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RESEARCH ARTICLE

Historical Geographic Dispersal of the Golden Snub-Nosed Monkey (*Rhinopithecus roxellana*) and the Influence of Climatic Oscillations

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Current understanding of historic climate oscillations that have occurred over the past few million years has modified scientific views on evolution. Major climatic events have caused local and global extinction of plants and animals and have impacted the spatial distribution of many species. The endangered golden snub-nosed monkey (*Rhinopithecus roxellana*) currently inhabits three isolated regions of China: the Sichuan and Gansu provinces (SG), the Qinling Mountains in Shaanxi province (QL), and the Shennongjia Forestry District in Hubei province (SNJ). However, considerable uncertainty still exists about their historical dispersal routes under the influence of environment change. To date, two dispersal routes have been proposed: (1) the QL and SNJ populations originated from the SG population; and (2) the SG population recolonized from the QL and SNJ populations. We used the mitochondrial DNA complete control region to perform statistical assessments of the relative probability of alternative migration scenarios and the role of environmental change on the geographic dispersal of *Rhinopithecus roxellana*. Thirty haplotypes were identified from the three geographic regions and a high degree of genetic structure was observed. The most recent common ancestor among the mitochondrial DNA haplotypes was estimated to live around 0.47–1.88 million years ago and five notable haplotype clusters were found. Phylogenetic analysis and historical gene flow estimates suggested that the QL and SNJ populations originated from the SG population, with at least two dispersal events from the SG population occurring during the Pleistocene (1.17 ± 0.70 and 0.53 ± 0.30 Ma). Composite dispersal history of the golden snub-nosed monkey can be explained by both environmental change inducing global climate change and the influence of the Tibetan Plateau uplift. Such range shifts involved considerable demographic changes, as revealed in the dramatic decreases in population size during the last 25,000 years. Am. J. Primatol. 74:91–101, 2012.

Key words: climatic oscillation; dispersal route; environment change; golden monkey; mtDNA

INTRODUCTION

Global climate and environment changes (e.g. tectonic uplift) have greatly influenced the surface of the earth during the past three million years, and inevitably changed the distributions of most living organisms through the alteration of vegetation [Hewitt, 2000]. The extinction, colonization, and recolonization of species throughout their distribution range depend largely on spatial factors unique to each locality (i.e. latitude) [Hewitt, 2004]. Dramatic demographic changes leave phylogeographical signatures [Flagstad & Roed, 2003], making observation of genetic structure patterns and inference of possible population history scenarios relatively straightforward [Jablonski & Pan, 1988]. Within China, for example, the widely distributed ring-necked pheasant (*Phasianus colchicus*) diverged into three clades (western clade, eastern clade, and Sichuan Basin clade) during the late Pleistocene due to climatic changes under the influence of the Qinghai-Tibet Plateau uplift and the existence of geographical

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barriers [Qu et al., 2009], while the white-rumped snowfinch (*Onychostruthus taczanowskii*) and Hume's ground tit (*Pseudopodoces humilis*) experienced rapid population expansion during the retreat of the last extensive glacial period (0.5–0.175 Ma) [Qu & Lei, 2009].

The golden snub-nosed monkey (*Rhinopithecus roxellana*) is a species of Asian colobine, which became widely dispersed in south and central China during the late Pleistocene and early Holocene [Gu & Hu, 1991; Gu & Jablonsk, 1989; Han, 1982; Jablonski, 1998a,b; Jablonski & Pan, 1988]. It has been postulated that the ancestor of Asian colobines, *Mesopithecus*, followed a wooded savanna 'corridor' from Africa to Asia [Jablonski, 1998a,b; Pan et al., 2004]. After arriving in the Qinghai-Tibet Plateau, the ancestors of the snub-nosed monkeys dispersed in different directions [Li et al., 2007]. Based on analysis of the mitochondrial cytochrome b gene, it is estimated that this divergence occurred 2.08–2.84 million years ago [Li et al., 2004]. Fossil studies show that after speciation, climatic oscillations in the Quaternary caused the repeatedeastward migration of *R. roxellana*into north and central China during glacial expansion, followed by westward recolonization during glacial retreat [Jablonski, 1998a,b].

Today, *R. roxellana* is restricted to three isolated regions at the edge of the Tibetan Plateau and in the mountains of central China: Sichuan and Gansu (SG), Qinling (QL) and Shennongjia (SNJ), with the total population numbering no more than 20,000 individuals [Li et al., 2007; Quan & Xie, 2002]. The golden snub-nosed monkey is recognized as an endangered species by the World Conservation Union [IUCN, 2010] and has Class I Protected Status under Chinese law. Limited by inappropriate genetic markers and insufficient sample sizes, little information is available regarding the relationships of these extant populations [Li et al., 2004, 2007; Pan et al., 2009]. To date, however, two hypotheses have been proposed. The mono-origin hypothesis posits that the QL and SNJ populations originated from the SG population [Li et al., 2004; Pan et al., 2009]. Under this hypothesis, climatic changes drove some populations southward and eastward while some populations survived the environmental changes and preserved their ancestral genetic structure [Li et al., 2004]. The multiorigin hypothesis states that the SG population is a fusion of the QL and SNJ populations [Pan et al., 2009]. This suggests that during glacial periods, all SG populations either moved southeast or perished. During interglacial periods, however, *R. roxellana* populations expanded and recolonized western areas and it is from here that the extant SG population originated. As *R. roxellana* is a threatened species of limited and restricted population, understanding their historical dispersal and the relationships between extant populations will aid appropriate conservation management strategies [Moritz, 1994].

