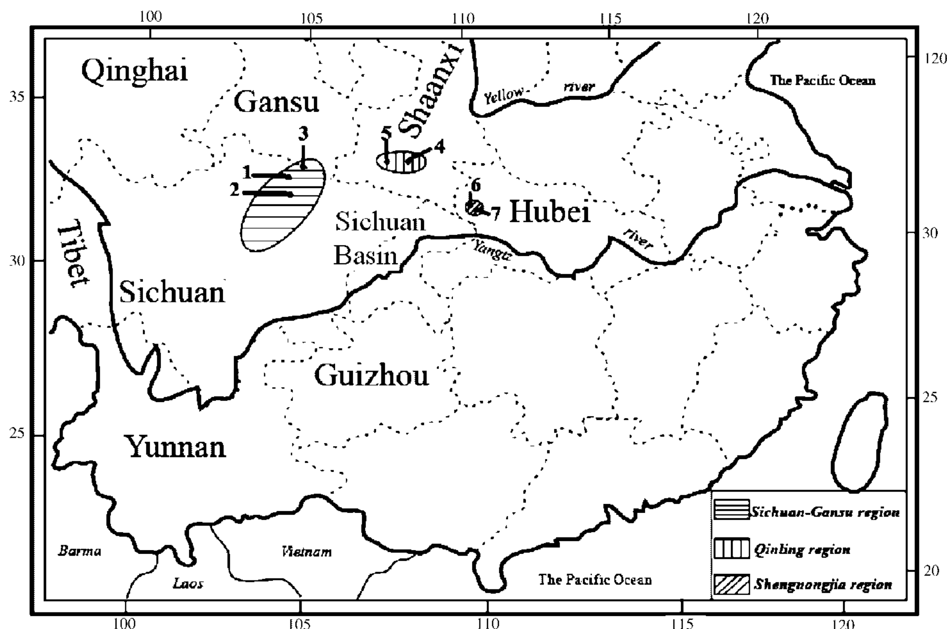

Fig. 1. Map of the present distribution and sampling locations. The Arabic numerals indicate sampling locations of the present study (sample sizes in parentheses): 1 Songpan (4), 2 Wolong (6), 3 Beishuijiang (8), 4 Zhouzhi (8), 5 Taibai (11), 6 Shennongjia location a (7), 7 Shennongjia location b (16).

subspecies could be defined within the species, namely *R. r. roxellana* in SG region; *R. r. hubeiensis* in SNJ region; and *R. r. qinlingensis* in the QL region [Wang et al., 1998]. However, analyses by Li et al. [2001] indicated that genetic divergence between populations from the three regions was low (0.001–0.005) based on analyses of 735 bp of mtDNA cytochrome b and 387 bp of 12S rRNA sequence (six samples from SG, eight from QL and four from SNJ). In another study using allozyme electrophoresis, 44 allozyme loci were surveyed but none was found to be polymorphic among 32 individuals (18 from SG, 13 from QL and 1 from SNJ), implying a low genetic diversity and a recent genetic bottleneck within the last 15,000 years [Li et al., 2003]. Thus, there may be low genetic diversity among golden monkey populations contradicting the designation of three subspecies [Li et al., 2001, 2003, 2004].

Small sample size used and the usage of low resolution allozyme markers [Li et al., 2003] have so far not allowed to identify genetic differences, phylogeographic patterns, evolutionary processes and genetic structure of the golden monkeys. To assess the genetic diversity among populations and explore the relationships among different geographic regions, we collected 85 samples throughout the current distribution of the species, and used the hypervariable segment I (HVI) of mtDNA control region (CR) to analyze the population genetic structure, phylogeographic pattern and evolutionary history of the golden monkey. Finally, a conservation strategy for the species is discussed based on the results.

