Journal of Biogeography (J. Biogeogr.) (2010) 37, 657–668

ORIGINAL ARTICLE

Allopatric divergence and phylogeographic structure of the plateau zokor (*Eospalax baileyi*), a fossorial rodent endemic to the Qinghai–Tibetan Plateau

Li-Zhou Tang[1,2,3], Liu-Yang Wang[1,3], Zhen-Yuan Cai[1], Tong-Zuo Zhang[1], Hai-Xin Ci[1,3], Gong-Hua Lin[1,3], Jian-Ping Su[1]* and Jian-Quan Liu[4]*

[1]*Key Laboratory of Evolution and Adaptation of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, Qinghai 810001, China,* [2]*Yunnan-Guizhou Plateau Institute of Biodiversity, Qujing Normal University, Qujing, Yunnan 655011, China,* [3]*Graduate University of the Chinese Academy of Sciences, Beijing 100039, China,* [4]*Key Laboratory of Arid and Grassland Ecology, Lanzhou University, Lanzhou, Gansu 730000, China*

*Correspondence: Jian-Quan Liu, Key Laboratory of Arid and Grassland Ecology, Lanzhou University, Lanzhou, Gansu 730000, China.
E-mail: liujq@nwipb.ac.cn

Jian-Ping Su, Key Laboratory of the Qinghai–Tibetan Plateau Ecosystem and Biological Evolution and Adaptation, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, Qinghai 810001, China.
E-mail: jpsu@nwipb.ac.cn

ABSTRACT

Aim Most species of temperate regions are believed to have shifted to lower latitudes or elevations during the glacial periods of the Quaternary. In this study we test whether this phylogeographic assumption is also true for the plateau zokor (*Eospalax baileyi*), a fossorial rodent endemic to the climate-sensitive Qinghai–Tibetan Plateau (QTP), which ranges in elevation from 2600 to 4600 m.

Location The QTP of western China.

Methods Phylogeographic analyses were conducted based on the mitochondrial cytochrome *b* gene sequences of 193 individuals from 20 populations over the entire range of the species.

Results A total of 54 haplotypes identified in the present study clustered into four geographically correlated clades located in the interior of the QTP (clade A) and at the plateau edge (B, C and D). Molecular calibrations suggest that the interior plateau (A) and plateau-edge (B–D) clades diverged at 1.2 Ma and that the three plateau-edge clades diverged between 0.85 and 0.80 Ma. These estimates are concordant with diastrophism and glaciation events in the QTP. Coalescent tests rejected both the hypothesis that all current populations originated from a single refugium at a low elevation during the Last Glacial Maximum (LGM) and the hypothesis that the two lineages diverged during the LGM. The tests instead supported the hypothesis that there were four refugia during the LGM, and that the four clades diverged prior to the late Pleistocene.

Main conclusions Our results suggest that Quaternary diastrophisms and glaciations repeatedly promoted allopatric divergence of the plateau zokor into geographical clades, and that these regional clades subsequently persisted at high elevations, rather than migrating to the low-elevation plateau edge during subsequent glacial ages.

Keywords
Cytochrome *b*, *Eospalax baileyi*, genetic divergence, phylogeography, plateau zokor, rodents, Qinghai–Tibetan Plateau, western China.

INTRODUCTION

Quaternary climatic oscillations have played an important role in shaping the current distribution of biodiversity in the Northern Hemisphere (Hewitt, 2000). In both Europe and North America, phylogeographical studies of contemporary genetic samples have provided evidence of glacial retreats and interglacial/post-glacial recolonizations by most temperate organisms (Avise, 2000). Both animal and plant species ranges have been shown to have shifted repeatedly in response to climatic oscillations, tracking favourable climate (Hewitt, 2000; Rowe *et al.*, 2004; Steele & Storfer, 2006).

© 2009 Blackwell Publishing Ltd

doi:10.1111/j.1365-2699.2009.02232.x

Journal of Biogeography

Shi *et al.* (1998) suggested that global climatic changes during the Quaternary affected environments on the Qinghai–Tibetan Plateau (QTP, an area of 2.5×10^6 km^2 with a mean elevation > 4000 m a.s.l.) more dramatically than in any other place on Earth, and that regional species were highly responsive to these climatic shifts. Trinkler (1930), Gupta *et al.* (1992) and Kuhle (1988) suggested that the entire plateau was covered by a huge ice sheet during the glacial ages, forcing most species to retreat to refugia at lower elevations on the edge of the plateau during glacial maxima, from which they recolonized the interior during interglacials (Grubov, 1963). Several recent phylogeographic studies support this hypothesis, suggesting that most current populations recolonized the plateau interior from refugia that existed on the plateau edge during the Last Glacial Maximum (LGM, *c.* 20 ka) and/or previous glacial periods (Qu *et al.*, 2005; Zhang *et al.*, 2005; Yang *et al.*, 2006a; Meng *et al.*, 2007). Shi *et al.* (1998) instead maintained that ice sheets were disjunctly distributed within high-elevation regions even during the glacial stages, such that some cold-tolerant animals could have persisted in ice-free areas of the central plateau region during glacial maxima.

Here we report the phylogeographic structure of a QTP endemic rodent, the fossorial plateau zokor, *Eospalax baileyi* (Thomas, 1911) (Rodentia, Spalacidae), which occurs in alpine meadows and prairies of the QTP (Zhang *et al.*, 1997; Wang, 2003; Smith & Yan, 2008) (Fig. 1). Until recently, *Eospalax* was included as a subgenus within *Myospalax* (e.g. Fan & Shi, 1982; Song, 1986; Lawrence, 1991). Zheng (1994) recognized two genera (*Myospalax* and *Eospalax*), subsequently supported by molecular studies that demonstrated reciprocal monophyly and distinct genetic divergence (Norris *et al.*, 2004; Zhou & Zhou, 2008). Similarly, *Eospalax* (*Myospalax*) *baileyi* has been considered as a subspecies of *Eospalax fontanierii* or *Eospalax rufescens* (see Musser & Carleton, 2005) or as a distinct species (Fan & Shi, 1982). Herein, we consider *E. baileyi* as a distinct species based on the unanimous support of recent molecular studies (Zhou *et al.*, 2004; Zhou & Zhou, 2008).

Eospalax baileyi is the only species of the genus that occurs in the high-elevation interior of the QTP, at elevations ranging from 2600 to 4600 m, although it is parapatric with other species (e.g. *Eospalax cansus*) on the north-eastern edges of the plateau (Fan & Shi, 1982). The plateau zokor feeds mainly on the roots and shoots of annual and perennial grasses, forbs and shrubs (Wang *et al.*, 2000; Zhang, 2000), and is highly adapted to a strictly subterranean lifestyle (Zhang & Liu, 2003): the species spends 85–90% of its lifetime in underground nests (1.5–2 m deep) and its feeding activities take place mainly at a depth of 3–20 cm from the soil surface (Zhou & Dou, 1990; Zhang, 1999). Each mature zokor occupies a private burrowing system and enters the territories of others only during mating periods (Zhou & Dou, 1990). The average length of tunnels is short (about 100 m) and the home range is restricted (1500 m^2 for males, 500 m^2 for females; Zhou & Dou, 1990). Because populations of this species occur in the interior of the QTP (Fan & Shi, 1982), the species is an ideal model animal for use in testing alternative hypotheses regarding the persistence of animals in the central plateau at high elevations. In the present study we sequenced the mitochondrial cytochrome *b* (cyt *b*) gene to determine the phylogeographical structure of this species. Although mtDNA markers are inferior to nuclear markers for clarifying gene flow among populations, they do have a number of advantages when tracing range shifts of animal species, including high rates of substitution, maternal inheritance, and relative ease of amplification (Wilson *et al.*, 1985). We addressed the following questions. (1) Did this species retreat to the plateau edge and then recolonize the interior of the plateau, like other species, or did the current populations in the interior of the plateau persist *in situ*