We collected 86 samples of *R. roxellana* across its current distribution. The complete mitochondrial DNA control region was used to analyze gene flow among the three populations and detect historical dispersal routes. The control region is generally considered a neutral or near-neutral molecular marker [Avise, 1991] and is relatively stable in mammals, especially primates [Zhang & Hewitt, 2003]. Population histories, including the timing of population divergences, the direction and extent of migration, and estimates of effective population sizes, can be inferred effectively based on genetic information with coalescent theory [Thalmann et al., 2011]. Our aims were (1) to assess the level and partitioning of genetic variation and genetic structure within *R. roxellana*; (2) to examine historical geographic dispersal routes; (3) to analyze population demographic history; (4) to discuss the reasons behind these evolutionary scenarios; and (5) to discuss management strategies for this species.

METHODS

Sample Collection and DNA Extraction

We collected 86 *R. roxellana* samples from the current distribution (SG population = 41, SNJ population = 22, and QL population = 23) (Fig. 1, Table I), including 51 samples from our lab (blood, muscle, and skin) and 35 sample sequences [Genebank: AY545036–AY545053] from a former study conducted by Pan et al. [2009]. All sample collections were carried out in compliance with the relevant institutions and laws of China. This research adhered to the American Society of Primatologists principles for the ethical treatment of primates. Muscle ($N = 38$) and skin ($N = 3$) samples were gathered from remains provided by local museums and nature reserves. Skin samples were stored dry. Muscle was stored in 95% ethanol. Ten blood samples were collected while trapping individuals for physical examination, and were stored in a refrigerator at −80°C. To prevent contamination

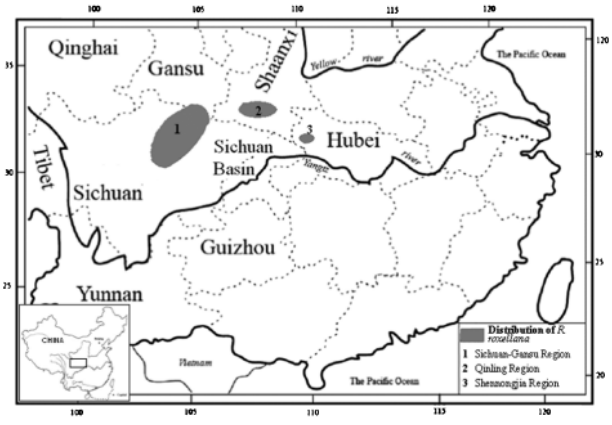

Fig. 1. Distribution of *Rhinopithecus roxellana* in China.

TABLE I. Summary of mtDNA Control Region Haplotypes Distributions

Population haplotype	SG population	QL population	SNJ population	Total
H01	1			1
H02	4			4
H03	7			7
H04	1			1
H05	1			1
H06	1			1
H07	1			1
H08	1			1
H09	1			1
H10	2			2
H11	4			4
H12	1			1
H13	1			1
H14	5			5
H15	9			9
H16			1	1
H17			1	1
H18			5	5
H19			1	1
H20			14	14
H21		4		4
H22		1		1
H23		1		1
H24		1		1
H25		1		1
H26		1		1
H27		4		4
H28		1		1
H29		8		8
H30		1		1

SG, Sichuan and Gansu; QL, Qinling; SNJ, Shennongjia.

during DNA extraction, benches and plastic ware were cleaned with 10% bleach and sterile water and then exposed to UV light for 30 min prior to handling. The surface of muscle, skin, and hair samples were also exposed to UV light for 30 min. DNA samples were extracted using the QIAgen DNA Stool Mini Kit (Qiagen, Germany). We used eight extraction controls and none produced positive amplification during subsequent PCR.