MATERIALS AND METHODS

Samples, DNA Extraction and Sequencing

Eighty-five DNA samples of golden monkeys were extracted from muscle, skin, hair, and faeces collected from Songpan (SP), Wolong (WL), and

Beishuijiang (BSJ) in Sichuan-Gansu (SG) region; Zhouzhi (ZZ), and Taibai (TB) in Qinling (QL) region; and the two locations (SNJa and SNJb) in Shennongjia (SNJ) region (Fig. 1). None of the samples were used in previous analyses [Li et al., 2001, 2004]. Muscle ($n = 18$) and skin ($n = 25$) samples were gathered from carcasses, which were provided by local museums and National Nature Reserves in 2002. Hair samples ($n = 15$) were collected from captured animals in 2002. Muscle, skin, and hair were sampled following a protocol approved by Institute of Zoology, Chinese Academy of Sciences Institutional Animal Care Committee and State Forestry Administration of China. Twenty-seven faecal samples from the two populations (SNJa and SNJb) in the SNJ region were collected under trees in August 2003, in which monkeys slept, according to the methods described by Bradley et al. [2004]. Skin and hair samples were stored dry. Muscle and faeces were stored in 95% ethanol. To prevent contamination during DNA extraction, benches, and plastic ware was cleaned with 10% bleach and sterile water and then exposed to UV light for 30 min. The surface of muscle, skin, and hair samples was also exposed to UV light for 30 min. We used eight extraction controls and none produced positive amplification during subsequent PCR. Genomic DNA was extracted from muscle, skin, and hair with the Chelex 100 method [Walsh et al., 1991]. Faecal DNA samples were extracted using QIAGEN DNA Stool Mini Kit (Qiagen GMBH, Germany).

Three hundred and seventy nine (379) bp of the control region HV I were PCR amplified with primer pairs L1 (5′-TCAAAGCTTACACCAGTCTTGT AAACC-3′), L76 (5′-TGGCATTCTATTTAAACTAC-3′), H486 (5′-TGCTTGTGCG GGATATTGAT-3′) and H578 (5′-TGATAATATGGCCCTGAGGTAAG-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, 1.5 units Taq DNA polymerase (Qiagen), 1 μg/μl BSA and $\leq$10 ng of genomic DNA. PCR amplification was performed on a Perkin-Elmer Cetus 9700 DNA Thermocycler with pre-denaturing at 94°C for 10 min, thirty-five cycles for denaturing at 94°C for 60 s, annealing at 56°C for 60 s, extending at 72°C for 60 s, and a final 10 min of extension at 72°C. Positive (muscle DNA) and negative (water) controls were used to check PCR performance and contamination for each separate batch of PCR. Negative controls never produced PCR products. PCR products were purified using Spin Column-PCR Purification Kit (Shanghai Huaxun Biology and Technology Ltd.) and sequenced with the Prism BigDye™ Terminator Ready Reaction kit (Applied Biosystem Inc.) and an ABI 377 or an ABI-PRISM™ 3100 Genetic Analyzer, according to manufacturer's manual.

Data Analysis

Forward and reverse sequencing was performed for each individual, and consistent sequences for all individuals were aligned using Clustal X [Thompson et al., 1997] and rechecked by eye. Genetic diversity within populations was estimated as haplotype diversities (h) and nucleotide diversities (π) [Nei, 1986] using DnaSP 3.0 [Rozas & Rozas, 1999]. The pair-wise sequence difference among the haplotypes was calculated by Kimura 2-parameter model using MEGA 2.1 [Kumar et a1., 2001]. Genetic differentiation between different regions was assessed by comparing average numbers of pair-wise differences between populations (πXY); average number of pair-wise differences within populations (πX and πY); and the corrected average pair-wise difference ($\pi XY-(\pi X+\pi Y)/2$) using Arlequin 2.000 [Schneider et al., 2000]. Arlequin 2.000 was also used to

carry out analysis of molecular variance (AMOVA) [Excoffier et al., 1992] to test the partitioning of the variation between geographical units. With AMOVA, the groupings that maximized values of Φ_{CT} (the proportion of genetic variation among groups of the populations relative to all samples) and statistical significance indicate the most parsimonious geographical subdivision.