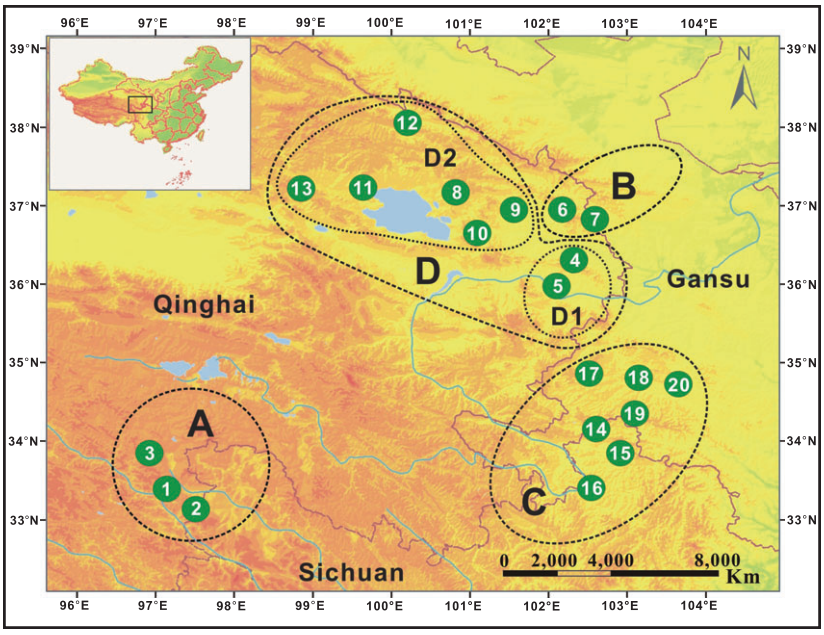

Figure 1 Locations of the 20 sampled populations of the plateau zokor (*Eospalax baileyi*) on the Qinghai–Tibetan Plateau (QTP) of western China (inset). Details of the populations are given in Table 1, and the phylogenetic clades and subclades are given in Fig. 2.

© 2009 Blackwell Publishing Ltd

throughout the Quaternary climatic oscillation? (2) Are the intraspecific divergences related to geographical distribution and did past glaciations promote the allopatric divergence of this species?

MATERIALS AND METHODS

Population samples

Samples for phylogeographic analyses totalled 193 individuals from 20 populations of *E. baileyi* from throughout its distribution, namely 187 individuals from 17 populations collected and sequenced for this study and six individuals from three populations (populations 7, 8 and 17, Table 1, Fig. 1) reported by Zhou & Zhou (2008). Three populations (1–3) were from the interior of the QTP (south Qinghai; elevations > 4000 m), eight populations (4–6, 9–13) were from north-east Qinghai (2600–3800 m), and six populations (14–16, 18–20) were from south Gansu and west Sichuan (3000–3500 m). Populations 4–20 are distributed around the north-eastern edge of the QTP. Except for those reported by Zhou & Zhou (2008), zokors were captured from points at least 50 m apart, and the sampled tissues were immediately preserved in 95% ethanol and transferred to the Northwest Plateau Institute of Biology (HNWP), Chinese Academy of Sciences, for storage at −20 °C.

DNA extraction, polymerase chain reaction amplification and sequencing

Total DNA was isolated from ethanol-fixed tissue after proteinase K digestion, followed by standard phenol–chloroform extraction and ethanol precipitation. A partial sequence of the mitochondrial cyt *b* gene was amplified by means of a polymerase chain reaction (PCR) using the primer pairs L14724 (5′-CGAAGCTTGATATGAAAAACCATCGTTG-3′) and H15917 (5′-CGGAATTCCATTTTTGGTTTACAAG-3′) (Zhou *et al.*, 2004). PCR amplifications were performed in total reaction volumes of 30 μL, containing 10 μM Tris–HCl (pH 8.0), 1.5 μM $MgCl_2$, 50 μM KCl, 150 μM of each dNTP, 0.3 μM of each primer (synthesized by Sangon, Shanghai, China), 0.4 μL (about 40 ng) of template DNA, and 1μ *Taq* DNA polymerase (Sangon). The reaction mixtures were denatured at 95 °C for 5 min and subjected to 31 cycles of 40 s at 95 °C, 1 min at 53 °C, 1.5 min at 72 °C, and a final extension step of 7 min at 72 °C. PCR products were purified using a CASpure PCR Purification Kit, following the company's recommended protocol (Casarray, Shanghai, China). Sequencing reactions were carried out in a Biometra thermocycler using a DYEnamic Dye Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech Inc., Sunnyvale, CA USA), following the manufacturer's protocol. Purified DNA

Table 1 Geographic origins, sample sizes and haplotypes of the 20 sampled populations of the plateau zokor (*Eospalax baileyi*) from the Qinghai–Tibetan Plateau (QTP) of western China.

Population	Location	*n*	°E longitude	°N latitude	Elev. (m)	Haplotypes (number of individuals)
1	Chenduo, QH	11	97°14.123′	33°21.213′	4390	H1(11)
2	Chenduo, QH	11	97°28.363′	33°12.092′	4450	H2(5), H3(5), H4(1)
3	Chenduo, QH	14	96°56.623′	33°46.178′	4550	H5(14)
4	Hualong, QH	16	102°18.282′	36°11.418′	3230	H17(3), H18(1), H19(2), H20(3), H21(1), H22(1), H23(1), H24(2), H25(1), H26(1)
5	Hualong, QH	8	102°11.850′	36°02.633′	2603	H19(4), H24(4)
6	Huzhu, QH	11	102°07.173′	36°54.070′	3040	H30(2), H31(3), H32(1), H33(2), H34(1), H35(1), H36(1)
7*	Ledu, QH	1	102°24.131′	36°28.798′		H53(1)
8*	Xihai, QH	2	100°46.779′	36°52.942′		H49(1), H50(1)
9	Datong, QH	11	101°40.677′	36°56.740′	3020	H6(4), H7(2), H8(1), H9(2), H10(1), H11(1)
10	Huangyuan, QH	10	101°06.848′	36°38.113′	3110	H11(3), H27(3), H28(1), H29(3)
11	Gangcha, QH	14	99°42.690′	37°10.157′	3230	H12(5), H13(2), H14(3), H15(3), H16(1)
12	Qilian, QH	9	100°13.023′	38°04.192′	3450	H30(8), H37(1)
13	Tianjun, QH	14	98°52.258′	37°10.778′	3840	H43(6), H44(4), H45(4)
14	Ruoergai, SC	8	102°39.438′	34°07.247′	3270	H38(8)
15	Ruoergai, SC	11	102°53.400′	33°54.892′	3450	H38(2), H39(7), H40(1), H41(1)
16	Ruoergai, SC	12	102°31.998′	33°24.615′	3490	H42(12)
17*	Sangke, GS	3	102°06.663′	35°05.914′		H51(1), H52(1), H54(1)
18	Zhuoni, GS	10	103°14.828′	34°44.977′	3160	H46(8), H47(2)
19	Zhuoni, GS	11	103°03.140′	34°22.042′	3270	H46(7), H47(3), H48(1)
20	Zhuoni, GS	6	103°33.117′	34°44.357′	3020	H46(6)

QH, Qinghai Province; SC, Sichuan Province; GS, Gansu Province.

Note: H1–H48 correspond to the accession numbers FJ358643–FJ358690.

*Sequences of populations reported by Zhou & Zhou (2008); accession numbers: H49, AF326256; H50, AF326255; H51, AF387081; H52, AF387083; H53, AF387082; H54, AF387084.

© 2009 Blackwell Publishing Ltd

fragments were sequenced directly using a MEGABACE 500 DNA Analysis System (Sangon). To ensure accuracy, strands were sequenced in both directions for each individual using the same primer pairs as for PCR amplification.