MtDNA Control Region Amplification and Sequencing

The complete mtDNA control region was amplified using primers following Pan et al. [2009]. We performed amplification in a total volume of 50 ml containing 50 mM KCl, 10 mM Tris–HCl, 1.5 mM Mg21, 200 mmol of each dNTP and 0.2 mmol of each primer, 1.5 units Taq DNA polymerase (Takura), and 1 mg/ml BSA and r10 ng of genomic DNA. The PCR amplification was performed on a 9700 DNA Thermocycler (Applied Biosystem Inc., Carlsbad, CA) with predenaturing at 94°C for 10 min, 35 cycles for denaturing at 94°C for 30 sec, annealing at 56°C for 60 sec, extending at 72°C for 60 sec, and a final 10-min extension at 72°C. Positive (DNA) and negative (water) controls were used to check PCR performance and contamination for each PCR separate batch. Negative controls never produced PCR products. The PCR products were purified using Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI). To avoid errors in amplification and sequencing, PCR amplifications of all samples were performed twice or more and the products were sequenced for both ends. Direct sequencing was performed on the Prism BigDyeTM Terminator Ready Reaction Kit (Applied Biosystem Inc.) and an ABI 377 or ABI-PRISMTM 3100 Genetic Analyzer (Applied Biosystem Inc.). Two independent PCR amplifications may exclude contaminations with nuclear integrations of mitochondrial fragments (numts). We also randomly sequenced five samples using the pGEM T-Easy Vector System (Promega). Recombinant clones were detected by blue/white screening and plasmid DNA minipreps were prepared with a Plasmid Mini Kit. Ten clones per individual containing inserts were sequenced. All obtained sequences were identical.

Genetic Diversity and Phylogeographical Structure

We aligned the sequences using Mega 4 software [Tamura et al., 2007] and rechecked them by eye. As in the previous study [Pan et al., 2009], the tandem T repeat was removed from all individuals according to Bandelt et al. [2000] because these fragments are not suitable for analyses owing to uncertainties of their origins [Bendall & Sykes, 1995; Budowle et al., 2003; Hayasaka et al., 1991]. We identified haplotypes and calculated fundamental genetic indices (for example, haplotype diversity (h) and nucleotide diversity (π)) using DNA SP 5.0 software [Librado & Rozas, 2009].

Two different methods were used to estimate evolutionary relationships of the haplotypes. First, a median-joining network was constructed using NETWORK software [Bandelt et al., 2000]. Second, a Bayesian analysis of the haplotypes was performed using BEAST v1.5.4 software [Drummond & Rambaut, 2007] with gaps treated as fifth states and *Pygathrix nemaeus*[Genbank: EU004481] and *Semnopithecus entellus*[Genbank: NC008215] as outgroups. We identified an appropriate model of molecular evolution with a likelihood ratio test using MODELTEST v3.06 [Posada & Crandall, 1998]. We selected the proposed split for the divergence between *R. roxellana* and *Pygathrix nemaeus* as 6.9 ± 0.65 million years ago [Kirstin et al., 2006]. Four Markov chain Monte Carlo (MCMC) simulations were run for 10^7 generations with sampling every 1,000 generations. The initial 15% of trees were discarded as burn-in. In Tracer v1.5, log files were analyzed and effective

sample sizes were used to evaluate MCMC convergence within chains.

Historical Geographic Dispersal Routes

Gene flows between the clades were estimated with MIGRATE 3.1 [Beerli, 1998, 2004, 2006, 2008; Beerli & Felsenstein, 1999] using the full data set of the 86 sequences. MIGRATE uses a Metropolis-Hastings algorithm to explore all possible genealogies [Beerli & Felsenstein, 1999]. We used both the maximum likelihood and Bayesian inference analysis to take into account history of mutations and genealogy uncertainty [Beerli, 2008]. The transition/transversion ratio 9.895 and nucleotide compositions of A (0.293), T (0.276), C (0.29), and G (0.141) were used for analysis. The ArlequinF_{ST} estimates (described above) were used as starting values for the initial analysis. We adopted the assumptions of the coalescent approach including asymmetrical immigration between the three populations with constant population sizes, constant migration, and mutation rates [Beerli, 1998; Beerli & Felsenstein, 1999]. In the ML strategy, ten short chains with 5,000 record steps and one long chain with 50,000 record steps were set. For each chain, the number of discarded trees (burn-in) was 10,000. Two independent replicate chains were run for estimates. The static heating scheme was set to four chains with default temperatures of 5.00, 3.00, 1.50, and 1.00 and the swapping interval set at one. In the Bayesian strategy, two long chains with 50,000 recorded steps were used. The sampled parameter value was 10,000,000. The first 100,000 trees per chain were discarded as burn-in. The same static heating scheme was used in Bayesian inference. The essence of the Bayesian viewpoint is there is no logical distinction between model parameters and data [Beaumont & Rannala, 2004]. The coverage of the Bayesian approach is rather conservative and includes the true values in former simulation research [Beerli, 2004].

Historical Demography

Using the full data set, we estimated major demographic changes using BEAST v1.5.4 software [Drummond & Rambaut, 2007]. The Bayesian Skyline Plot (BSP) model was applied to examine a number of different population sizes through time and to run a smoothing procedure to visualize historical population size changes [Drummond et al., 2005]. Standard MCMC sampling is used by BSP to estimate posterior distribution of effective population size through time from a sample of gene sequences, which gives a specified nucleotide-substitution model. Compared with previous methods, the BSP includes credibility intervals for the estimated effective population size at every point in time, back to the most recent common ancestor of the gene sequences [Drummond et al., 2005]. The prior setting was the same as described in the Bayesian analysis above.

