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Regular research paper

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PHYLOGENETIC DISCONTINUITY OF PLATEAU ZOKOR (*MYOSPALAX BAILEYI* THOMAS) POPULATIONS IN QINGHAI-TIBETAN PLATEAU

ABSTRACT: Mitochondrial cytochrome *b* gene sequences and morphological data (body length, hind foot length, etc.) for twelve populations with pairwise distances 27–600 km in Qinghai-Tibetan Plateau (distributed in Qinghai, Gansu and Sichuan Province, and at the altitude 3020–4550 m) in Western China were used to investigate the phylogeographical pattern of Plateau zokor (*Myospalax baileyi* Thomas). There was a little disparity between mtDNA genetic distance and morphological Euclidean distance on population relationships. However, there is a significant correlation ($P < 0.001$) calculated by Mantel's tests was validated between mtDNA and morphology distances. Analysis of Molecular Variance showed that most of the observed genetic variations occurred between populations, indicating little maternal gene flow between them, as a result of geographical restrictions. Phylogenetic analysis coupled with cluster analysis together showed that the substantial population structuring and phylogenetic discontinuities existed within this species. The evident allopatric population structuring of this subterranean rodent may mostly result from its specialized subterranean excavating behavior with high energy costs, predation from grassland raptors and also the influences of perennial tundra and environmental desiccation in the Qinghai-Tibetan Plateau.

KEY WORDS: Plateau zokor, population structuring, cytochrome *b*, morphological characters, Qinghai-Tibetan Plateau, phylogeography

1. INTRODUCTION

The core of phylogeography mainly involves in the historical characteristics of phylogeographic space distribution and the phylogenetic relationship trying to recognize the current population pattern within species (Avise 2000). In addition, phylogeography, couples with the geographical distribution of populations, further detects and validates the interrelated geological events, and infers the possible course of population evolution (Avise *et al.* 1987, Avise *et al.* 1998). Five major types of population phylogeographic pattern were generalized by Avise *et al.* (1987), followed by many species in different distribution ranges being successively studied. First, the populations with phylogenetic discontinuousness occurred in different geographical ranges. This category proposed for many species, such as European woodmouse (*Apodemus sylvaticus* L.) (Michaux *et al.* 2003), hedgehogs (*Erinaceus europaeus* L.) (Seddon *et al.* 2001) and the Eurasian beaver (*Castor fiber* L.) (Durka *et al.* 2005). Second,

the populations with phylogenetic discontinuousness distributed in the same region. For example, the phylogeographic structure of Hume's Ground Tit (*Pseudopodoces humilis* Hume) endemic to the Qinghai-Tibetan Plateau (QTP) should belong to this pattern (Yang *et al.* 2006b), owing to long time period of cessation of gene flow and reproductive segregation. Third, the populations with phylogenetic continuity distributed in different geographical regions. This pattern is approximated to the *Bufo terrestris* (Bonnaterre) (Bermingham *et al.* 1986). Fourth, the populations with phylogenetic continuity occurred by continuous space distribution. The Red-necked snow finch (*Pyrgilauda ruficollis* Blanford) (Qu *et al.* 2005) and the White-rumped snow finch (*Onychostruthus taczanowskii* Przevalski) (Yang *et al.* 2006a), both endemic to the QTP region, correspond to this pattern with high gene flow and strong migration. Lastly, the populations with phylogenetic continuity occurred by partial continuity of space distribution. This situation also observed in the *Peromyscus polionotus* (Wagner) (Wooten *et al.* 1999), a beach mouse with medium gene flow.