Phylogenetic analyses

All newly obtained sequences for *E. baileyi* were submitted to GenBank (accession numbers FJ358643–FJ358690, Table 1). We downloaded from GenBank cyt *b* sequences of the four other *Eospalax* species and two *Myospalax* species for the phylogenetic analyses (Zhou & Zhou, 2008; accession numbers: *Rhizomys sinensis*, AF326274; *Myospalax aspalax*, AF326272; *Myospalax psilurus* 1, AF326270; *Myospalax psilurus* 2, AF326271; *Eospalax fontanierii* 1, AF326264; *Eospalax fontanierii* 2, AF326265; *Eospalax fontanierii* 3, AF326266; *Eospalax cansus* 1, AF326261; *Eospalax cansus* 2, AF326262; *Eospalax cansus* 3, AF326260; *Eospalax cansus* 4, AF326263; *Eospalax rufescens*, AF326269; *Eospalax rothschildi* 1, AF326267; *Eospalax rothschildi* 2, AF326268). Following Zhou & Zhou (2008), we used the closely related genus *Rhizomys* (represented by *Rhizomys sinensis*) as an outgroup. All sequences were aligned using CLUSTALX (Thompson *et al.*, 1997) with the default settings, and refined manually. The number of variable sites and the number of parsimony-informative sites were determined using DNASP (version 4.0; Rozas *et al.*, 2003). Phylogenetic relationships were assessed by maximum parsimony (MP) and maximum likelihood (ML) methods using PAUP* 4.0b10 (Swofford, 2002). MP trees were constructed using a heuristic search, 100 random additions of sequences, equally weighted characters and nucleotide transformations, with the tree bisection–reconnection (TBR) branch swapping, MULTREES and COLLAPSE options switched on (Swofford, 2002). The ML analysis was performed using the HKY+I+G model, as determined using the Akaike information criterion implemented in MODELTEST 3.06 (Posada & Crandall, 1998). The robustness of MP and ML trees was tested by 1000 bootstrap replicates. We used TCS 1.21 to construct a parsimony network for all the recovered haplotypes with a statistical parsimony of 95% probability (Templeton *et al.*, 1992; Clement *et al.*, 2000).

Molecular calibration and divergence estimation

A molecular clock hypothesis was tested with a likelihood-ratio (LR) test (Huelsenbeck & Rannala, 1997) using PAUP*, comparing the log likelihood of the ML trees with and without assuming a molecular clock. The hypothesis of rate constancy was evaluated with the LR test by calculating the log likelihood score of the chosen model with the molecular clock enforced and comparing it with the log likelihood score without the molecular clock enforced. We used Bayesian analysis to estimate the divergence times of each clade recovered within *E. baileyi* by the program BEAST (Drummond *et al.*, 2002; Drummond & Rambaut, 2003). Two population models (constant size and exponential growth) were tested, and the final rate estimates from the two models were compared. The mean substitution rate of the model that yielded the highest posterior probability was chosen. Following a burn-in of 500,000 cycles, all parameters were sampled once every 100 generations from 5,000,000 Markov chain Monte Carlo (MCMC) steps. Convergence of the chains to the stationary distribution was checked by visual inspection of plotted posterior estimates using the program TRACER (Rambaut & Drummond, 2003), and the effective sample size for each parameter sampled from the MCMC analysis was almost always found to exceed 100, usually by an order of magnitude. The divergence between *Eospalax* and *Myospalax* was assumed to start before 4 Ma, from which time distinctly differentiated fossils of the two genera are known (Zheng, 1994). We also used a penalized likelihood approach to calculate the clade divergences with the aid of R8S (Sanderson, 2002). To obtain standard deviations for estimated divergence times, the data set was bootstrapped 100 times using the SEQBOOT module from PHYLIP (Felsenstein, 1989), and branch lengths were re-estimated for each node under the constrained initial topology in PAUP*. The dating analyses were then repeated for each tree, and node statistics were summarized using the profile command of R8S (Sanderson, 2002).

Population genetic analysis

We use the Mantel test (Mantel, 1967), implemented by IBD 1.52 (Bohonak, 2002) with 10,000 matrix randomizations, to detect the significance of correlations between genetic distance and geographical distance. Hierarchical analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) was used to characterize population structure and genetic variation using the program ARLEQUIN 3.0 (Excoffier *et al.*, 2005), with permutation tests of significance being used to test genetic variance by comparison to null distributions with 10,000 random permutations. For the hierarchical analysis, populations were grouped according to the mtDNA lineages recovered in the phylogenetic analyses as well as to geographic proximity. Nucleotide diversity, haplotype diversity, and pairwise measures of F_{ST}-values for all populations and each lineage were also calculated in this program.

To test the signature of demographic expansion (Rogers, 1995), mismatch distributions and Fu's F_S-tests (Fu, 1997) (with 10,000 permutations) were conducted for each lineage/clade and all samples using ARLEQUIN 3.0 (Excoffier *et al.*, 2005). The sum-of-squared deviations (SSD) between observed and expected mismatch were used to compute the proportion of simulations producing a larger SSD than the observed SSD. The raggedness index (Harpending, 1994) and its significance were also calculated to quantify the smoothness of the observed mismatch distribution.

Coalescent analyses and simulations

We used the gene-tree and population-tree methods with coalescent simulations to test the fit of the observed gene tree

© 2009 Blackwell Publishing Ltd

to different phylogeographic hypotheses (Knowles, 2001; Knowles & Maddison, 2002). The tests were performed using the program MESQUITE 2.5 (Maddison & Maddison, 2008). We simulated DNA sequences with the same parameters as the empirical data for each genealogy on each of the replicate gene trees. The model of observed sequence evolution was selected using MODELTEST 3.06 (Posada & Crandall, 1998) but using only samples from *E. baileyi* (excluding the outgroups). For each simulation, 100 coalescent genealogies constrained within different hypotheses of population history were simulated using MESQUITE 2.5. We used PAUP* 4.0b10 (Swofford, 2002) to reconstruct trees from the simulated gene matrices, and the *S*-values for these trees (the minimum number of sorting events required to explain the population subdivision; Slatkin & Maddison, 1989) were recorded. For all coalescent simulations, absolute time (years) was converted to coalescent time (generations) assuming a generation time of 1 year for *E. baileyi* (Wei *et al.*, 1998). We tested whether the observed genealogies were consistent with the given models by comparing the *S*-value of the empirical ML genealogy with those of the simulated genealogies.

For all simulations, we estimated the effective population size (N_e) using the θ-values calculated by the ML and coalescent-theory approach in the program MIGRATE 2.4.4 (Beerli, 2002). This approach assumes the Wright–Fisher model, and in order to find the best-fit model with which to obtain convergent and consistent results, we ran the program for several replicates and used different parameter combinations. The results of analyses were accepted if the 95% confidence intervals (CIs) of one run included the estimated ML value of the other runs and results of multiple runs were similar. Finally, the following parameters were used for our analyses: 10 short chains of 1,500,000 steps followed by three long chains of 15,000,000 steps; chains were sampled every 100 steps following a burn-in of 100,000 steps, and default settings were used for the initial estimate of the θ-value. We converted the θ-value to N_e using the formula $\theta = 2N_e\mu$, with a substitution rate of $\mu = 4.12\%$ Myr^{-1}, estimated by molecular calibrations based on the fossil records as described previously. When running the coalescent simulations, we set the overall N_e to equal the empirically estimated values, and constrained the N_e of the putative refugial population to a size proportional to the overall empirically estimated N_e.

RESULTS

Sequence variation and phylogenetic analyses

A total of 54 haplotypes were identified within the 193 individuals (Table 1, Fig. 2). Of the 709 sites aligned in all individuals, 146 were observed to vary. The base composition was deficient in G (11.41%), whereas similar frequencies were observed for the other three nucleotides (A, 30.37%; T, 30.36% and C, 27.86%). All variation resulted from transitions and transversions (ti/tv ratio: 6.500); no deletions or insertions were observed.

Maximum likelihood and MP phylogenetic analyses produced highly consistent tree topologies, differing only in bootstrap support of the major nodes (Fig. 2). All haplotypes of *E. baileyi* comprised a monophyletic group apart from four other *Eospalax* species. Within *E. baileyi*, four distinct clades were recovered. The earliest diverged clade A (Lineage I) was found in the interior of the QTP, and the other three clades (Lineage II: B, C and D) were distributed on the edge of the plateau (Figs 1 & 2). Two subclades (D1 and D2) were recovered within clade D. Clades B–D and the two subclades were allopatrically distributed around the north-eastern margin of the QTP (Fig. 1). As shown in the minimal spanning network (Fig. 2), the two major lineages identified by the phylogenetic analyses were separated by five unidentified (or extinct) haplotypes, whereas the three clades within lineage II were separated from one another by two or three unidentified (or extinct) haplotypes.