We estimated standard population demographic statistics to detect demographic history of *R. roxellana*. Tajima's *D* [Tajima, 1989] and Fu's *Fs* [Fu, 1997] neutrality tests, as well as the mismatch distributions for all samples were performed in Arlequin 3.1 [Excoffier et al., 2005]. Fu's *Fs* test was selected as it is the most powerful coalescent-based neutrality test against population growth for larger sample sizes [Ramos-Onsins & Rozas, 2002]. The shape of the mismatch distribution of pairwise differences is usually multimodal in samples drawn from populations at demographic equilibrium as it reflects the highly stochastic shape of gene trees, while a unimodal distribution is generally found in populations having passed through a recent demographic expansion [Richard et al., 1992; Rogers & Harpending, 1992] or though a range expansion with high levels of migration between neighboring demes [Excoffier, 2004; Ray et al., 2003]. Additionally, FLUCTUATE 1.4 was used to estimate the exponential growth rate for each clade [Kuhner et al., 1998]. It was initiated with a Watterson [1975] estimate of θ and random topology, performing ten short chains (sampling every 20 genealogies for 2,000 steps) and two long chains (sampling every 20 genealogies for 200 000 steps). For each data set, analyses were repeated five times, and the mean with standard deviation of θ and "*g*" were calculated from the results of the five separate runs.

RESULTS

Genetic Diversity and Phylogeographical Structure

Removal of the tandem T's from the mtDNA complete control region resulted in a 1,080-bp fragment, which was used for analyses. Among the sequences, 100 (9.26%) variable sites including 85 transitions and 15 transversions, defined 30 haplotypes among 86 samples [Genbank: HQ831477–HQ831506] (Fig. 1, Tables I and II). In the nucleotide composition, a strong bias against guanine (mean G = 14.1%, A = 29.3%, T = 27.6%, and C = 29.0%) was found, consistent with studies showing the control region to be an A–T rich region of the genome [Saccone et al., 1987]. Diversity indices are summarized in Table III. Haplotype diversity for all samples was relatively high (0.939 ± 0.012), whereas nucleotide diversity (π) was relatively low (0.021 ± 0.010). The SNJ population showed the lowest nucleotide diversity (0.002 ± 0.001) among all populations. All haplotypes displayed a very strong geographical specificity, consistent with patch clustering based on geographical partitioning. No haplotype was shared among the populations.

TABLE II. Variable Sites of the Control Region in *Rhinopithecus Roxellana*

	Variable sites									
										1111
Haplo-type	111111	1111111111	2222222222	2222222222	3333333333	3333334444	4444455556	6677777777	7777888888	9999990000
	188123445	6666789999	0012222223	4445556678	0000123345	5557784567	7888912296	6700111116	6689001337	3445792333
	8869727033	0169951358	2540134890	1791252704	1456391290	1695746925	9139600362	7989234583	4512390465	8161482689
H01	AATCTCACAT	TCTGTATACA	CCCCTACATA	GAACCCCCCT	CACCTTACAC	CAACAGCAGC	TAATCACTAT	TATATACCCA	TTCATGCCCA	ACAAGGGGTC
H02	.....T....	..C.....	....	A.G.....	.GTTC....T	.T.....	.G.....	......T...	....	.....T....
H03	....	....	....	A.......T.	.G.....T	....	....	....C.....	....	....
H04	.......T..	...A.....	.....G....	A.....	.G..C....T	.T.....	....	..C.....	....	....
H05	..C.C..T.C	.T...G.GT.	T.TTCGT.CG	A..ATTT...	T.T.CCGT.T	T.C.GATGC.	......TC.C	C....G.TTG	...G..TT..	..TGA.AA..
H06	....C..T..	.T..CG..T.	T.TTCGT.CG	A..ATTT...	T.T.CCGT.T	T.C.GATGC.	......TC.C	C....G.TTG	...GC.TT..	..TGA.AA..
H07	....C..T..	.T...G..T.	T.TTCGT.C.	A..ATTT...	T.T.CCGT.T	T.C.GATGC.	......TC.C	C....G.TTG	...GC.TT.C	..TGA.AA..
H08	....C..T..	.T..CG..T.	T.TTCGT.CG	A..ATTT...	T.T.CCGT.T	T.C.GATGC.	......TC.C	C....G.TTG	...GC.TT.C	..TGA.AA..
H09	....	....	....	A.....	.........T	....G..G..	....	....	....	....
H10	..CGC..T.C	.T...G..T.	T.TTCGT.CG	A..ATTT...	T.T.CCGTGT	T.C.GATGC.	......TC.C	C....G.TTG	...GC.TT..	..TGA.AA..
H11	......G...	C.....	....	.....C.	A.....	....C....T	....	....	...T......	....
H12	......G...	C.....	....	.....C.	A.....	....C....T	....	....	....	....
H13	....	....	T.....	....	.....T.	.........T	....	....	....	....
H14	....C..T..	.T...G..T.	T.TTCGT.C.	A..ATTT...	T.T.CCGT.T	T.C.GATGC.	......TC.C	C....G.TTG	...GC.TT..	..TGA.AA..
H15	....	......C...	.....G....	A.....	.G..C....T	.T.....	....	....	....	....
H16	........G.	....	.....C.	..C.....C	T.TT.....T	.G.T..TG..	..G.G.....	.G.....	.....A....	....
H17	....	....	.....C.	..C.....C	T.TT.....T	.G.T..TG..	..G.....	.G.....	.....A....	....
H18	....	....	.....C.	A.C.....C	T.TT.....T	.G.T..TG..	..G.....	.G.....	.....A....	....
H19	....	....	.....G.GC.	A.C.....C	T.TT.....T	.G.T..TG..	..G..C..G.	.G.....	....	....
H20	....	....	.....G.GC.	A.C.....C	T.TT.....T	.G.T..TG..	..G.....	.G.....	....	....
H21	....	...AC.....	.T.TC...C.	AG.....C	..TTC....T	......TG..	....	....	.CT.......	....
H22	.....T....	...AC.....	.T.TC...C.	AG.....C	..TTC....T	....	....	...T......	....	....
H23	.G.....T..	.....TG	.....C.	A.....T..	..TT.....T	......T..T	C..A.....	......TT..	....	CT.C.....
H24	....	....	.T..C...C.	AG.....C	T.TTC.G..T	......TG..	....	.....G....	C.T.......	....
H25	.G.....T..	.....TG	.....C.	.....T..	..TT.....T	......T..T	C..A.....	......TT..	.......AT.	....
H26	T....T....	...AC.....	.T.TC...C.	AG.....C	..TTC....T	......TG..	....	....	.CT.......	....
H27	.....T....	...AC.....	.T.TC...C.	AG.....C	..TTC....T	......TG..	....	....	.CT.......	....
H28	.....T....	...AC.....	.T.TC...C.	AG.....C	..TTC....T	......T..T	C..A.....	......TT..	....	....
H29	.G.....T..	.....TG	.....C.	A.....T..	..TT.....T	......T..T	C..A.....	......TT..	....	....
H30	.G.....T..	.....TG	.....C.	A.....T..	..TT.....T	......T..T	C..A.....	....	.CT.......	.....CT