Plateau zokor (*Myospalax baileyi* Thomas) (According to Mammal Species of the World, Wilson DE and Reeder DM (ed), 1985, *baileyi* is a subspecies or synonym of *Eospalax fontanierii* Milne-Edwards, however, the latest studies support it to be an independent species (Norris *et al.* 2004, Zhou and Zhou 2008).) of the family Myospalacinae are highly specialized subterranean rodents widely distributed in meadow, prairie and alpine prairie habitats in the QTP (Zhang *et al.* 1999) at an altitudes of 2800–4200 meters or higher above sea level. Its distributional areas include Qinghai, southern Gansu, and northwestern Sichuan provinces (Northwest Plateau Institute of Biology Site, The Chinese Biodiversity Information Center, <http://www.haibei.org>). Its food mainly comes from the roots and shoots of annual and perennial grasses, forbs or shrubs (Wang *et al.* 2000, Zhang 2000). Like other subterranean rodents, Plateau zokors are highly adapted to the strictly subterranean lifestyle, which means that their primary diet depends on excavating the burrow systems (Zhang and Liu 2003). The digging behavior is undoubtedly a sig-

nificant process of energy cost. As previous researches showed, the burrow inhabitants need face more challenges from high energy cost for digging than on aboveground (Kennerly 1964, Contreras and McNab 1990, Nevo 1999). Therefore, the high energy costs of digging underground may largely restrict the movement of subterranean Plateau zokor. Their weak dispersal ability and endemism in the plateau promote us to investigate whether the phylogeographical pattern of this species is congruent with the second category of Avise *et al.* (1987).

The important uplifts of the QTP and Quaternary glaciations could be responsible for the convergency and divergence of population of Plateau zokor. The fleetly holistic uplift of the plateau occurred at 3.6–1.7 million years ago (mya) named Qingzang movement, followed by Kunlun-Huanghe movement (1.1–0.6 mya) and Gonghe movement (0.15 mya) (Li and Fang 1998, Li 1999). The permanent tundra appeared as the altitude increased during the course of the uplift of the plateau. When altitudes increased to almost 4000–5000 meters or higher, the insular and continuous permafrost may occur in the western alpine regions of the QTP (Zhou and Guo 1982). Otherwise, the maximum glaciations (0.8–0.6 mya) appeared with the gradually increased altitude of almost 3500 meters during the period of the Kunlun-Huanghe movement (Shi 1998). The climate changes induced by the uplift should also bring recurrent retreats and advances of the Plateau zokor' ranges during glacial and interglacial cycles.

The interests of researchers about the Plateau zokor were mainly focused on behavior, physiology and macroecology, the studies on population structure or phylogeographical pattern are lacking. The limited dispersal ability of this species should facilitate the detection of phylogeographical patterns because of the reduction of the homogenizing effects of long-distance dispersal (Brito 2005).

In this paper, mitochondrial gene sequences and morphological data were used to study the population structuring of Plateau zokor in the QTP. In addition, we will analyze the causes for population differentiation and its relationships with the geological events in the QTP.

2. MATERIAL AND METHODS

2.2. Mitochondrial DNA analysis

2.1. Population samples

A total of 131 individuals of Plateau zokor were collected from 12 populations (Table 1) across three provinces (Qinghai/Gansu/Sichuan) of west China. We assured that these sampling sites covered most distribution areas of the species. Among these populations, six belong to Qinghai region, three from Sichuan and three from Gansu (Fig. 1).

Tissue samples derived from muscle of field caught zokors were preserved in 95% ethanol. Total DNA was isolated from ethanol-fixed tissue by proteinase K digestion followed by standard phenol-chloroform extraction and 70% ethanol precipitation. The partial sequence of mitochondrial cytochrome *b* (*cyt b*) gene was amplified via the polymerase chain reaction (PCR) using the primers pair L14724 (5'-C GAA GCT TGA

Table 1. The geographic location, number of samples and designated haplotypes for twelve populations (see Fig. 1) of Plateau zokor (*Myospalax baileyi*).