Molecular calibration and divergence estimation

The molecular clock could not be rejected because constrained and unconstrained analyses did not differ significantly (HKY+I+G, 2 ln LR = 81.409, d.f. = 70, $P = 0.166$). Therefore, all parameters for divergence estimates were set to conform to the molecular clock. Based on the assumption that *Eospalax* and *Myospalax* diverged about 4.0 Ma, the time since divergence between two lineages was estimated by BEAST and R8S analyses to have occurred from 1.184 (CI: 0.865–1.556) to 1.242 (± 0.13) Ma (Table 2). Estimates of the divergence of the three clades (B–D) within lineage II varied from 0.792 (± 0.08) to 0.869 (0.610–1.124) Ma. The divergence of two subclades (D1 versus D2) within clade D was estimated to range from 0.580 (± 0.06) to 0.596 (0.401–0.791) Ma (Table 2).

Genetic structure and population dynamics

The Mantel test indicated a highly significant correlation ($r = 0.55$; $P < 0.001$) between genetic differentiation of populations and geographic distances across the whole range of the species. The hierarchical AMOVAs revealed that most of the variation was among regions/populations (Table 3), indicating restricted maternal gene flow and substantial geographical differentiation. The haplotype diversity of each clade was relatively high ($h = 0.7365$–0.9603), whereas the nucleotide diversity ranged from 0.0038 to 0.0229 (Table 4). Mismatch distributions for all four clades (A–D) and all samples were multimodal. One or both of these two tests of SSD and raggedness index values as well as Fu's F_S-tests rejected the sudden expansion hypotheses for each clade and all samples (Table 4).

Coalescent analyses and simulations

We conducted coalescent simulations to test three hypotheses concerning the glacial refugia of *E. baileyi*: (1) all current populations of the species were derived from a single refugium

© 2009 Blackwell Publishing Ltd

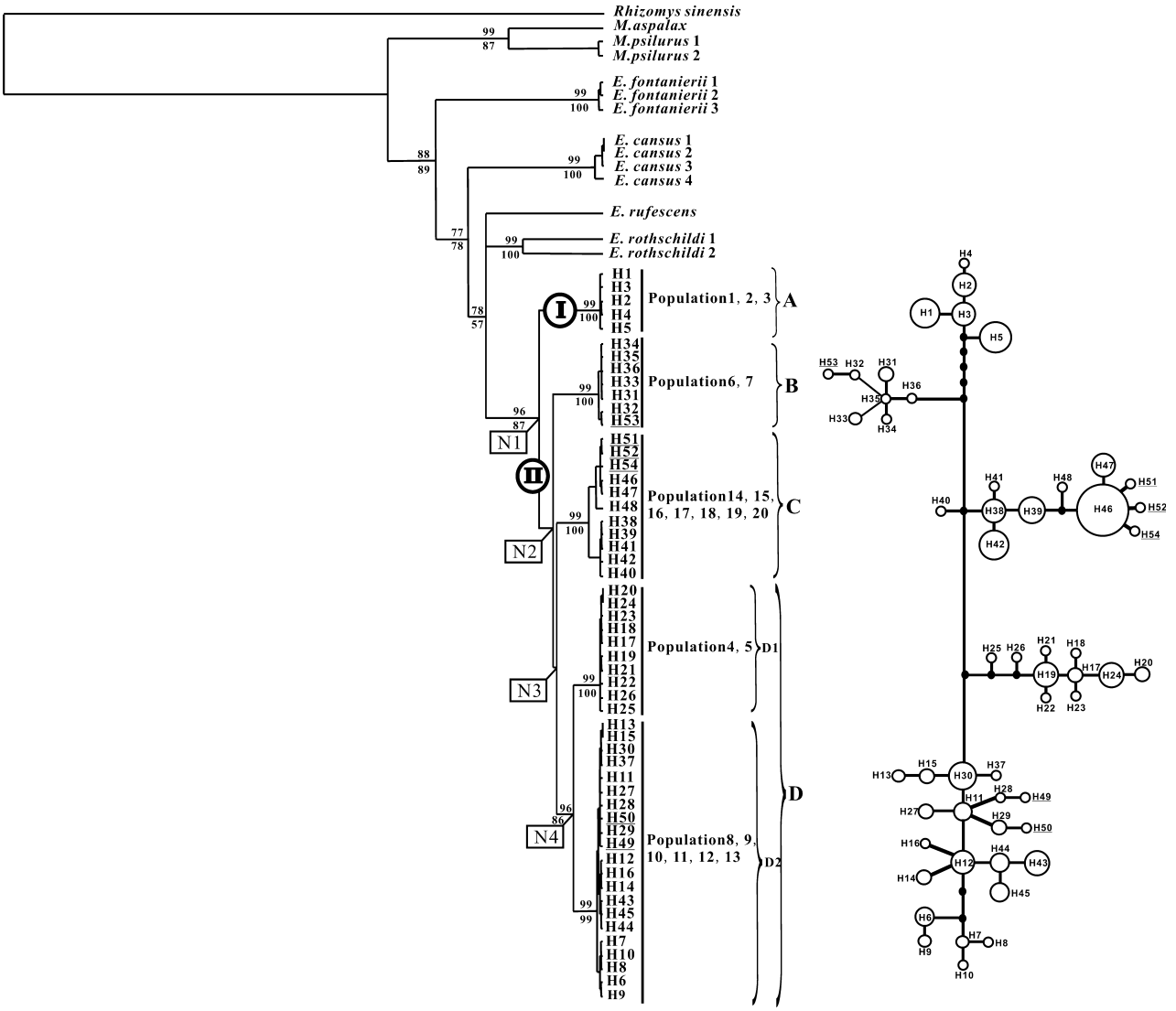

Figure 2 Maximum likelihood (ML under the molecular clock constraint) tree (left) and network (right) of the 54 recovered haplotypes of the plateau zokor (*Eospalax baileyi*). Values above and below the branches represent bootstrap values calculated, respectively, by ML and maximum parsimony analyses. The relative sizes of the circles in the network are proportional to haplotype frequencies, and the black dots represent missing haplotypes (not sampled or extinct). The two major lineages (from the interior of the Qinghai–Tibetan Plateau and the plateau edge) are indicated by I and II in the ellipses. N1–N4 indicate the internal nodes for which divergence times were estimated by BEAST and R8S (Table 2).

Table 2 The estimated divergences (Ma) of each node (Fig. 2) of the plateau zokor (*Eospalax baileyi*) based on BEAST and R8S approaches under fossil calibration.

	Fossil calibration BEAST		
Node	Constant size (CI)	Exponential growth (CI)	R8S (± SD)
N1	1.209 (0.874–1.567)	1.184 (0.865–1.556)	1.242 (± 0.13)
N2	0.869 (0.610–1.124)	0.845 (0.604–1.085)	0.852 (± 0.10)
N3	0.818 (0.550–1.068)	0.809 (0.556–1.054)	0.792 (± 0.08)
N4	0.596 (0.401–0.791)	0.585 (0.410–0.800)	0.580 (± 0.06)

CI, confidence interval of 95%; SD, standard deviation.

Table 3 Results of hierarchical analyses of molecular variance (AMOVAs) of genetic variations of the plateau zokor (*Eospalax baileyi*).