Dots (.) indicate nucleotide identity.

TABLE III. Genetic Diversity Indices of *Rhinopithecus roxellana* Populations

Clades	N	N	$h \pm$SD	$\pi \pm$SD	$g \pm$SD	$\theta \pm$SD	Tajima's D (P-value)	Fs (P-value)
SG	41	15	0.90 ± 0.02	0.02 ± 0.01	-28.82 ± 27.59	0.013 ± 0.002	1.45 (0.95)	7.67 (0.97)
QL	23	10	0.84 ± 0.05	0.01 ± 0.006	49.36 ± 46.37	0.017 ± 0.003	1.02 (0.87)	3.29 (0.90)
SNJ	22	5	0.56 ± 0.10	0.002 ± 0.001	529.87 ± 406.65	0.003 ± 0.001	-0.08 (0.51)	1.04 (0.76)
All	86	30	0.94 ± 0.01	0.02 ± 0.01	14.44 ± 20.64	0.029 ± 0.002	0.42 (0.72)	2.64 (0.86)

Note: *n*, number of individuals; *N*, number of haplotypes; *h*, gene diversity; π, nucleotide diversity; *g*, growth parameter; θ, population parameters of theta; SG, Sichuan and Gansu; QL, Qinling; SNJ, Shennongjia.

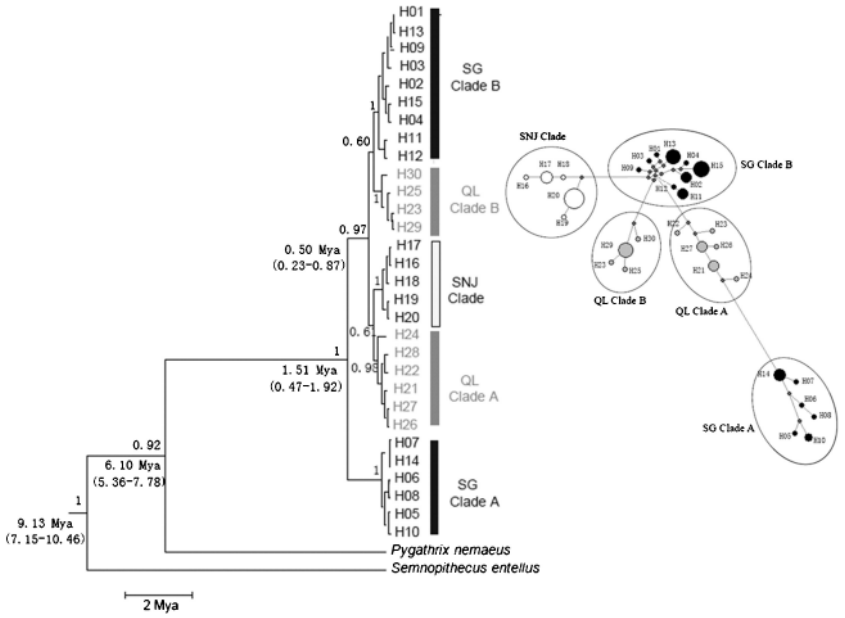

Fig. 2. Bayesian tree and median-joining network generated from entire control region haplotypes of *Rhinopithecus roxellana*. Labels are haplotype identification numbers (Tables I and II). Values above branches indicate support for each node.