For phylogenetic inference, we performed maximum-likelihood and neighbor-joining analyses using the program PAUP* 4.0 [Swofford, 2000] and Bayesian analysis using the program MRBAYES V.3.0 [Huelsenbeck & Ronquist, 2001]. MODELTEST 3.06 [Posada & Crandall, 1998] was applied to determine the appropriate model of molecular evolution in a likelihood ratio test framework. The homologous sequences of *R. brelichi*, *Macaca thibetana*, and *Trachypithecus poliocephalus* were used together as outgroups. Gaps were treated as a fifth state. Bootstrap analyses were performed with 5,000 replicates for neighbor-joining and 100 full heuristic bootstrap replicates were used for maximum-likelihood. For Bayesian phylogenetic inference four Monte Carlo Markov Chains were analyzed for 100,000 generations and sampled every ten generations; the initial 5% of trees were discarded as burn-in. As a haplotype network is more effective than a tree in visually portraying the relationships between sequences for populations with low sequence diversity [Crandall & Templeton, 1993], a network was constructed using TCS 1.18 [Clement et al., 2000]. On the basis of the mutation rate for CR HVI of 11.5–17.3% per million years, estimated from the human–chimpanzee split [Vigilant et al., 1991], which was also used in a study on the Japanese macaque [Marmi et al., 2004], we also estimated the most recent common ancestor (TMRCA) of the golden monkey mtDNA haplotypes.

Signatures of population demographic changes (e.g., bottlenecks or expansions) were examined using two different approaches. First, we investigated the demographic history by comparing mismatch distributions for all the samples, each geographic region, and the observed phylogenetic clade A using DnaSP [Rozas & Rozas, 1999]. The shape of the mismatch distribution of pair-wise differences is usually multimodal in samples drawn from the populations at demographic equilibrium, whereas a unimodal distribution is generally found in populations having passed through a recent demographic expansion [Rogers and Harpending, 1992]. It is important to note, however, that unimodal distribution could also be generated by a bottleneck, and that distinguishing between these demographic events remains difficult [Rogers and Harpending, 1992]. The overall validity of the estimated demographic model is tested by obtaining the distribution of a test statistic SSD (the sum of squared differences) between observed and expected mismatch distributions. A significant SSD value is taken as the evidence of departure from the estimated demographic model of a sudden population expansion. In addition, the Tajima's D statistic (DnaSP 3.0) and the Fu's *Fs* (Arlequin 2.000) statistic were used to test whether CR 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 [Fu, 1997; Tajima, 1989].

RESULTS

Genetic Diversity and Differentiation

A total of 379 bp of HV I region DNA sequence was generated from 60 samples, 18 of which were from SG, 19 from QL and 23 from SNJ. Of the total

379 nucleotide positions, 55 variable sites were observed, comprising 52 transitions (ts), four transversions (tv) (both ts and tv at the 32nd and 50th variable site), and one insertion/deletion (Table I). Twelve haplotypes (AY589715–AY589726) were identified from 60 samples. Of 12 haplotypes, eight were found in SG (SG1–SG8), two in QL (QL1 and QL2) and two in SNJ (SNJ1 and SNJ2), respectively (Table I). Within regions, SG4 was shared between the SP and WL locations in SG region; QL2 was shared between the TB and ZZ locations in QL region; and SNJ2 between the SNJa and SNJb locations in SNJ region (Table I). No haplotype was found to be shared between any two of the three regions. There were three diagnostic sites (32nd, 50th, and 53rd of the 55 variable sites) which differentiated SNJ from SG and QL sequences (Table I). Diversity indices, h and π, are summarized in Table I. In total, h was 0.845 and π was 0.0331, showing a high-haplotype diversity and low nucleotide diversity. h and π (0.876 and 0.0406) for SG was higher than for QL (0.281 and 0.0096) and for SNJ (0.474 and 0.0038). Average pair-wise sequence difference among all haplotypes was estimated to be 5.5%, ranging from 0.3 to 9.6% (Tables II and V). With regard to regions, average percentage of pair-wise difference was highest within the SG region (5.5%), followed by QL (3.6%) and SNJ (0.8%) (Table V).

AMOVA based both on genetic distance and haplotype frequency indicated several possible population groupings with significant Φ_{CT} values ($P<0.05$; Table V). The grouping pattern "[BSJ, SP, WL] [TB, ZZ] [SNJa, SNJb]" gave the highest Φ_{CT} value (0.567, $P<0.001$). These results suggested that "[BSJ, SP, WL] [TB, ZZ] [SNJa, SNJb]" is the most parsimonious geographical subdivision. Additionally, AMOVA partitioned 56.7% of molecular variance between these groups (Table IV). Finally, the test of population differentiation showed that these three regions are significantly differentiated one from the other in pair-wise comparisons ($P<0.001$) (Table III).