	Variance component (%)		
Clade	Among populations	Within populations	F_{ST}
Clade A	92.77	7.23	0.9278
Clade B	93.68	6.32	0.9368
Clade C	83.38	16.62	0.8338
Clade D	88.11	11.89	0.8811
Clade A versus B versus C versus D	96.60	3.40	0.9660

© 2009 Blackwell Publishing Ltd

Table 4 Results of mismatch analysis for each clade and corresponding haplotype diversity and nucleotide diversity of the major clades of the plateau zokor (*Eospalax baileyi*)

Clade	*n*	Haplotype diversity	Nucleotide diversity	Fu's F_S (*P*-value)	SSD (*P*-value)	RAG (*P*-value)
A	36	0.7365	0.0038	2.722 (0.891)	0.058 (0.100)	0.1728 (0.0579)
B	10	0.9111	0.0137	0.799 (0.631)	0.114 (0.064)	0.2815 (0.1620)
C	61	0.8077	0.0133	6.196 (0.952)	0.058 (0.035)	0.0574 (0.0547)
D	86	0.9603	0.0229	−0.087 (0.557)	0.043 (0.262)	0.0141 (0.4763)
All samples	193	0.9640	0.0530	5.189 (0.858)	0.009 (0.109)	0.0055 (0.0025)

SSD, sum-of-squared deviations; RAG, raggedness index.

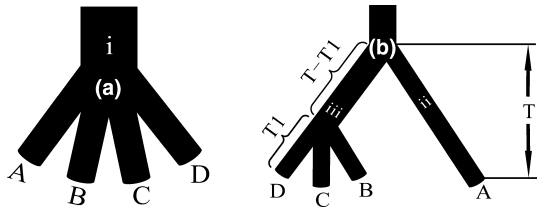

Figure 3 Coalescent simulations were used to test hypotheses about population structures of the plateau zokor (*Eospalax baileyi*). (a) The single-refugium hypothesis, in which all populations were derived from a single refugium at the end of the Last Glacial Maximum (LGM). (b) Two- or multiple-refugia hypotheses: two lineages split at the beginning of the LGM (T = 20 ka) and all current populations are derived from them with the coalescence time of T_1 = 12 ka, at the end of the LGM; under the four-refugia hypothesis, the two deep lineages split T = 0.7 Ma and the three clades from the plateau edge diverged T = 0.2 Ma.

present towards the end of the LGM (*c.* 12 ka) and located either at the eastern edge of the QTP or in the interior of the plateau (Fig. 3a); (2) the species persisted during the LGM in at least two refugia, accounting for the interior plateau lineage and the plateau-edge lineage (divergence beginning 12 ka and coalescence occurring at 12 ka; Fig. 3b); and (3) divergence between the two lineages and among the three clades (B–D) occurred long before 20 ka, as estimated using BEAST in this study.

Several empirical estimates of the θ-value were calculated as follows: $\theta_{total} = 3.46 \times 10^{-2}$ (CI: 2.16×10^{-2}–4.52×10^{-2}); $\theta_{lineage\ A} = 3.13 \times 10^{-3}$ (CI: 1.76×10^{-3}–4.71×10^{-3}); $\theta_{lineage\ B} = 5.48 \times 10^{-3}$ (CI: 3.62×10^{-3}–7.90×10^{-3}); $\theta_{lineage\ C} = 7.62 \times 10^{-3}$ (CI: 2.01×10^{-3}–1.42×10^{-2}); and $\theta_{lineage\ D} = 1.63 \times 10^{-2}$ (CI: 1.26×10^{-2}–1.97×10^{-2}). Based on these θ-values and the fixed cyt *b* substitute rates, we were able to estimate each N_e: $N_{e\ total}$ = 419,903; $N_{e\ lineage\ A}$ = 37,985; $N_{e\ lineage\ B}$ = 66,505; $N_{e\ lineage\ C}$ = 92,476; $N_{e\ lineage\ D}$ = 197,816. The model of evolution for ingroup sequences calculated by MODELTEST 3.06 and used in the coalescent simulations was: GTR+G model, πA = 0.3008, πC = 0.2763, πG = 0.1229, πT = 0.3000; gamma shape parameter = 0.2942; rA-C = 3.5849, rA-G = 19.7154, rA-T = 1.3727, rC-G = 0, rC-T = 45.1986, rG-T = 1.0000. These θ-values were estimated based on the presence of gene flow and the assumed effective population sizes, and are therefore smaller than the actual ones because the geographical distribution of haplotypes suggested restricted gene flow within this species. In order to test whether θ-values estimates obtained from MIGRATE analysis could discriminate among the empirical predictions, we adopted two approaches ('Coalescent within current tree' and 'Coalescent in current tree with migration') to simulate coalescent times under different scenarios.

We simulated the coalescent times of the current trees under the assumption 'without migration' using MESQUITE 2.5. The results of the coalescent tests rejected the single-refugium hypotheses of the current populations recolonizing from either the QTP edge or an interior refugium following the LGM ($P < 0.01$); we also rejected the hypothesis of two isolated refugia for the species at the start of the LGM ($P < 0.01$). However, the coalescent tests supported the hypothesis that there were four refugia for the species during the LGM, with two main divergences between the two lineages and among the three plateau-edge clades occurring during the middle and late Pleistocene (Fig. 3). The highest *P*-value (0.49) occurred at T = 0.70 Ma and T_1 = 0.20 Ma. We then performed simulations under continuous migrations using the module 'Coalescent in current tree with migration' in MESQUITE. All probabilities were simulated with automatically set varying migration rates (probabilities/individual/generation, 1×10^{-3}, 1×10^{-6} and 1×10^{-8}). All simulated datasets similarly rejected the first and second hypotheses ($P < 0.01$), but supported the four-refugia assumption, and the highest *P*-value (0.46) occurred at T = 0.70 and T_1 = 0.20 Ma.

DISCUSSION

Gene flow and allopatric divergence

The four major clades (A–D) recovered by phylogenetic analyses are distributed allopatrically (Fig. 2), and hierarchical AMOVAs revealed that *c.* 96% of the total variation is distributed among clades (Table 3). Both of these results indicate highly restricted maternal gene flow and fragmented divergence throughout the distributional range of the species. Two dependent life-history factors may contribute to this genetic pattern. First, this species rarely emerges from its subterranean habitat; aboveground activity entails a high risk of predation owing to the rodent's poor eyesight and slow movement (Zhou & Dou, 1990). Second, its geographic distribution is highly precinctive: the lifetime range of an individual has been estimated to be an area of only 1500 m^2

© 2009 Blackwell Publishing Ltd

(Zhou & Dou, 1990); the species harvests food mainly through excavating extensive burrow systems and the average excavating speed tends to decline with increased digging time (Su, 1992); mature individuals live alone and are aggressively territorial (Zhang & Liu, 2003); populations are spatially clumped; and individuals within populations use vegetation patches at different spatial and temporal scales (Zhang & Liu, 2003). In contrast, other rodents characterized by a higher rate of gene flow between regional groups often colonize aboveground habitats and have wider individual ranges [e.g. the wild naked mole-rat, *Heterocephalus glaber* (Braude, 2000); red-tailed chipmunk, *Tamias ruficaudus* (Good & Sullivan, 2001); common vole, *Microtus arvalis* (Haynes *et al.*, 2003); Japanese field mouse, *Apodemus speciosus* (Hirota *et al.*, 2004); sand-dune tuco-tuco, *Ctenomys australis* (Mora *et al.*, 2006); and cururos, *Spalacopus cyanus* (Opazo *et al.*, 2008)].