Code of population	Province	Longitude (°)	Latitude (°)	Altitude (m)	Sample size	Haplotypes
Pop1	Qinghai	97.2354	33.3536	4390	11	Hap1
Pop2	Qinghai	97.4727	33.2015	4450	11	Hap2, 3, 4
Pop3	Qinghai	96.9437	33.7696	4550	14	Hap5
Pop4	Qinghai	99.7115	37.1693	3230	14	Hap6, 7, 8, 9, 10
Pop5	Qinghai	98.8710	37.1796	3840	14	Hap18, 19, 20
Pop6	Qinghai	100.2171	38.0699	3450	9	Hap11, 12
Pop7	Sichuan	102.7213	34.1031	3230	8	Hap13
Pop8	Sichuan	102.8900	33.9149	3450	11	Hap13, 14, 15, 16
Pop9	Sichuan	102.5333	33.4103	3490	12	Hap17
Pop10	Gansu	103.2472	34.7496	3160	10	Hap21, 22
Pop11	Gansu	103.0523	34.3674	3270	11	Hap21, 22, 23
Pop12	Gansu	103.5519	34.7393	3020	6	Hap21

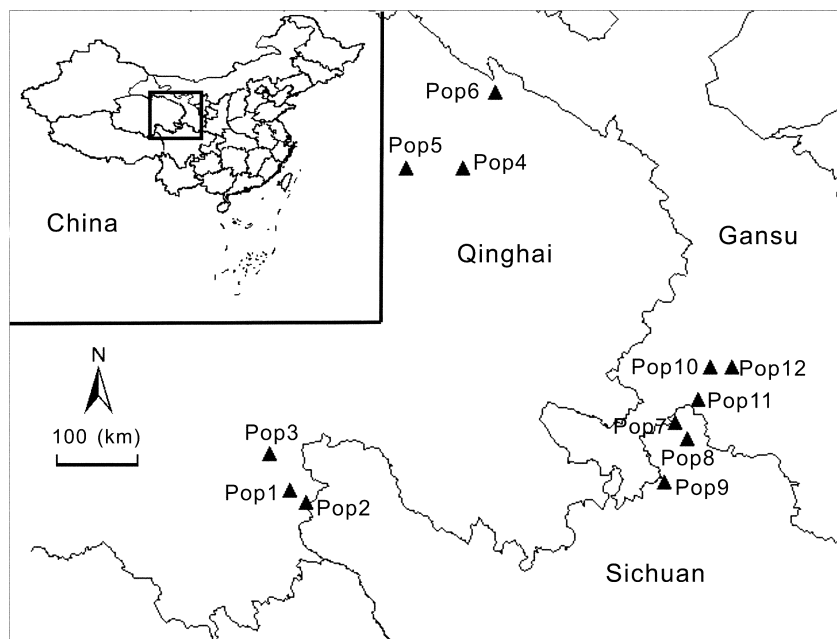


Fig. 1. Sampling sites of twelve populations of Plateau zokor.

TAT GAA AAA CCA TCG TTG-3') and H15917 (5'-C GGA ATT CCA TTT TTG GTT TAC AAG-3') (Zhou *et al.* 2004). PCR amplifications were performed in total reaction volumes of 30 μ L, containing 10 uM Tris-HCl (pH 8.0), 1.5 uM MgCl₂, 50 uM KCl, 150 uM of each dNTP, 0.3 uM of each primer (synthesized by Sangon), 0.4 μ l (about 40 ng) template DNA, and 1U *Taq* DNA polymerase (Sangon, China). 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 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.) following the manufacturer's protocol. Purified DNA fragments were directly sequenced using a MegaBACE 500 DNA Analysis System (Shanghai, China) following the manufacturer's instructions. To ensure accuracy, strands were sequenced in both directions for each individual using the same primer pairs for PCR amplification.

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 (version

4.0; Rozas *et al.* 2003). Pairwise measures of Nei's genetic distance (Nei 1978) between populations were calculated and used to construct a neighbor-joining tree using the computer program MEGA version 3.1 (Kumar *et al.* 2004). Isolation by distance was examined in the *cyt b* DNA data by plotting genetic divergence against geographical distance. The significance of correlations between genetic distance and geographical distance was determined using the Mantel test (Mantel 1967) with 10,000 matrix randomizations, as executed by the program IBD 1.52 (Bohonak 2002).

An analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) was implemented using the ARLEQUIN version 3.1 (Excoffier *et al.* 2006). Permutation tests of significance were used to test genetic variance by comparisons to null distributions with 10,000 random permutations. Nucleotide diversity and haplotype diversity were also calculated in ARLEQUIN.

2.3. Morphologic characteristics analysis

All matured individuals from twelve populations were chosen for analyses. Seventeen morphological characters (body length, hind foot length, tail length, profile length, skull basilar length, basal length, braincase height, greatest length of the nasals, least breadth between the orbits, zygomatic breadth, occipital

Table 2. Descriptive names of the seventeen morphological characters of Plateau zokor.

ID number	Abbreviation	Description for criterion of measurement
1	BL	body length (mm)
2	HFL	hind foot length (mm)
3	TL	tail length (mm)
4	PL	profile length (mm)
5	SBL	skull basilar length (mm)
6	BL	basal length (mm)
7	BH	braincase height (mm)
8	NGL	greatest length of the nasals (mm)
9	OLB	least breadth between the orbits (mm)
10	ZB	zygomatic breadth (mm)
11	OB	occipital breadth (mm)
12	DLB	least breadth of the diastema (mm)
13	MPL	median palatal length (mm)
14	TBB	breadth between the tympanic bulla (mm)
15	GMB	greatest mastoid breadth (mm)
16	PRLD	least distance between the parietal ridges (mm)
17	FRLD	least distance between the frontal ridges (mm)

Table 3. Analysis of molecular variation of twelve populations (Fig. 1) of Plateau zokor.

Source of variation	df	Sum of squares	Variance components	Percentage of variation
Among populations	11	2243.722	18.73421 Va	98.27
Within populations	119	39.210	0.32949 Vb	1.73
Total	130	2282.931	19.06371	100
Fixation Index			Fst = 0.98272 (P < 0.001)	

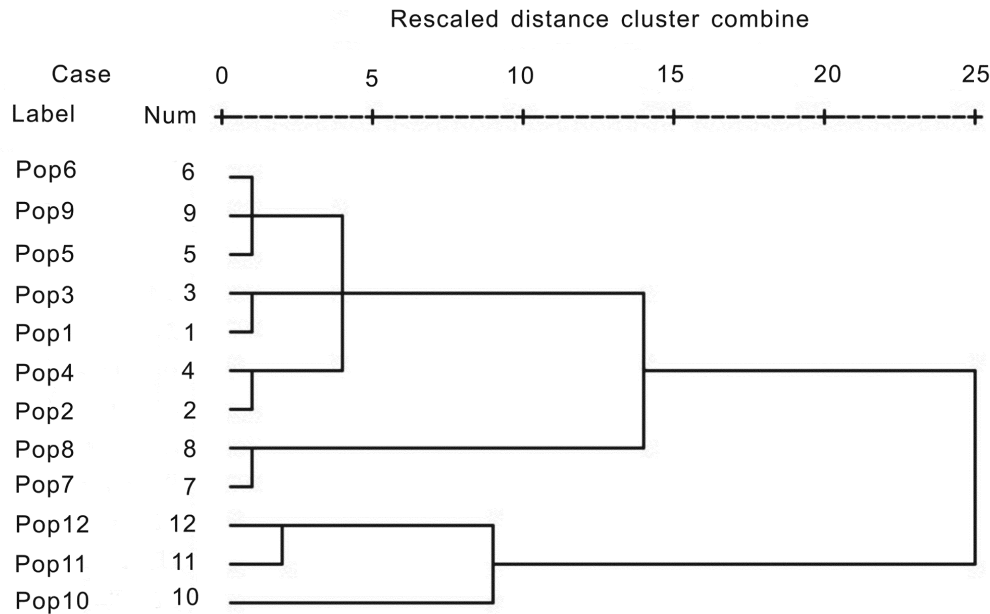


Fig. 3. Cluster analysis of seventeen morphological characters for 12 populations of Plateau zokor (see Fig. 1).

separated Pop10, Pop11 and Pop12 from all the others as a group. The second group included Pop7, Pop8 and Pop9, which all distributed in Sichuan province. The third group included Pop1, Pop2 and Pop3. The remaining three populations (Pop4, Pop5 and Pop6) were grouped together as the fourth group. This divergence pattern was intimately associated with the distributional areas.