Phylogeny and Phylogeography

The HKY-G model with a gamma shape correction of 0.0148 was identified by MODELTEST as the best-fitting distance estimator. Bayesian inference, maximum-likelihood and neighbor-joining analyses resulted in almost identical tree topologies (Fig. 2) with high bootstrap values and posterior probabilities for the two main clades (A and B). The distinction between the two *R. roxellana* clades was also evident in network analysis (Fig. 3). Clade A including all three regions contains eight haplotypes and Clade B involving only SG region contains only four haplotypes. Wthin Clade A, haplotypes from each region clustered into three groups, respectively (Figs. 2 and 3). Each of the three sample sites in the SG region contained haplotypes of both clades. The haplotype network also showed that the split was clear between clades A and B and within clade A (Fig. 3). According to the average difference of pair-wise sequence among all the haplotypes (5.5%), using a mutation rate range of 11.5–17.3% per million years in HVI, the divergence time of Clades A and B was estimated to be 0.48–0.32 Mya, which corresponds to the time of the most recent common ancestor (TMRCA) of extant haplotypes.

Demographic History

Mismatch distribution analyses of the overall dataset, each region, and clade A each produced different patterns (Fig. 4). The shapes of the functions for the overall dataset, SG and QL populations and clade A were ragged and multimodal,

TABLE I. A Summary of mtDNA CR Haplotype Distributions, and Haplotype Diversity and Nucleotide Diversity Indices for All Sampling Locations Within the SG, QL and SNJ Regions

Haplotype	Variable sites	SG: BSJ	SG: SP	SG: WL	QL: ZZ	QL: TB	SNJ: SNJa	SNJ: SNJb	Total
SNJ1	ATTCACTTCTGTATACCACCCCCTACACAGACCCCCCTCTATTTTACACGATTAG							8	8
SNJ2	........................G.G..A........................						7	8	15
QL1	G....T..........TG...........A.A....T.TC.........A..C..					3			3
QL2	...T......AC........T.TC.....AGA.......C...C.....A..C..				11	5			16
SG1	..C..T..T...G...T..T.TTCGT...A.AATTT..T...CCCGT.TAC.CGA	2							2
SG2	....G..C.....................A.A......TC.CCC.....A..C..	5							5
SG3	.............C..........G..T.A.A......TCGCCC.....T..C..	1							1
SG4	.CC..TC.T...G...T..T.TTCGT..GA.AATTT..T...CCCGT.TAC.CGA		1	1					2
SG5	.CC..TC..C....G.T.TT.T.C.T..GA.G.TTT.CT...CCCGTG.ACCCGA		1						1
SG6	....G..C........T............A.A......TC.CCC.....A..C..		2						2
SG7	.CC..TC..C....GTT.TT.TTC.T..GA.A.TT...T...CCCGTG.AC.CGA			1					1
SG8	...........................T.A-A......TC.CC......A..CG.			4					4
	Number of samples	8	4	6	11	8	7	16	60
	Number of haplotypes	3	3	3	1	1	1	2	12
	Haplotype diversity (h)	0.607	0.833	0.600	0	0.536	0	0.533	0.845
	Nucleotide diversity (π)	0.0344	0.0586	0.0428	0	0.0203	0	0.0042	0.0331

Dots (.) indicate nucleotide identity and hyphens (-) means gap. The nucleotides (shaded) presented the three diagnostic sites which differentiated SNJ from SG and QL sequences.