Our coalescent simulations indicated that four clades had diverged before 0.20 Ma and the molecular calibrations based on the fossil records suggested that the first deep divergence between two lineages (A versus B–D) probably occurred around 1.2 Ma, and the remaining three clades and two subclades (respectively) diverged 0.80–0.85 and 0.58–0.59 Ma (Table 2). These estimates may be distorted by two factors. First, the historical population dynamics of *E. baileyi* remain unknown and this may affect coalescent tests of the different hypotheses concerning the glacial refugia of the species, although the coalescent tests based on both assumptions with or without gene flow rejected the post-glacial recolonization hypotheses. Second, the estimated substitution rate may affect the accuracy of coalescent tests and direct divergence estimates among lineages and clades. The substitution rate (μ = 4.12% Myr^{-1}) estimated here for *E. baileyi* based on the fossil record is higher than those estimated for other rodent groups (0.5–4.0% Myr^{-1}; Smith & Patton, 1993; Lessa & Cook, 1998; Conroy & Cook, 1999; Spradling *et al.*, 2001; Suzuki *et al.*, 2003). This high substitution rate may result from rapid mutations of the *Eospalax* species in subterranean habitats, and/or the late fossil calibration point. The actual divergence between *Eospalax* and *Myospalax* may be earlier than the 4.0 Ma adopted here, as fossils of two genera were well recognizable by this time (Zheng, 1994). Lower estimates of substitution rate owing to an earlier fossil calibration or in comparison with other rodents both lead to an increase in divergence times between lineages/clades in both coalescent simulations and direct estimations, similarly supporting the hypothesis that the four clades diverged before the late Pleistocene. Furthermore, the divergence times of the two lineages, three clades, and the two subclades within lineage II estimated here based on fossil calibration (Table 2) were highly consistent with the geological uplift and glaciation events recorded for the QTP during the middle–late Pleistocene (Zheng *et al.*, 2002). The Kun-Huang diastrophisms occurred on the QTP around 1.2, 0.8 and 0.6 Ma (Li *et al.*, 1995). Around 1.2 Ma, the Yellow River drainage appeared in response to regional uplift, and the Naynayxungla Glaciation started around 1.2 Ma and reached its maximum between 0.8 and 0.6 Ma (Shi *et al.*, 1990; Zhou & Li, 1998; Zheng *et al.*, 2002). During this most extensive glacial advance, an ice sheet covered an area five to seven times larger than it does today (Shi *et al.*, 1990; Wu *et al.*, 2001; Zheng *et al.*, 2002; Owen *et al.*, 2006), and ice coverage exceeded an estimated 500,000 km^2 across the QTP at that time. Subsequent glaciations were thought to have occurred between 0.5 and 0.6 Ma (Jr, 2008), and regional uplift, development of glaciers, and/or extremely low temperatures at high elevations (> 4500 m) during these glaciations may have impeded gene flow and resulted in divergences between lineages and among clades and subclades of the plateau zokor (Fig. 1). A limited number of other animal and plant species that have been studied on the QTP have exhibited intraspecific diversifications partly corresponding to regional uplift and Quaternary glaciations of the middle and late Pleistocene (Yang *et al.*, 2006b; Qi *et al.*, 2007; Jin *et al.*, 2008; Wang *et al.*, 2009).

Persistence during subsequent glacials

The interior plateau populations (1–3) are currently restricted to the high-elevation plateau (Fig. 1). Similarly, within the plateau-edge lineage, major clades and subclades are geographically restricted, and population genetic analyses indicated limited gene flow among clades (Table 3). The four groups exhibit no significant expansion or contraction of populations following initial divergence, despite subsequent glacial cycles. As the clade nodes in the phylogenetic analysis are dated to earlier than 0.20 Ma, whether simulated by coalescent analyses or estimated directly from the fossil calibration under the molecular clock constraint, at least four refugia must have existed for this species during the LGM. The persistence of populations at high elevations during the LGM is further supported by the fact that most populations within the four clades (and all populations of clade A) are currently located at high elevations (> 3000 m). The existence of high-elevation glacial refugia is also supported by palaeobotanical data: pollen of a few plant species was deposited in the interior plateau during the LGM and previous glacials (Tang *et al.*, 1998).

Most regionally differentiated clades of animals in both North America and Europe appear to have undergone distinct demographic expansions because of repeated population bottlenecks during the Quaternary climatic oscillations (Seddon *et al.*, 2001; Fedorov *et al.*, 2003; Lessa *et al.*, 2003; Durka *et al.*, 2005). Such a scenario was also the case for two previously studied animal species occurring on the QTP: the red-necked snow finch (*Pyrgilauda ruficollis*) and the Qinghai toad-headed lizard (*Phrynocephalus vlangalii*). For both of these species, the origin of the haplotypes of most clades could be described by a star-shaped pattern (Qu *et al.*, 2005; Jin *et al.*, 2008), reflecting a recent (post-glacial and/or interglacial) expansion within each clade (Hudson, 1990). In contrast, we failed to detect such a pattern in the network of haplotypes in *E. baileyi* (Fig. 2). The molecular clock tests of these haplotypes suggested that the cyt *b* gene evolved at a constant

© 2009 Blackwell Publishing Ltd

rate in *Eospalax* and *Myospalax*, and mismatch distributions and Fu's F_S-tests rejected the demographic expansions of each geographically restricted clade and all sampled individuals (Table 4). There have been more than five distinct glacial ages (including the Penultimate Glaciation and the LGM) since the origin of the interior plateau lineage 1.2–0.7 Ma as estimated here, and at least one glaciation (the LGM) since the origin of the three clades in the plateau-edge lineage (Shi *et al.*, 1998). Despite this, all of our analyses indicate that the climatic oscillations following the origins of these lineages did not result in distinct population shrinkage or expansion of each regional clade. This finding suggests that the decreased temperature during the subsequent glacial ages was insufficient to reduce the survival of this zokor species, perhaps because of the relatively stable subterranean conditions. However, there is robust evidence that the dominant plant species on the QTP changed in response to the lower temperatures during the subsequent and particularly later climatic oscillations (Tang & Shen, 1996; Tang *et al.*, 1998). During both the Penultimate Glaciation and the LGM glacial ages, cold- and drought-resistant plant species (e.g. *Artemisia*-like species) replaced the grasses and other species in the high-elevation regions of the QTP. Why did the change in plant species not result in a distinct population shrinkage–expansion of each lineage/clade or shift of the overall distribution of *E. baileyi*? One possible answer is that this species feeds on the roots of a variety of plant species (Wang *et al.*, 2000; Zhang, 2000), and a change in the composition of plant communities may have had little effect on their potential food resource.

Overall, our results suggest that the mountain building and climatic oscillations of the Quaternary promoted allopatric divergence within *E. baileyi*. Once differentiated, these clades survived at high elevations and were able to maintain stable populations during subsequent climatic oscillations. Thus, the phylogeographic pattern within *E. baileyi* exhibits neither the population shrinkage or expansion nor the signal of post-glacial recolonization observed in other species of the QTP (Yang *et al.*, 2006b; Qi *et al.*, 2007; Jin *et al.*, 2008). Although it is still unknown how many animal species may exhibit a phylogeographic history similar to *E. baileyi*, or whether the persistence of this species in central parts of the QTP can be confirmed by fossil evidence, these findings demonstrate that the demographic histories of animal species in the QTP are more complex and variable than previously assumed.

ACKNOWLEDGEMENTS

We thank Ryan Norris (Pennsylvania State University) for comments on an earlier draft, and John Blackwell for improving the English of the final manuscript. This work was supported by the Chinese Academy of Science Innovation Program (CXLY-2003-3 to J-P. Su), the Training Qualified People Plan 'Hope of West China' of the Chinese Academy of Sciences, the NSFC (30725004 to J-Q.L.) and the Biodiversity Conservation Research Group of Qujing Normal University.

REFERENCES

Avise, J.C. (2000) *Phylogeography: the history and formation of species*. Harvard University Press, Cambridge, MA.

Beerli, P. (2002) *Migrate: documentation and program, part of LAMARC. Version 1.5*. Available at: http://evolution.genetics.washington.edu/lamarc/index.html (accessed 20 January 2005).

Bohonak, A.J. (2002) IBD (isolation by distance): a program for analyses of isolation by distance. *Journal of Heredity*, **93,** 153–154.

Braude, S. (2000) Dispersal and new colony formation in wild naked mole-rats: evidence against inbreeding as the system of mating. *Behavioral Ecology*, **11,** 7–12.

Clement, M., Posada, D. & Crandall, K.A. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9,** 1657–1659.

Conroy, C.J. & Cook, J.A. (1999) MtDNA evidence for repeated pulses of speciation within arvicoline and murid rodents. *Journal of Mammalian Evolution*, **6,** 221–245.

Drummond, A.J. & Rambaut, A. (2003) *BEAST. Version 1.3*. University of Oxford, Oxford. Available at: http://evolve.zoo.ox.ac.uk/beast (accessed 31 January 2006).

Drummond, A.J., Nicholls, G.K., Rodrigo, A.G. & Solomon, W. (2002) Estimating mutation parameters, population history and genealogy simultaneously from temporally spaced sequence data. *Genetics*, **161,** 1307–1320.