According to the hierarchical-likelihood ratio test, and under the Akaike information criterion in Modeltest 3.06, the HKY+I+G model was identified as the best-fitting distance estimator, with a γ-shape correction of 0.608. All phylogenetic analyses resulted in identical tree topologies, identifying five main clades (clade SG-A, clade SG-B, clade QL-A, clade QL-B, and clade SNJ) (Fig. 2). The highest pairwise distance was between Clade SG-A and SNJ ($F_{ST} = 0.951$, $P < 0.001$), and the lowest pairwise distance was between SG-B and QL-A ($F_{ST} = 0.675$, $P < 0.001$), followed by SG-B and QL-B ($F_{ST} = 0.712$, $P < 0.001$) (Table III). Pairwise distances demonstrated that the difference within the SG and QL populations was equal to or even greater than that between populations (Tables III and IV).

Historical Geographic Dispersal Route

Historical gene flow using both ML and Bayesian references showed identical gene flow direction between populations (Fig. 3). The most remarkable gene flow was found from the SG to the QL population and from the QL to the SNJ population. We detected gene flow signatures from the QL to SG population. However, no significant gene flow was found between the SNJ and SG populations. These results suggest that historical geographic dispersal was from the SG to QL and SNJ populations.

Historical Demography

Among the five clades, SG-A was the oldest and diverged from other clades 1.17 ± 0.70 million years ago, which corresponds to the time of the most recent common ancestor. For all samples, the BSP suggested a sharp decrease in population size about 0.025 million years ago (Fig. 4), which was supported by the non-optimistic population growth parameter g (14.44 ± 20.64) test for all the populations (Table III). For all samples Tajima's D [Tajima, 1989] and Fu's Fs [Fu, 1997] were -0.72 ($P = 0.34$) and 0.37 ($P = 0.55$), respectively (Table III), with no population expansion indicated. Consistent with these results, the mismatched distribution in all populations of golden snub-nosed monkeys (Fig. 5) and for each population showed an atypical distribution shape.

DISCUSSION

Population Structure and Historical Geographic Dispersal Route

We observed a distinct phylogeographical structure among the three populations of snub-nosed monkey. The lack of shared haplotypes within the five clades indicated a long period of isolation among extant golden monkey populations. However, the most remarkable finding was that the QL and SNJ populations originated from the SG population and that at least two major historical migration events have taken place.

All analyses revealed genetic signals of these historical dispersal events. First, both the SG and QL populations were found to have two deeply divergent haplotype clades. Yet there is no record of geographic barriers such as large rivers that might prevent gene flow between different populations, which suggests historic population subdivision [Li et al., 2007]. Researchers have previously reported on the genetic structure within SG [Li et al., 2007; Pan et al., 2009]. This was confirmed by our data, and we observed that QL haplotypes also showed subdivision. Second, nucleotide diversity in the SG population was higher than any other population, with most haplotypes belonging to SG followed by QL. This supports SG as the ancestral population as previous research indicates that older haplotypes are more broadly distributed geographically and older populations have higher genetic diversity [Crandall & Templeton, 1993; Templeton et al., 1992]. Furthermore, if SG was recolonized by QL and SNJ populations, it should have lower genetic diversity, as shown in postglacially colonized populations [Hewitt, 1996, 2000]. Climatic oscillations have affected the distribution and divergence of other taxa, leaving a signature of lower genetic diversity in postglacial populations [Arctander et al., 2000; Bernatchez & Wilson, 1998].

Most importantly, estimates of population size and bidirectional migration rates between populations are key factors for identifying the ancestral population of a species [Manier & Arnold, 2005]. We detected that historical gene flow mainly came from the SG to the QL population and the QL to the SNJ population than from the QL to the SG population (Fig. 3). Migration between SNJ and SG populations was not significant (0.000 038 9 and 0.000 008 77, respectively, see Additional file 1), which is consistent with the geographic distance between populations (Fig. 1). Since there was little gene flow detected from the SNJ to the SG and QL populations, the direction of historical gene flow was most likely from the SG to the QL and then to the SNJ. Moreover, estimates of effective population size supported SG as the oldest population since effective population size was largest in SG (Fig. 3).

TABLE IV. Genetic Distance Represented by Pairwise F_{ST} (lower diagonal) and $F_{ST}/(1F_{ST})$ (upper diagonal) ($^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$)

	SG-A	SG-B	QL-A	QL-B	SNJ
SG-A		7.576	12.644	18.139	19.563
SG-B	0.884***		2.076	2.467	2.594
QL-A	0.927***	0.675***		5.784	5.362
QL-B	0.948***	0.712***	0.853***		8.653
SNJ	0.951***	0.722***	0.843***	0.896***	

SG, Sichuan and Gansu; QL, Qinling; SNJ, Shennongjia.