TABLE II. The Pair-Wise Sequence Difference Among the mtDNA CR Region Haplotypes

	SNJ1	SNJ2	QL1	QL2	SG1	SG2	SG3	SG4	SG5	SG6	SG7
SNJ1											
SNJ2	0.008										
QL1	0.030	0.033									
QL2	0.033	0.036	0.036								
SG1	0.083	0.080	0.074	0.077							
SG2	0.030	0.033	0.027	0.030	0.074						
SG3	0.035	0.033	0.036	0.038	0.076	0.019					
SG4	0.092	0.089	0.083	0.086	0.008	0.083	0.086				
SG5	0.092	0.095	0.086	0.096	0.044	0.086	0.095	0.036			
SG6	0.033	0.036	0.024	0.033	0.071	0.003	0.022	0.080	0.083		
SG7	0.089	0.092	0.080	0.083	0.038	0.080	0.089	0.030	0.016	0.077	
SG8	0.027	0.030	0.024	0.033	0.071	0.013	0.016	0.080	0.083	0.016	0.077

TABLE III. Pair-Wise Population Differentiation for Control Region Sequences Among Different Regions

	SG	QL	SNJ
SG	15.7	18.2***	18.1***
QL	8.4***	4.0	13.3***
SNJ	9.6***	10.7***	1.4

Above diagonal: average number of pair-wise differences between populations in different regions (πXY). Diagonal elements: average number of pair-wise difference within population (πX). Below diagonal: corrected average pair-wise difference $(\pi XY)-(\pi X+\pi Y)/2$). (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

revealing a complicated demographic history. Within the SG region, which contained haplotypes from the two distinct clades A and B (Fig. 2), multimodality was likely caused by the treatment of a genetically subdived populations as one population. Our assumption of one population was based on the co-occurrence of haplotypes of both clades in all three sampling areas within SG. Both Tajima's D value and Fu's Fs value support the results of mismatch distribution and indicated no significant population expansion or bottleneck ($P>0.1$) for the total dataset or for any of the geographic populations (Table V).

DISCUSSION

Genetic Variation

The genetic variation among golden monkeys detected in this study indicated that this species should not to be regarded as a taxon with low genetic diversity. Within the 379 bp segment of mtDNA CR HVI examined, 55 polymorphic sites and 12 haplotypes were found. h and π (0.845 and 0.0331) were almost equal to the level of variation found in Barbary macaques (*Macaca sylvanus*, $h = 0.872$ and $\pi = 0.026$) [Modolo et al., 2005] and baboons (*Papio hamadryas hamadryas*, $h = 0.856$ and $\pi = 0.023$) [Winney et al., 2004], and the h of the golden monkey is higher than that of the Japanese macaque (*Macaca fuscata yakui*, $h = 0.305$) [Hayaishi & Kawamoto, 2006]. However, the low π and average percentage of pair-wise difference suggests a gloomy status for the population in SNJ,