Durka, W., Babik, W. & Ducroz, J.F. (2005) Mitochondrial phylogeography of the Eurasian beaver *Castor fiber* L. *Molecular Ecology*, **14,** 3843–3856.

Excoffier, L., Smouse, P.E. & Quattro, J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131,** 479–491.

Excoffier, L., Laval, G. & Schneider, S. (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1,** 47–50.

Fan, N.C. & Shi, Y.Z. (1982) A revision of the zokors of subgenus *Eospalax*. *Acta Theriologica Sinica*, **2,** 183–199.

Fedorov, V.B., Goropashnaya, A.V., Jaarola, M. & Cook, J.A. (2003) Phylogeography of lemmings (*Lemmus*): no evidence for postglacial colonization of Arctic from the Beringian refugium. *Molecular Ecology*, **12,** 725–731.

Felsenstein, J. (1989) PHYLIP – phylogeny inference package (version 3.2). *Cladistics*, **5,** 164–166.

Fu, Y.X. (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147,** 915–925.

Good, J.M. & Sullivan, J. (2001) Phylogeography of the red-tailed chipmunk (*Tamias ruficaudus*), a northern Rocky Mountain endemic. *Molecular Ecology*, **10,** 2683–2695.

Grubov, V.I. (1963) *Rasteniya Central'noj Azii*. Nauka, Moscow.

Gupta, S.K., Sharma, P. & Shah, S.K. (1992) Constraints on ice sheet thickness over Tibet during the last 40,000 years. *Journal of Quaternary Science*, **7,** 283–290.

© 2009 Blackwell Publishing Ltd

Harpending, R.C. (1994) Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human Biology*, **66,** 591–600.

Haynes, S., Jaarola, M. & Searle, J.B. (2003) Phylogeography of the common vole (*Microtus arvalis*) with particular emphasis on the colonization of the Orkney archipelago. *Molecular Ecology*, **12,** 951–956.

Hewitt, G.M. (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405,** 907–913.

Hirota, T., Hirohata, T. & Mashima, H. (2004) Population structure of the large Japanese field mouse, *Apodemus speciosus* (Rodentia: Muridae), in suburban landscape, based on mitochondrial D-loop sequences. *Molecular Ecology*, **13,** 3275–3282.

Hudson, R.R. (1990) Gene genealogies and the coalescent process. *Oxford surveys in evolutionary biology*, Vol. 7 (ed. by D. Futuyma and J. Antonovics), pp. 1–44. Oxford University Press, Oxford.

Huelsenbeck, J.P. & Rannala, B. (1997) Phylogenetic methods come of age: testing hypotheses in an evolutionary context. *Science*, **276,** 227–232.

Jin, Y.T., Brown, R.P. & Liu, N.F. (2008) Cladogenesis and phylogeography of the lizard *Phrynocephalus vlangalii* (Agamidae) on the Tibetan plateau. *Molecular Ecology*, **17,** 1971–1982.

Jr, W.D. (2008) Stadial subdivisions of early Pleistocene glaciations in central United States – a developing chronology. *Boreas*, **1,** 55–61.

Knowles, L.L. (2001) Did the Pleistocene glaciations promote speciation? Test of explicit refugial models in montane grasshoppers. *Molecular Ecology*, **10,** 691–701.

Knowles, L.L. & Maddison, W.P. (2002) Statistical phylogeography. *Molecular Ecology*, **10,** 2623–2635.

Kuhle, M. (1988) The Pleistocene glaciation of Tibet and onset of ice age: an autocycle hypothesis. *Geographical Journal*, **17,** 581–595.

Lawrence, M.A. (1991) A fossil *Myospalax* cranium (Rodentia: Muridae) from Shanxi, China, with observations on zokor relationships. *Contributions to mammalogy in honor of Karl F. Koopman* (ed. by T.A. Griffiths and D. Klingener), pp. 261–286. Bulletin of the American Museum of Natural History, New York.

Lessa, E.P. & Cook, J.A. (1998) The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) suggests an early burst of speciation. *Molecular Phylogenetics and Evolution*, **9,** 88–99.

Lessa, E.P., Cook, J.A. & Patton, J.L. (2003) Genetic footprints of demographic expansion in North America, but not Amazonia, during the Late Quaternary. *Proceedings of the National Academy of Sciences USA*, **100,** 10331–10334.

Li, J.J., Shi, Y.F. & Li, B.Y. (1995) *Uplift of the Qinghai-Xizang (Tibet) Plateau and global change* [in Chinese]. Lanzhou University Press, Lanzhou.

Maddison, W.P. & Maddison, D.R. (2008) *Mesquite: a modular system for evolutionary analysis. Version 2.5*. Available at: http://mesquiteproject.org (accessed 10 September 2008).

Mantel, N. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27,** 209–220.

Meng, L., Yang, R., Abbott, R.J., Miehe, G., Hu, T. & Liu, J. (2007) Mitochondrial and chloroplast phylogeography of *Picea crassifolia* Kom. (Pinaceae) in the Qinghai-Tibetan Plateau and adjacent highlands. *Molecular Ecology*, **16,** 4128–4137.

Mora, M.S., Lessa, E.P., Kittlein, M.J. & Vassallo, A.I. (2006) Phylogeography of the subterranean rodent *Ctenomys australis* in sand-dune habitats: evidence of population expansion. *Journal of Mammalogy*, **87,** 1192–1203.

Musser, G.G. & Carleton, M.D. (2005) Superfamily Muroidea. *Mammal species of the world, a taxonomic and geographic reference* (ed. by D.E. Wilson and D.M. Reeder), pp. 894–1531. Johns Hopkins University Press, Baltimore, MD.

Norris, R.W., Zhou, K.Y., Zhou, C.Q., Yang, G., Kilpatrick, C.W. & Honeycutt, R.L. (2004) The phylogenetic position of the zokor (Myospalacinae) and comments on the families of muroids (Rodentia). *Molecular Phylogenetics and Evolution*, **31,** 972–978.

Opazo, J.C., Bugueno, M.P., Carter, M.J., Palma, R.E. & Bozinovic, F. (2008) Phylogeography of the subterranean rodent *Spalacopus cyanus* (Caviomorpha, Octodontidae). *Journal of Mammalogy*, **89,** 837–844.

Owen, L.A., Finkel, R.C., Haizhou, M. & Barnard, P.L. (2006) Late Quaternary landscape evolution in the Kunlun Mountains and Qaidam Basin, Northern Tibet: a framework for examining the links between glaciation, lake level changes and alluvial fan formation. *Quaternary International*, **154–155,** 73–86.

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

Qi, D., Guo, S., Zhao, X., Yang, J. & Tang, W. (2007) Genetic diversity and historical population structure of *Schizopygopsis pylzovi* (Teleostei: Cyprinidae) in the Qinghai–Tibetan Plateau. *Freshwater Biology*, **52,** 1090–1104.

Qu, Y.H., Ericson, P.P., Lei, F.M. & Li, S.H. (2005) Postglacial colonization of the Tibetan plateau inferred from the matrilineal genetic structure of the endemic red-necked snow finch *Pyrgilauda ruficollis*. *Molecular Ecology*, **14,** 1767–1781.

Rambaut, A. & Drummond, A.J. (2003) *Tracer. Version 1.2*. University of Oxford, Oxford. Available at: http://evolve.zoo.ox.ac.uk/tracer (accessed 31 January 2006).

Rogers, A.R. (1995) Genetic evidence for a Pleistocene population explosion. *Evolution*, **49,** 608–615.

Rowe, K.C., Heske, E.J., Brown, P.W. & Paige, K. (2004) Surviving the ice: Northern refugia and postglacial colonization. *Proceedings of the National Academy of Sciences USA*, **101,** 10355–10359.

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.

Sanderson, M.J. (2002) Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution*, **19,** 101–109.

© 2009 Blackwell Publishing Ltd

Seddon, J.M., Santucci, F., Reeve, N.J. & Hewitt, G.M. (2001) DNA footprints of European hedgehogs, *Erinaceus europaeus* and *E. concolor*: Pleistocene refugia, postglacial expansion and colonization routes. *Molecular Ecology*, **10,** 2187–2198.

