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Full Length Research Paper

Genetic differentiations between habitat edges and interiors of Plateau Zokor (*Eospalax baileyi*) in the Qinghai-Tibetan Plateau

Lizhou Tang[1,2], Long Yu[1] Junjie Wang[1], Wei Ding[1], Xiaodong Shi[1], Tongzuo Zhang[2*] and Jianping Su[2*]

[1]Yunnan-Guizhou Plateau Institute of Biodiversity, College of Biologic Resource and Environment Science, Qujing Normal University, Qujing, Yunnan 655011, China.
[2]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.

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Nucleotide variations in the mitochondrial cytochrome *b* and control region gene sequences were used to investigate genetic differentiations between edge habitats and the interiors of *Eospalax baileyi*. There were 187 individuals for cytochrome b gene analysis and 155 individuals for control region from 16 populations over the total distribution range. Genetic analysis showed that the habitat edges present a substantial higher genetic diversity than the interiors, which were supported by significant values of haplotypes diversity (t = 69.634, *P* = 0.009; t = 85.780, *P* = 0.007) and nucleotide diversity (t = 14.100, *P* = 0.045; t = 17.146, *P* = 0.037). Mismatch distribution analyses indicated that there were more stable population pattern in the habitat edges than in the interiors, and that the latter may have undergone a population explosion recently (0.086 to 0.103 million years ago) which may have occurred at the interglacials of the Qinghai-Tibetan Plateau (QTP). These results can be explained by the different extent of disturbances induced by geological events in the QTP. The transition zone between QTP and Loess Plateau colonized by the edges could provide sufficient food and suitable subsistence conditions because of relatively low disturbance. However, the interiors of this species have undergone serious challenges from deep environmental changes.

Key words: *Eospalax baileyi*, mitochondrial gene, genetic differentiation, Qinghai-Tibetan Plateau.

INTRODUCTION

Plateau zokor (*Eospalax baileyi*), identified as a species of the genus *Eospalax* (Norris et al., 2004; Zhou and Zhou, 2008; Tang et al., 2010), is a highly specialized subterranean rodent widely distributed in meadow, prairie and alpine prairie habitats in the Qinghai-Tibetan Plateau (QTP) (Zhang et al., 1999) at an altitude range of 2800 to 4200 meters or higher above sea-level. This species has also been validated as the only species of the genus that occurs in the high-elevation interior of the QTP, although its distribution area is parapatric with other sister species (for example, *Eospalax cansus*) on the north-eastern edges of the plateau (Fan and Shi, 1982). Recent phylogeographic studies have suggested that the Quaternary diastrophisms and glaciations of the QTP repeatedly promoted allopatric divergence of *E. baileyi* into four geographical clades and also supported a hypothesis that there are four refugia during the Last Glacial Maximum (LGM) located in the interior and edge regions of the QTP (Tang et al., 2010). The north-eastern edges of the QTP are transition zones between adjacent

*Corresponding author. E-mail: jpsu@nwipb.ac.cn. zhangtz@nwipb.ac.cn. Tel: +86-971-6143748. Fax: +86-971-6143282.

Abbreviations: QTP, Qinghai-Tibetan plateau; **LGM,** last glacial maximum; **LP,** loess plateau; **DNA,** deoxyribonucleic acid; **PCR,** polymerase chain reaction; **MCMC,** markov chain Monte Carlo; **CI,** confidence interval; **Hd,** haplotype diversity; **Nd,** nucleotide diversity; **Pi,** pair wise differences; **ACT,** auto-correction time; **ESS,** effective sample size.

Table 1. The geographic origin, altitude and haplotypes of habitat interiors and edges of plateau zokor.