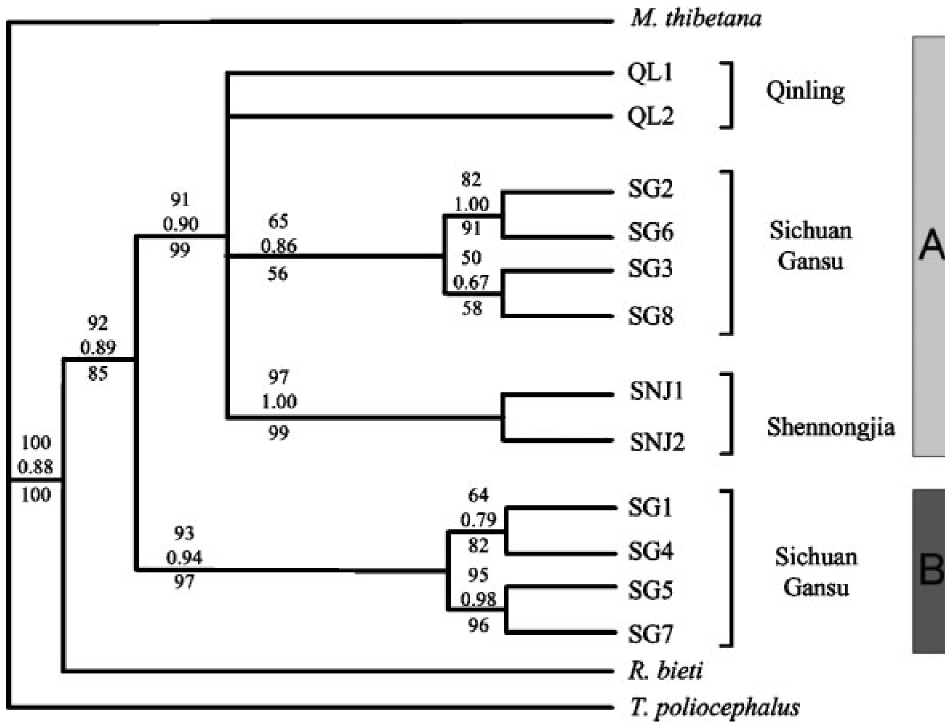

Fig. 2. Maximum-likelihood (ML) tree for all 12 haplotypes of the *R. roxellana* and for three outgroup taxa. The haplotype codes refer to those in Table I. Values above branches are, first, bootstrap support for the ML analysis and, second, posterior probabilities for the Bayesian inference. Bootstrap support for the corresponding neighbor-joining tree is given below branches.

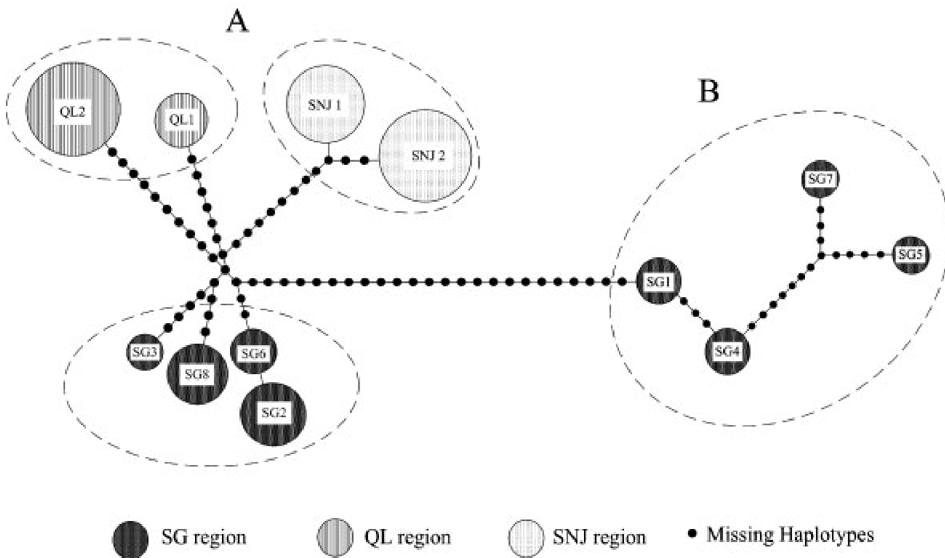

Fig. 3. Median-joining network of the haplotypes. The size of the circle indicates the relative frequency of the corresponding haplotype in the whole data set. Missing haplotypes in the network are represented by black dots.

TABLE IV. The Results of AMOVA for Groupings of Populations Estimated Using Φ-Statistics Based on Control Region Sequences

	Among groups	Among pops within groups	Within pops	Percentage of variation (%)			
Groups	Φ_{CT}	Φ_{SC}	Φ_{ST}	Among groups	Among pops within groups	Within pops	*P*
[BSJ, SP, WL] [TB, ZZ] [SNJa, SNJb]	0.567	0.165	0.638	56.7	7.1	36.2	0.00000 ± 0.0000
[BSJ, SP, WL, TB, ZZ] [SNJa, SNJb]	0.352	0.465	0.653	35.2	30.5	34.3	0.00000 ± 0.00000
[BSJ, SP, WL] [TB] [ZZ] [SNJa] [SNJb]	0.477	0.244	0.605	50.2	14.3	35.5	0.02542 ± 0.00528
[BSJ, SP, WL] [TB, ZZ, SNJa, SNJb]	0.290	0.507	0.650	28.1	37.1	34.8	0.03030 ± 0.00466
[BSJ, SP, WL, SNJa, SNJb] [TB, ZZ]	0.300	0.500	0.650	30.1	35.3	34.6	0.09775 ± 0.00918

Sampling locations SNJa and SNJb are from the Shengnongjia, SG region. Sampling locations for BSJ, SP and WL are from the SG region and sampling locations for TB and ZZ are from the QL region.