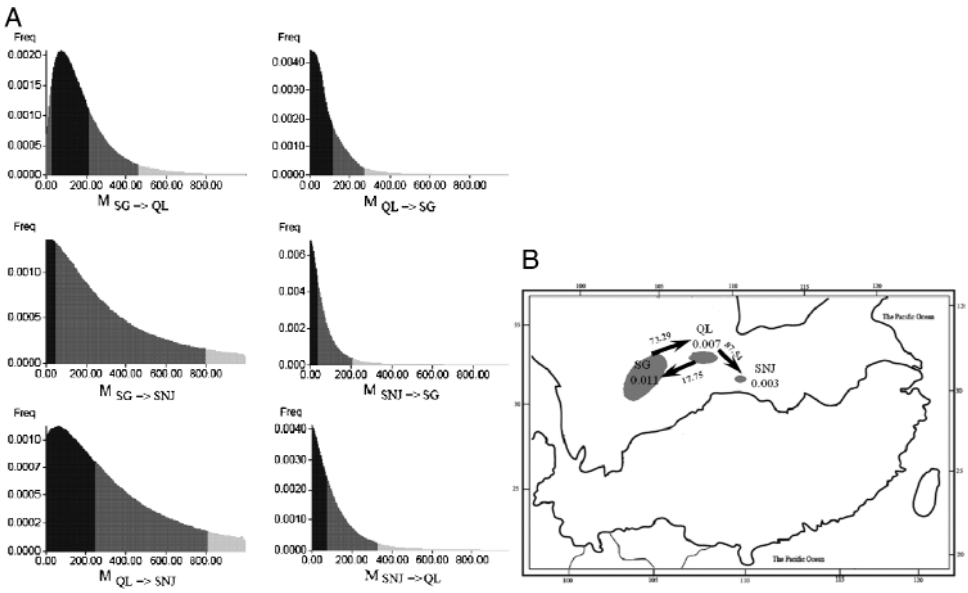

Fig. 3. Gene flow estimations among populations: (**A**) posterior distribution of historical gene flow over all loci using Bayesian inference; (**B**) historical migration route inferred from ML inference: arrows represent the direction of gene flow; the results from ten independent runs were averaged.

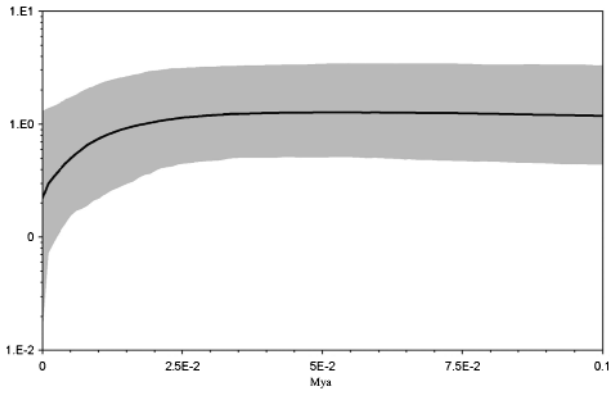

Fig. 4. Bayesian skyline plot of past demographic trends in mitochondrial lineages: *x*-axis: time in 10^6 ya; *y*-axis: estimated population size (units = Neτ, the product of effective population size and generation length in years, log-transformed). The mean estimate and the 95% HPD limits are indicated.

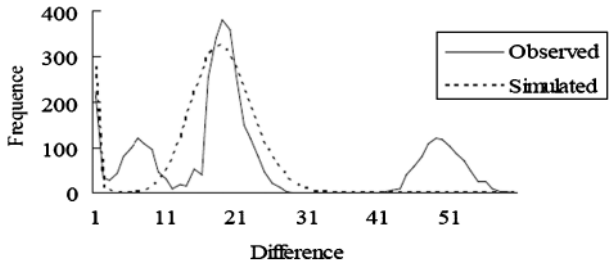

Fig. 5. Observed (solid pillar) and expected (discontinuous line) spatial mismatch distributions showing the frequencies of pairwise differences within *Rhinopithecus roxellana.*

We concluded that QL and SNJ originated from the SG population and that at least two major migration events occurred previously. Initial population differentiation took place about 1.17 ± 0.70 million years ago, after the first SG population migration arrived at the QL area and became the predecessor of clade QL-A. Our results suggest that the two groups did not remain isolated from each other. For a period of time after they arrived in the QL Mountains, gene flow occurred between the two populations, most likely during periods of glacial retreat. This probably caused the differentiation within the SG population (clade SG-A and clade SG-B): the initial unmoved ones formed clade SG-A while clade SG-B was the ones that recolonized from QL population. The last differentiation occurred approximately 0.53 ± 0.30 million years ago after the golden snub-nosed monkeys moved from the QL area to the southeastern SNJ area [Jablonski & Gu, 1991] and recolonization from SNJ caused the divergence within the QL population (clade QL-A and clade QL-B).