Habitat	Population	Longitude (E)	Latitude (N)	Altitude (m)	Cyt *b* haplotypes	D-loop haplotypes
I1	P1	97.24°	33.35°	4390	C1	D1,2,3,4,5
	P2	97.47°	33.20°	4450	C2,3,4	D6,7,8,9,10,11
	P3	96.94°	33.77°	4550	C5	D12,13
I2	P4	99.71°	37.17°	3230	C12,13,14,15,16	D18,19,20,21,22,23, 24,25,26,27
	P5	98.87°	37.18°	3840	C43,44,45	D55,56,57,58,59,60
	P6	100.22°	38.07°	3450	C30,37	D37,38,39,40,41
E1	P7	102.72°	34.10°	3230	C38	D42,43,44,45
	P8	102.89°	33.91°	3450	C38,39,40,41	D46,47,48
	P9	102.53°	33.41°	3490	C42	D49,50,51,52,53,54
	P10	103.25°	34.75°	3160	C46,47	D61,62
	P11	103.05°	34.37°	3270	C46,47,48	D61,62,63,64,65
	P12	103.55°	34.74°	3020	C46	D62,66
E2	P13	102.30°	36.19°	3230	C17,18,19,20,21, 22,23,24,25,26	D28,29
	P14	102.12°	36.90°	3040	C30,31,32,33,34, 35, 36	D34,35,36
	P15	101.68°	36.95°	3020	C6,7,8,9,10,11	D14,15,16,17
	P16	101.11°	36.64°	3110	C11, 27, 28, 29	D30,31,32,33

Note: I, habitat interior; E, habitat edge.

habitats actually, and they could be a type of ecotone that connected two important natural habitats, the QTP and the Loess Plateau (LP) (Magura et al., 2001; Lin et al., 2008). Previous theoretics about edge effect emphasized that the edge habitats could support a higher abundance and diversity of colonized species than interior habitats (Denys and Tscharntke, 2002; Magura, 2002; Harper and Macdonald, 2002; Kitahara and Watanabe, 2003; Klein et al., 2003; Major et al., 2003; Harper et al., 2005). The most reasonable explanation for this trend is that the habitat edges could accumulate more distinct colonizing areas and greater overall species richness (Magura, 2002; Ewers and Didham, 2006). Therefore, this classical theory impassioned us a hypothesis to address the following question. (1) Are genetic diversity of *E. baileyi* higher in the habitat edge of QTP than in the habitat interior? (2) Are environmental variations affecting the distribution of *E. baileyi*? If so, do they influence edge effect on *E. baileyi?*

MATERIALS AND METHODS

Population samples

A total of 187 individuals of *E. baileyi* were collected from four geographic populations, there into two habitat edges and other two habitat interiors (Table 1 and Figure 1) across the whole distribution area of west China. Tissue samples collected from the muscles of field caught zokors were preserved in 95% ethanol.

Deoxyribonucleic acid (DNA) extraction, polymerase chain reaction (PCR) amplification and sequencing

Total DNA was isolated from ethanol-fixed tissue by proteinase K digestion followed by standard phenol-chloroform extraction and 70% ethanol precipitation (Joe and David, 2001). The partial sequence of mitochondrial cytochrome *b* (cyt *b*) gene and control region (D-loop) were amplified using the primers pair L14724 (5'-C GAA GCT TGA TAT GAA AAA CCA TCG TTG-3'), H15917 (5'-C GGA ATT CCA TTT TTG GTT TAC AAG-3') (Zhou et al., 2004) and the primers pair FR (5'-TAC CAT CCT CCG TGA AAC CA-3'), RV (5'-CTA ATA ATA- AGG CCA GGA CC-3') (Reyes et al., 2003), respectively. The detailed techniques of PCR amplifications, sequencing reactions, purified and sequencing were consulted to Tang et al. (2010) and Lin et al. (2008). To ensure accuracy, strands were sequenced in both directions for each individual using the same primer pairs of PCR amplification.

Data analysis

Sequences were aligned using CLUSTAL X (Thompson et al., 1997) with default settings, and refined manually. Number of variable sites and number of parsimony informative sites were computed using DnaSP (vision 4.0; Rozas et al., 2003).