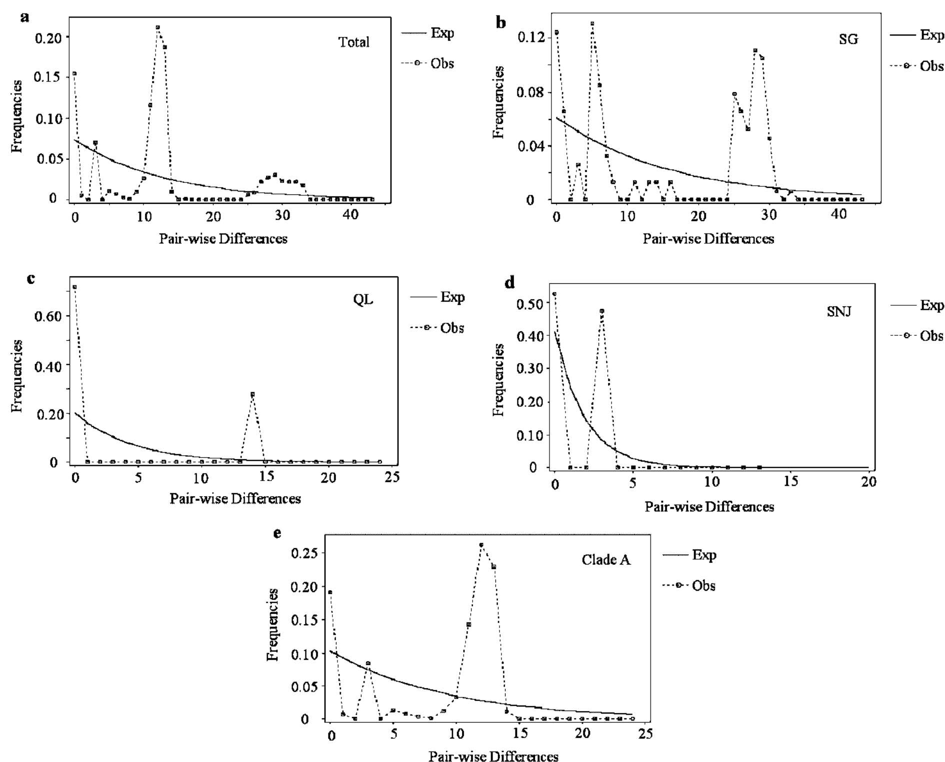

Fig. 4. Mismatch distribution of the golden monkey mtDNA control region for the total samples (a), SG (b), QL (c) and SNJ (d) population and Clade A (e).

consisting of a population of only about 600 individuals in wild [Li et al., 2002; Quan and Xie, 2002].

Population Structure, Phylogeography, and Demographic History

AMOVA analysis detected a largest Φ_{CT} value (56.7%, $P<0.001$) for the population grouping pattern: [BSJ, SP, WL] [TB, ZZ] [SNJa, SNJb] (Table IV), implying "[SG] [QL] [SNJ]" as the most parsimonious geographical subdivision within *R. roxellana*. Additionally, the test of pair-wise population differentiation showed that these three regions are significantly differentiated from each other ($P<0.001$) (Table IV). It should also be noted that no common haplotype was shared among the three geographic regions. This suggests some degree of spatial genetic structure within *R. roxellana* and limited dispersal of individuals among these three regions.

Recent history may have shaped the pattern of genetic differentiation observed in the golden monkey. Phylogenetic tree and network analysis clustered haplotypes from QL and SNJ into respective groups, which are separated from haplotypes found in SG (Figs. 2 and 3). Phylogenetic analysis identified two, statistically well-supported clades A and B. Clade A showed clear geographical partitioning of haplotypes according to the three regions and in accordance with the AMOVA analysis, whilst haplotypes of clade B were solely found in SG. The co-occurrence of hapotypes of both clades in SG indicates a complex demographic

TABLE V. Gene diversity (*h*), Nucleotide Diversity (π), Average Pair-Wise Sequence Difference (*A*), Tajima's (*D*), Fu's (*Fs*), and Mismatch Distribution Tests (SSD) for CR Sequences