Reasons for Differentiations Within Populations and Population Decline

During the late Tertiary, *R. roxellana* occupied tropical and subtropical broadleaf deciduous and evergreen forest habitats, which were common vegetation types in Asia during that time [Jablonski, 1998a,b; Jablonski & Pan, 1988; Pan & Jablonski, 1987; Quan & Xie, 2002]. During the Quaternary, however, glaciers advanced extensively, destroying vegetation types and impacting fauna and flora [Jablonski, 1998b]. During the Last Glacial Maximum (0.0265–0.019 Ma) [Firestone et al., 2007; Owen et al., 2002], many mega-fauna populations diminished in size or became extinct under the influence of significant climate change [Barnosky et al., 2004; Comes & Kadereit, 1998; Peter et al., 2009]. This extinction was pronounced in North America where at least 35 mammal genera disappeared [Grayson, 2007], including mammoths, mastodons, ground sloths, and endemic horse and camel species [Barnosky et al., 2004; Comes & Kadereit, 1998]. Glaciations were less severe in East China than in Europe and North America [Ju et al., 2007], however, and primates were somewhat less affected due to their ability to survive harsh environments and produce offspring quickly [Jablonski, 1998a]. Although *R. roxellana* did not become extinct at this time, populations did begin to decline in size about 0.025 Ma (Fig. 4), with previous dental morphology research suggesting that a population bottleneck occurred during this period [Jablonski 1992]. Conversely, however, our study using mitochondria DNA does not detect a bottleneck. Another reason behind the disagreement is likely due to the sampling strategy. In Jablonski's study, just 24 samples were examined and all of them came from Sichuan province while the samples in our study covered three major distribution areas.

Quaternary climate changes in the distribution areas of *R. roxellana* were influenced not only by global climate change but also by climate change under the influence of the Tibetan Plateau [Jablonski, 1998b]. The current climate of China emerged gradually during the Cenozoic followed by rapid development during the Quaternary [Liu, 1996]. The uplift of the Tibetan Plateau in southwestern China dramatically influenced the evolution of East Asian biota and climate in the late Tertiary and Quaternary [Whyte, 1984]. It affected summer paleomonsoon circulation as well as strengthened the winter paleomonsoon [An et al., 2001; Clift et al., 2008]. Though much of the present elevation of the Tibetan plateau was attained about 8–12 million years ago [Harrison et al., 1992], its influence on restricting the southwest paleomonsoon has been intensive since the Late Pleistocene [Kusky et al., 2011; Zhang, 1981]. The height of the Tibetan Plateau stopped warm and moist air currents from the Indian Ocean entering many areas of China, leaving many regions of China colder in late Pleistocene than at other times during the Quaternary [Ju et al., 2007; Kusky et al., 2011; Molhar et al., 1993; Zheng et al., 1998]. Grasslands replaced forests

as the climate gradually became drier, colder, and windier, leading to the development of massive glaciers and vast deserts [Wu et al., 2001]. These changes in vegetation types increased the fragmentation of *R. roxellana* habitat, preventing gene flow between habitat areas and leading to the current genetic structures of the extant golden snub-nosed monkey populations.

CONCLUSIONS

Genetic consequences of environmental changes have been studied extensively in many areas, such as the genetic consequences of Quaternary climatic oscillations in the Alps of Beringia [reviewed in Hewitt, 2004] and the influence of the Tibetan Plateau uplift in Southeast China [Qu & Lei, 2009; Qu et al., 2009]. The pattern of genetic variation in a species can clarify its evolutionary history and the influencing forces involved. Our study showed how climatic and environmental changes affected the distribution and genomic divergence of the golden snub-nosed monkeys and clarified the relationships among the three extant populations, knowledge of which will aid appropriate conservation management strategies. Five mtDNA haplotype groups (SG-A, SG-B, QL-A, QL-B, and SNJ) were found. Differences within SG and QL populations merit particular attention and should be further studied to assist conservation and prevent a significant decrease in genetic diversity. The 30 haplotypes (0.94 ± 0.01) found indicated a moderate level of genetic diversity; however, the level of genetic diversity was no higher in *R. Roxellana*than in *R. bieti*although the population size of *R. roxellana* is much greater than *R. bieti* (about 20,000 individuals compared to about 1,500 individuals) [Li et al., 2007; Liu et al., 2007; Quan & Xie, 2002]. It is important, however, that *R. roxellana* research and conservation focus not just on population size but on the preservation of genetic diversity. As the ancestral population of *R. roxellana*, SG should be monitored closely with priority placed on maintaining its genetic diversity. Consistent with earlier studies [Li et al., 2001, 2003, 2007; Pan et al., 2009], we found that genetic diversity in the SNJ population was still very low, which suggests the need for special attention to ensure the conservation of monkeys inhabiting the SNJ National Nature Reserve.

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