Permutation tests of significance were used to test genetic variance by comparisons to null distributions with 10,000 random permutations. Nucleotide diversity, haplotype diversity and expected heterozygosity were also calculated in ARLEQUIN 3.1 (Excoffier et al., 2006). The

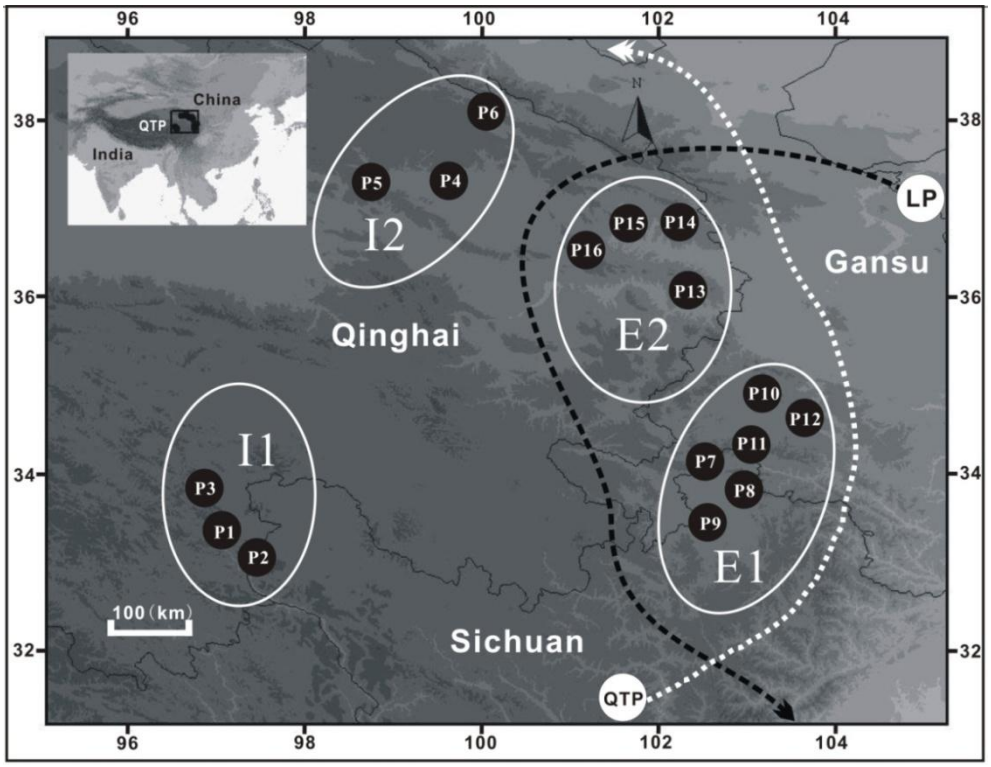

Figure 1. Locations of sampled populations in the interior and edge of Qinghai-Tibetan Plateau for plateau zokor. The names of populations and habitats are corresponding to Table 1 and Figure 2, respectively. QTP and LP indicate the Qinghai-Tibetan plateau and the Loess Plateau.

hypotheses of selective neutrality were tested by both Tajima's D (Tajima, 1989a, b) and Fu's *Fs* (Fu, 1997) tests using ARLEQUIN with 10,000 permutations. Mismatch distribution analyses were conducted for interior habitats and edge habitats using ARLEQUIN. The sum of squared deviation, harpending's raggedness index and their p-values were estimated to test the significance level from population expansion model.

Time since population expansion (T) was calculated using the formula Tau (τ) = 2μkT, where μ is the mutation rate of per nucleotide and k is the number of nucleotides assayed (Rogers and Harpending, 1992; Gaggiotti and Excoffier, 2000). The mean substitution rate of Cyt *b* and D-loop combined data was estimated by the program BEAST (Drummond and Rambaut, 2007a, b). The time of most recent common ancestor of the *E. baileyi* monophyletic group could be set about 1.15 to 1.25 million years ago (Mya) (Tang et al., 2010). 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. Lastly, the convergence of the chains to the stationary distribution, stage of burn in, mean mutation rate and its 95% confidence interval (CI) were checked using the program TRACER (Drummond and Rambaut, 2007c).