Shi, Y.F., Zheng, B.X. & Li, S.J. (1990) Last glaciation and maximum glaciation in Qinghai–Xizang (Tibet) plateau. *Journal of Glaciology and Geocryology*, **12,** 1–15.

Shi, Y.F., Li, J.J. & Li, B.Y. (1998) *Uplift and environmental changes of Qinghai–Tibetan plateau in the Late Cenozoic* [in Chinese]. Guangdong Science and Technology Press, Guangzhou.

Slatkin, M. & Maddison, W.P. (1989) A cladistic measure of gene flow inferred from the phylogenies of alleles. *Genetics*, **123,** 603–613.

Smith, A.T. & Yan, X. (2008) *A guide to the mammals of China*. Princeton University Press, Princeton, NJ.

Smith, M.F. & Patton, J.L. (1993) The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. *Biological Journal of the Linnean Society*, **50,** 149–177.

Song, S.Y. (1986) A revision of the two species of the zokors on subgenus *Eospalax*. *La Animals Mondo*, **3,** 31–39.

Spradling, T.A., Hafner, M.S. & Demastes, J.W. (2001) Differences in rate of cytochrome-*b* evolution among species of rodents. *Journal of Mammalogy*, **82,** 65–80.

Steele, C.A. & Storfer, A. (2006) Coalescent-based hypothesis testing supports multiple Pleistocene refugia in the Pacific Northwest for the Pacific giant salamander (*Dicamptodon tenebrosus*). *Molecular Ecology*, **15,** 2477–2487.

Su, J.P. (1992) Energy cost of foraging and optimal foraging in the fossorial rodent (*Myospalax baileyi*). *Acta Theriologica Sinica*, **12,** 117–125.

Suzuki, H., Sato, J.J. & Tsuchiya, K. (2003) Molecular phylogeny of wood mice (*Apodemus*, Muridae) in East Asia. *Biological Journal of the Linnean Society*, **80,** 469–481.

Swofford, D.L. (2002) *PAUP*: phylogenetic analyses using parsimony (*and other methods). Version 4*. Sinauer Associates, Sunderland, MA.

Tang, L.Y. & Shen, C.M. (1996) Late Cenozoic vegetational history and climatic characteristics of Qinghai-Xizang Plateau. *Acta Micropalaeontologica Sinica*, **13,** 321–337.

Tang, L.Y., Shen, C.M., Kong, Z.Z., Wang, F.B. & Liu, K.B. (1998) Pollen evidence of climate during the Last Glacial Maximum in eastern Tibetan plateau. *Journal of Glaciology and Geocryology*, **20,** 133–140.

Templeton, A.R., Crandall, K.A. & Sing, C.F. (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction site endonuclease mapping and DNA sequence data. (III) Cladogram estimation. *Genetics*, **132,** 619–633.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The Clustal_X windows interface: flexible strategies for multiple alignment aided by quality analysis tools. *Nucleic Acids Research*, **25,** 4876–4882.

Trinkler, E. (1930) The ice-age on the Tibetan Plateau and in the adjacent regions. *Geographical Journal*, **75,** 225–232.

Wang, L.Y., Abbott, R.J., Zheng, W. & Liu, J.Q. (2009) History and evolution of alpine plants endemic to the Qinghai-Tibetan Plateau: *Aconitum gymnandrum* (Ranunculaceae). *Molecular Ecology*, **18,** 709–721.

Wang, Q., Zhang, Y., Wei, W. & Bian, J. (2000) Food habit of the plateau zokor. *Acta Theriologica Sinica*, **20,** 198–210.

Wang, Y. (2003) *A complete checklist of mammal species and subspecies in China. A taxonomic and geographic reference* [in Chinese]. China Forest Publishing House, Beijing.

Wei, W.H., Wang, Q.Y., Zhou, W.Y. & Fan, N.C. (1998) Effects of predatory disturbance on population reproductivity of *Myospalax baileyi*. *Acta Ecologica Sinica*, **18,** 176–180.

Wilson, A.C., Cann, R.L., Carr, S.M., George, M., Gyllensten, U.B., Helm-Bychowski, K.M., Higuchi, R.G., Palumbi, S.R., Prager, E.M., Sage, R.D. & Stoneking, M. (1985) Mitochondrial DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnean Society*, **26,** 375–400.

Wu, Y.Q., Cui, Z.J. & Liu, G.N. (2001) Quaternary geomorphological evolution of the Kunlun Pass areas and uplift of the Qinghai-Xizang (Tibet) plateau. *Geomorphology*, **36,** 203–216.

Yang, S.J., Lei, F.M., Qu, Y.H. & Yin, Z.H. (2006a) Intraspecific phylogeography of the white-rumped snowfinch (*Onychostruthus taczanowskii*) endemic to the Tibetan Plateau based on mtDNA sequences. *Journal of Zoology*, **268,** 187–192.

Yang, S.J., Yin, Z.H., Ma, X.M. & Lei, F.M. (2006b) Phylogeography of ground tit (*Pseudopodoces humilis*) based on mtDNA: evidence of past fragmentation on the Tibetan Plateau. *Molecular Phylogenetics and Evolution*, **41,** 257–265.

Zhang, Q., Chiang, T.Y., George, M., Liu, J.Q. & Abbott, R.J. (2005) Phylogeography of the Qinghai-Tibetan Plateau endemic *Juniperus przewalskii* (Cupressaceae) inferred from chloroplast DNA sequence variation. *Molecular Ecology*, **14,** 3513–3524.

Zhang, Y. (1999) Effect of plateau zokor on characters and succession of plant communities in alpine meadow. *Zoological Research*, **20,** 435–440.

Zhang, Y. (2000) *Studies on the pattern of animal–plant interaction: the effects of plateau zokor on the biogeochemical cycling of alpine meadow ecosystem and its response to the chemical defence of plants* [in Chinese]. PhD Thesis, Chinese Academy of Science, Beijing.

Zhang, Y. & Liu, J. (2003) Effects of plateau zokor (*Myospalax fontanierii*) on plant community and soil in an alpine meadow. *Journal of Mammalogy*, **84,** 644–651.

Zhang, Y., Jin, S., Quan, G., Li, S., Ye, Z., Wang, F. & Zhang, M. (1997) *Distribution of mammalian species in China* [in Chinese]. China Forest Publishing House, Beijing.

Zheng, B., Xu, Q. & Shen, Y. (2002) The relationship between climate change and Quaternary glacial cycles on the Qinghai–Tibetan Plateau: review and speculation. *Quaternary International*, **97/98,** 93–101.

Zheng, S.H. (1994) Classification and evolution of the Siphneidae. *Rodents and lagomorph families of Asian origins and diversification* (ed. by Y. Tomida, C.K. Li and T. Setoguchi), pp. 57–76. National Science Museum Monographs, Tokyo.

© 2009 Blackwell Publishing Ltd

Zhou, C.Q. & Zhou, K.Y. (2008) The validity of different zokor species and the genus *Eospalax* inferred from mitochondrial gene sequences. *Integrative Zoology*, **3,** 290–298.

Zhou, C.Q., Zhou, K.Y. & Zhang, S.L. (2004) Molecular authentication of the animal crude drug Sailonggu (bone of *Myospalax baileyi*). *Biological and Pharmaceutical Bulletin*, **27,** 1850–1858.

Zhou, S.Z. & Li, J.J. (1998) The sequence of Quaternary glaciation in the Bayan Har Mountains. *Quaternary International*, **45/46,** 135–142.

Zhou, W. & Dou, F. (1990) Studies on activity and home range of plateau zokor. *Acta Theriologica Sinica*, **10,** 31–39.

BIOSKETCH

The authors collaborated to study the population dynamics of alpine animals and plants on the Qinghai–Tibetan Plateau.

Author contributions: J-Q.L. and J-P.S. conceived the research project; L-Z.T., Z-Y.C. and T-Z.Z. collected the data; L-Z.T., L-Y.W., H-X.C. and G-H.L analysed the data; J-Q.L., L-Z.T. and L-Y.W. wrote the manuscript.

Editor: David Hafner

© 2009 Blackwell Publishing Ltd