Analysis of molecular variance (AMOVA) showed significantly population structuring and restricted gene flow. Most (98.27%) of the total variation was distributed among populations, with only 1.73% of variation within populations (Table 3). Genetic distances were also calculated for each pair of populations to determine the extent of their divergence (Table 4). Most pairwise genetic distances among populations reached higher level (0.40–1.00), except for the values among Pop10, Pop11 and Pop12 (Table 4). The tests

(10,000 permutations) of pairwise genetic distance values among all twelve populations were significant. A significant, positive correlation ($r = 0.553$; $P < 0.001$) was observed between genetic distance and geographical distance among pairwise populations.

3.2. Morphological characters analysis

Based on the cluster analysis of 17 morphological characters, a dendrogram of haplotypes covered twelve populations formed as illustration in Fig. 3. This dendrogram is divided into two large groups. The first group included Pop10, Pop11 and Pop12 with same distribution habitat in Gansu region. The remaining populations constituted the second largest group including all individuals from Qinghai and Sichuan areas. Although two subgroups diverged from the second group incompletely restricted to local region, this

population pattern could reflect to the regional differentiation basically. The Euclidean distance among each pair population nearly occurred from 1.00 to 11.00 (Table 4). The values of Euclidean distance among Pop10, Pop11 and Pop12 were highly lesser than others. The correlation coefficient and significance of the correlation of the matrices based on mitochondrial DNA and morphological data tested by the Mantel test, showed that a significant correlation existed between *cyt b* genetic distance and morphological Euclidean distance ($r = 0.557$, $P < 0.001$). A significant correlation ($r = 0.255$; $P < 0.05$) was also observed between morphological Euclidean distance and geographical distance.

4. DISCUSSION

The AMOVA analysis revealed a significant genetic differentiation within Plateau zokor, with nearly 98% of variations distributed among populations (Table 3). The phylogenetic analyses can provide important evidence that four major groups distributed mostly allopatrically (Fig. 2). This significant population structure was also supported by the results of morphological cluster analysis that showed fragmentation mainly throughout all sampled region (Fig. 3). In fact, the substantial phylogeographical structure with deep allopatric divergence was common in many rodents, such as Dusky-footed woodrat (*Neotoma fuscipes* Baird) (Matoq 2002), large Japanese field mouse (*Apodemus speciosus* Temminck) (Hirota *et al.* 2004), and true lemmings (*Lemmus* Link) (Fedorov *et al.*

2003). It may result from two crucial aspects: intrinsic factors, such as life history characteristics and dispersal ability; extrinsic factors that include historical events and physical nature of the habitat especially isolation by distance (Hurwood and Hughes 2001).

This population structure, corresponding to the second pattern of Avise *et al.* (1987), might be the results from long-term cessation of gene flow or the reproductive segregation. It is expected that two important factors appear to be responsible for the markedly restricted gene flow among geographical regions. First, as a rodent strictly adapted to subterranean lifestyle, Plateau zokor harvests food largely depend on excavating extensive burrow systems (Zhang and Liu 2003). Therefore, its territory could be mostly restricted by the particular burrowing activity. It is reported that the home range size for Plateau zokor is currently tens to hundreds square meters, with tunnel total length of males around two hundred meters (Zhou and Dou 1990). In addition, research on the speed of Plateau zokor's burrowing showed that the average speed of excavating tends to decline with increased digging time (Su 1992). These all means high energy cost underground, which badly restrict the dispersal ability and bidirectional intercourse among individuals in different geographical populations. By contraries, some rodents colonized habitats aboveground can further expand their colonies with energy cost unlimited correspondingly, such as the large Japanese field mouse (*Apodemus speciosus* Temminck) (Hirota *et al.* 2004), Red-tailed chipmunk

Table 4. The matrixes of Euclidean distance and Genetic distance among twelve populations (Fig. 1) of Plateau zokor (above diagonal is Euclidean distance; below diagonal is Genetic distance).