RESULTS

A total of 48 haplotypes estimated with 709 bp Cyt *b* gene sequence segments were identified from the 187 individuals of *E. baileyi*. And 66 haplotypes of 630 bp D-loop gene segments from 155 samples can be separated for further study (Tables 1 and 2). Important genetic differentiation between the habitat interior and the edges from Cyt *b* and D-loop gene estimation was the variation in haplotype diversity (Hd), nucleotide diversity (Nd), mean number of pair wise differences (Pi) and the expected heterozygosity (Het) (Table 2). The general trend from two genes calculations was that the habitat edges showed higher values of Pi, Hd and Nd than the interior habitats, but with lower Het values than the latter (Table 2). For Cyt *b* gene analysis, there were significant differences at all four values of Pi (t = 14.055, P = 0.045), Het (t = 15.794, P = 0.040) Hd (t = 69.634, P = 0.009) and Nd (t = 14.100, P = 0.045) between habitat interior and the edges of QTP (Table 2).

The results estimated from D-loop gene showed that there are significant differences at three values of Het (t = 25.762, P = 0.025), Hd (t = 85.780, P = 0.007) and Nd (t = 17.146, P = 0.037) between them, but except for the Pi values (t = 5.930, P = 0.106) (Table 2). Based on the assumption that the time of most recent common ancestor of *E. baileyi* occurred at 1.15 to 1.25 Mya, the mean substitution rate estimated by BEAST for the combined data (Cyt *b* and D-loop) was 3.49% (CI: 2.99 to4.10%) per site per million year. The stationary level of convergence was undoubtedly supported by the other estimated values of auto-correction time (ACT = 3927.82) and effective sample size (ESS = 1006.16). Mismatch distribution analysis indicated that the habitat

Table 2. Genetic diversity of habitat interior and edges of plateau zokor.

	Pop.	Ind.	Haplo.	Ps.	Pi	Het	Hd	Nd
Cyt *b*								
Habitat interior (I1, I2)	P1-6	73	15	63	27.8417 ± 12.3270	0.4419 ± 0.1418	0.9094 ± 0.0144	0.0393 ± 0.0193
Habitat edge (E1, E2)	P7-16	114	34	111	32.1071 ± 14.1086	0.3893 ± 0.1676	0.9359 ± 0.0118	0.0453 ± 0.0220
t value (*P*)					14.055 (0.045)	15.794 (0.040)	69.634 (0.009)	14.100 (0.045)
D-loop								
Habitat interior (I1, I2)	P1-6	80	33	79	16.6317 ± 7.4871	0.3198 ± 0.1694	0.9453 ± 0.0125	0.0331 ± 0.0132
Habitat edge (E1, E2)	P7-16	75	40	52	23.3791 ± 10.3910	0.2959 ± 0.2026	0.9676 ± 0.0089	0.0372 ± 0.0183
t value (*P*)					5.930 (0.106)	25.762 (0.025)	85.780 (0.007)	17.146 (0.037)

Pop. Populations; Ind., Number of Individuals; haplo, number of haplotypes; Ps, Number of polymorphic sites; Pi, mean number of pair wise differences; Het, expected heterozygosity; Hd, Haplotype diversity; Nd, nucleotide diversity.

Table 3. Mismatch distribution analyses of Cyt *b* and D-loop combined between habitat interior and edges of plateau zokor.

Habitat	Tau	Theta0	Theta1	Age (mya)	SSD (*P* value)	HRI (*P* value)	Tajima's D (*P* value)	Fu' *Fs* (*P* value)
I1	8.031	0.011	18.311	0.086	0.056 (0.050)	0.117 (0.030)	-0.398 (0.039)	-0.443 (0.346)
I2	9.592	0.000	28.325	0.103	0.004 (0.700)	0.011 (0.720)	-0.470 (0.470)	-5.368 (0.028)
E1	0.102	14.581	19.290	-	0.036 (0.430)	0.022 (0.470)	1.711 (0.966)	0.542 (0.774)
E2	0.385	56.772	59.859	-	0.021 (0.620)	0.018 (0.900)	0.602 (0.786)	3.954 (0.925)