Region/Clade	*n*	*h*	π	*A*	SSD (*P* value)	*D* (*P* value)	*Fs* (*P* value)
SG	18	0.876	0.0406	5.5%	0.0686 (0.000)	1.052 (0.892)	4.963 (0.969)
QL	19	0.281	0.0096	3.6%	0.1114 (0.000)	−0.070 (0.521)	8.786 (0.997)
SNJ	23	0.474	0.0038	0.8%	0.0243 (0.000)	1.914 (0.977)	4.224 (0.963)
Clade A	54	/	/	/	0.0815 (0.000)	1.211 (0.237)	8.525 (0.001)
Total	60	0.845	0.0331	5.5%	0.0647 (0.000)	0.308 (0.694)	8.302 (0.973)

history. Either clade B haplotypes were lost by random sorting in all regions except SG or differentiated populations with distinct haplotypes were admixed only in SG. Mismatch analysis also supports that the SG region represents a subdivided population. However, as haplotypes from both clades co-occurred in all three sampling areas within SG, the population subdivision is historic.

The ancestor of Asian colobines, *Mesopithecus,* is postulated to have followed a wooded savanna 'corridor' to Asia from Africa [Jablonski, 1998a,b; Pan et al., 2004]. After having arrived at the Qinghai-Tibet Plateau, the ancestors of the odd-nosed monkeys spread out in different directions. According to Pan et al. [2004], *R. bieti* remained in almost the same area. Other populations migrated southward along the river banks and mountain ridges and resulted in *R. avunculus* in Vietnam, Laos, Cambodia and Southeast Asia (Borneo and the Mentawais) and *Pygathrix*, *Nasalis* and *Simias* in South East Asia. The dispersal routes used by *R. roxellana*, however, were quite different from those chosen by other taxa. They might have moved eastward and inhabited north and central China [Jablonski, 1998a,b]. Therefore, the golden monkeys might have subsequently migrated eastward to Gansu, Sichuan, Shaanxi, and Hubei. The climatic oscillations during this period are widely regarded as one of the most important factors influencing population structure and phylogeographic patterns [Avise, 1998; Hewitt, 1996, 1999], and may have contributed to the extinction of some populations in northern China and to the southward migration of other populations where isolated populations were formed [Zhou, 1964]. The phylogeographic divergence among SG, QL, and SNJ populations may possibly be related to geographic barriers. The three habitats of *R. roxellana* are isolated mountainous regions cut by Sichuan basin, valleys and complicated water system, which might be natural barriers that separated the SG, QL, and SNJ populations.

High haplotype diversity, mismatch analysis, and Tajima's D and Fu's *Fs* values (Table II) indicate there was no single expansion or contraction in all populations of the golden monkey. Furthermore, the mismatch distribution analysis showed atypical distribution shapes, revealing a complicated demographic history for the populations. This may be related to the effects of climate oscillations during glaciations in the late Pleistocene and Holocene and to postglacial population expansions. The topographical diversity of the Qinghai-Tibetan Plateau and Qinling Mountain might have created both, networks of refuges during the glaciations and complex barriers to subsequent expansion [Hewitt, 2004]. The mismatch distribution deviation of the overall population could be the result of high sequence diversity among individuals from different geographic locations. As the geographic features changed, climate oscillated and

vegetation varied during glaciations in the late Pleistocene, the species might have suffered significant population fluctuations, but no single expansion or contraction in all populations.

Implication for Conservation

It should be appropriate to define SG, QL, and SNJ as three management units (MUs) according to the phylogeographic analysis and the significant genetic differentiation among those three regions. According to the model proposed by Moritz [1994], ESUs are designated on the basis of reciprocal monophyly at mitochondrial markers, whereas 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. Additionally, the haplotypes found in QL and SNJ were identified as independent clades, and are both different from haplotypes from SG within Clade A in the phylogeograhic tree. This indicates that the phylogeograhic pattern and genetic structure of the populations in SNJ and QL are different from that in SG. Furthermore, some studies based on morphologic characters of the populations in the three regions have described some significant differences [Wang et al., 1998]. Thus, we believe these three regions should be regarded as separate MUs, respectively, under the condition of further shrinking distribution, increasing isolation and decreasing haplotype diversity. Finally, special attention should be paid to the conservation of the SNJ region because of its very low-genetic diversity, small habitat area, significant genetic distance from other two regions and smallest population number (600 individuals) [Li et al., 2002; Quan and Xie, 2002].

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