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10	Pop11	Pop12
Pop1		4.27	2.86	6.19	5.46	5.34	5.88	7.57	5.85	7.86	8.23	9.45
Pop2	0.88		3.17	4.62	5.62	5.44	7.58	10.03	6.18	5.81	8.68	10.91
Pop3	1.00	0.83		5.00	5.02	4.37	5.91	8.16	5.59	7.04	7.94	9.72
Pop4	0.98	0.97	0.98		3.63	4.62	6.79	9.14	4.18	5.68	7.93	9.57
Pop5	0.99	0.98	0.99	0.52		3.33	4.88	6.45	4.21	6.38	6.53	6.99
Pop6	1.00	0.99	1.00	0.524	0.85		4.23	6.13	4.20	6.46	6.19	7.40
Pop7	1.00	0.99	1.00	0.98	0.99	1.00		3.43	4.61	8.15	6.08	6.52
Pop8	0.99	0.98	0.99	0.97	0.98	0.99	0.42		6.72	10.20	6.65	5.71
Pop9	1.00	0.99	1.00	0.98	0.99	1.00	1.00	0.82		6.71	6.76	7.92
Pop10	0.99	0.99	1.00	0.98	0.99	0.99	0.99	0.95	0.99		1.16	2.67
Pop11	0.99	0.98	0.99	0.96	0.97	0.98	0.94	0.90	0.95	-0.03		1.72
Pop12	1.00	0.99	1.00	0.98	0.99	1.00	1.00	0.96	1.00	0.11	0.06	

(*Tamias ruficaudus* Howell) (Good and Sullivan 2001) and the Common vole (*Microtus arvalis* Pallas) (Haynes *et al.* 2003). Second, although Plateau zokor can disperse to the suitable habitats aboveground more speedy and farther than excavating tunnel underground usually during summer or autumn, aboveground dispersal would suffer a high risk of predation by natural enemy for absence of eyesight and slow movement (Zhou and Dou 1990). A study about the food habits of some grassland raptors reported that the skulls of Plateau zokor existed in the nests of the predators (Cui *et al.* 2003). Zhou and Dou (1990) also presumed that Plateau zokor could be caught by eagles aboveground, based on the investigation that transmitters and bowels remains from 4 of the 40 marked zokors have been found in the study areas. Thus, predation from natural enemies aboveground could also restrict the gene flow among populations.

There was perennial tundra in interior of the plateau ever since the middle Pleistocene (Shi *et al.* 1998) which might largely restrict the excavating movement of the zokor for foraging diets, colonization or dispersal. Moreover, the areas around the Gonghe basin (E98.7667°–101.3667°, N35.4500°–36.9333°) in Qinghai province should also not be fit for subsistence of the zokor, as a result of intense desiccation, serious desertification and increasing degradation of the vegetation imposed by overgrazing in recent fifty years (Sun *et al.* 2004). So the distributional ranges of the Plateau zokor may be highly influenced by the geographical barriers, with cambered distribution pattern in the northeastern edges of the QTP. Therefore, the isolation by geographical distance becomes natural barrier inducing the genetic differentiation and morphological variation among regional populations.

Overall, our results suggest significantly fragmented population pattern within Plateau zokor supported by mitochondrial DNA and morphological data. However, this study just analysed the current population divergence of this subterranean rodent, and carefully considered the possible historical events for interpretation. It is apparent that population pattern of many rodent species distributed in the QTP region is still under unknown. Therefore,

it is important to assess the population structure of other high altitude taxa, and to explore the extent of congruent patterns for them and analyze these shared pattern if can reflect common biogeographic events.

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