Note: I, habitat interior; E, habitat edge; SSD, sum of squared deviation; HRI, harpending's raggedness index; mya, million years ago.

interiors (I1 and I2) could have undergone a recent population expansion, with significantly Tajima's D (-0.398, P = 0.039) for I1 and Fu's *Fs* (-5.368, P = 0.028) for I2 (Table 3) and the unimodal distribution curves (Figure 2). The calculation results estimated by the formula Tau (τ) = $2\mu k$T showed that these two regions (I1 and I2) on the interior of QTP had expansion ages that ranged from 0.086 to 0.103 Mya (Table 3). However, one of the two or both tests of SSD and HRI values as well as Tajima's D or Fu's *Fs*, coupled with multimodal distribution, all rejected the sudden expansion hypotheses for these two regions on the habitat edges (E1 and E2) (Table 3 and Figure 2).

DISCUSSION

Genetic analysis revealed a significant genetic differentiation between the habitat interiors and the edges within *E. baileyi*. The habitat edges of QTP showed a substantial higher genetic diversity than the interiors, which was supported by the significant values of Pi, Hd and Nd (Table 2). These results can be explained by the different extent of disturbances induced by environmental factors between these two habitat types (the edges and interiors). As a subterranean rodent, *E. baileyi* has been strictly adapted to underground lifestyle, whose foods must largely depend on excavating extensive burrow systems (Zhang and Liu, 2003). Therefore, these hard behaviors which relate to finding food, expanding colonies and others demand high energy cost (Su, 1992). So its territory is most likely to be restricted by its particular burrowing activities. It has been reported that the home range size for *E. baileyi* is currently tens to hundreds of square meters, with tunnel total length of males around two hundred meters (Zhou and Dou, 1990). All of these have compelled the zokors of different population that are colonizing QTP interior habitats or the edges to use vegetation patches at different spatial and temporal scales (Wang et al., 2000; Zhang and Liu, 2003). At the transition zone of QTP and Loess Plateau, edge habitats can provide sufficient food and suitable environment for species colonization because of its relatively low altitudes (Tang et al., 1998; 2010; Lin et al., 2008).

A variety of plant species could be used by the zokors distributed in the QTP edges in spite of climatic oscillations, because of the quick rate of regeneration or replacement of plant communities (Tang and Shen, 1996; Tang et al., 1998; 2010). On the other hand, the findings of high genetic diversity and stable population distribution in edge populations (Table 2; Fig. 2) indicate that climatic oscillations of QTP did not result in population shrinkage or expansion in the edges, perhaps because of the relatively stable subterranean conditions (Zhang and Liu, 2003; Tang et al., 2010). Therefore, edge populations could have the time to accumulate more nucleotide mutations in spite of other disturbances, although human activities could also influenced the stabilization and dispersal of *E. baileyi*. On the contrary, the interior of the inner QTP colonized by *E. baileyi* has undergone serious challenges from environmental changes induced by the

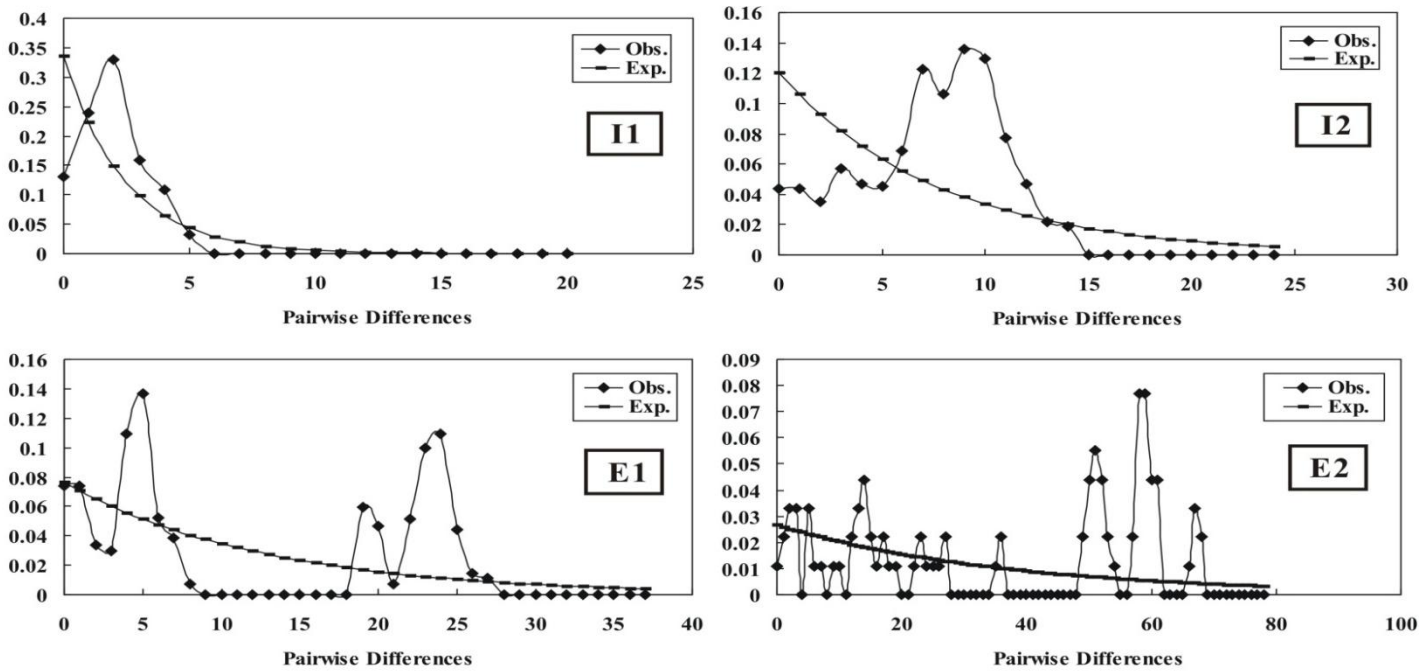

Figure 2. Mismatch distributions for the habitat edges and the interiors of plateau zokor.

uplift of QTP (Tang et al., 2010). According to geological researches, there are three fleetly holistic uplifts of QTP from the age of 3.6 Mya, which were named Qingzang, Kunhuang and Gonghe movements, respectively (Li and Fang, 1998; Li, 1999). These geologic movements consequently increased the emergence of permanent tundra and the altitudes to nearly 4000 to 5000 meters or higher, coupled with a series of glacial and interglacial cycles (Zhou and Guo, 1982; Shi et al., 1990; 1998; Shi, 1996, 1998).

These important events that occurred in the interior of QTP could be responsible for the low genetic diversity and population expansion of the inner populations of *E. baileyi* (Table 3 and Figure 2). This is because intense desiccation, serious desertification and increasing degradation of the vegetation might induce some inner habitats not to be fit for the subsistence of zokors (Sun et al., 2004). So the interior populations may have undergone more serious environment pressure and may have used much time to colonize the adaptive habitats at the interglacials than the edge populations (Tang et al., 2010). As a result, the interiors of *E. baileyi* presented a distinctly unstable distribution pattern and low genetic differentiation (Table 3 and Figure 2), which could have been principally imposed by the drastically changed environments as a result of disturbance, instead of effects from human activities.

In conclusion, this study has shown evidence of edge effects in *E. baileyi* and that the interior geographic populations have higher genetic diversity and more stable population pattern than the edges. In addition, all the results obtained supported the fact that genetic differentiation between the habitat interiors and the edges could be mainly affected by disparate geological variations and environmental conditions. However, this study just carefully analyzed important geological factors as the cause of genetic differentiation in various habitats of this species. In the future, it is necessary to explore genetic variation among different vegetation patches of this species, or investigate genetic diversity and distribution of other sister species in the same genus as well as compared them with the others in an attempt to provide biological evidences for the interrelated geology hypotheses.

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