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Population genetic structure and historical demography of the ground beetle *Chlaenius costiger* in the Tsinling-Dabashan Mountains of central China

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ABSTRACT. Population genetic structure and demographic history of the ground beetle *Chlaenius costiger* (Coleoptera: Carabidae) in the Tsinling-Dabashan Mountains of central China were estimated using mitochondrial DNA sequences (Cox1-tRNALeu-Cox2) of 144 individuals from 16 local populations. The high haplotype diversity was accompanied by low nucleotide diversity. Phylogenetic analysis (Bayesian inference) of the 43 haplotypes revealed no phylogeographic structure. Analysis of molecular variance suggested that most of the variation was attributed to within population variation (79.26%). Mantel test results showed a significant correlation between the genetic distance and geographical distance of the populations with a correlation coefficient equal to 0.216964 ($P = 0.0471 < 0.05$), indicating the presence of isolation by distance. Spatial AMOVA and PERMUT analyses showed no phylogeographic structure. Gene flow calculated

through the number of migrants was high between many pairs of populations. The results of a neutrality test, mismatch distribution analyses, and Bayesian skyline plot analysis together showed a demographic expansion. The estimated expansion time of the whole sampled population was 0.125 million years. The complex topography in the Tsinling-Dabashan Mountains area led to the high level of genetic diversity, and migratory flight resulted in the high level of gene flow, leading to the lack of a phylogeographic structure.

Key words: Population genetic structure; Historical demography; *Chlaenius costiger*; Mitochondrial DNA; China

INTRODUCTION

The Tsinling and the Dabashan Mountains are located in the central part of China; they parallelly stretch roughly from west to east. The middle parts of these two mountain systems are separated from each other by the Hanzhong Basin (Figure 1).

Landscape affects habitat, migration, and the genetic structure of populations (Manel et al., 2003; Palo et al., 2003). The phylogeographical tools could be used to analyze the effects of landscape on species' distributions and the geographical pattern of genetic diversity (Avise, 2000). Rapid population expansion may erase the previous geographical differences of the genetic diversity (Lessa et al., 2003). The complex topographic structure, the high diversity of vegetation, and climatic factors of the Tsinling-Dabashan Mountains could increase the genetic diversity of animals that inhabit these areas, while a rapid expansion in population size and migration could lead to the absence of phylogeographic structure.

Carabid beetles are usually considered incapable of flight. However, studies indicated the existence of migratory flight in a variety of carabid species (Matalin, 2003; Chapman et al., 2005; Feng et al., 2007; Zhang et al., 2008). Migratory flight increases the level of gene flow between populations, disturbing phylogeographic structure. The flight ability in the ground beetle *Chlaenius costiger* (Schaller) (Coleoptera, Carabidae) has not been reported.

This study aims to explore the effect of the landscape characteristics, population expansion, and migratory flight on the genetic diversity and phylogeographical pattern of *C. costiger* in the Tsinling-Dabashan mountainous areas using the mitochondrial DNA (mtDNA; Cox1-tRNALeu-Cox2) as the molecular marker. The demographic history of this species was also inferred.

MATERIAL AND METHODS

Sampling, DNA extraction, polymerase chain reaction (PCR) amplification, sequencing, and flight-wing configuration

A total of 144 adults of the ground beetle *C. costiger* were collected from 16 locations in the Tsinling-Dabashan Mountains (Table 1, Figure 1). The sample sizes range from 3 to 21 individuals. The beetles were preserved in 100% ethanol and stored at -20°C. A continuous fragment (1318 bp) of the mitochondrial cytochrome oxidase I and II (Cox1-tRNALeu-Cox2) was amplified using PCR with primers co1s 5'-AAAGGAAACTTTTGGATCATTAGGAA-3' and TK-N-3782 5'-GAGACCATTACTTGCTTTCAGTCATCT-3' (Emerson et al., 1999). The forward

primer co1s 5'-AAAGGAAACTTTTGGATCATTAGGAA-3' was designed by the authors of this paper. The PCR was carried out using a TIANquick Midi Purification Kit following the protocol recommendations (Tiangen, Beijing, China). Sequencing reactions were carried out with the PCR primers using ABI Prism Bigdye™ Terminator Cycle Sequencing Ready Reaction Kit on an ABI 3730XL sequencer. Of the fragment length of 1318 bp, a total of 647 bp was sequenced for the mtCoI gene, 63 bp for the intervening tRNA Leu gene, and 608 bp for the mtCoII gene. All sequences have been deposited in the GenBank database under accession Nos. JN944265-JN944307 (*C. costiger*), JN944308-JN944309 (*C. bengalensis*), and JN644074-JN644075 (*C. pallipes*).

Table 1. Sampling locations, sample size, GPS coordinates, and CoI-tRNALeu-CoII haplotypes of *Chlaenius costiger*.

LPNs	Sampling location	SS	GPS coordinates		Elevation (m)	Haplotypes (number of individuals)	P_i	H_d
			Latitude	Longitude				
1	Guangyuan, Si	11	32.44N	105.84E	494	T3(7), T22(1), T39(1), T40(1), T43(1)	0.00204 ± 0.00065	0.618 ± 0.164
2	Lueyang, Sh	4	33.52N	105.91E	715	T3(4)	0	0
3	Shiquan, Sh	14	33.17N	108.16E	388	T3(10), T33(1), T36(1), T37(1), T40(1)	0.00053 ± 0.00020	0.505 ± 0.158
4	Shiquan, Sh	4	33.27N	108.09E	526	T3(2), T37(1), T42(1)	0.00114 ± 0.00042	0.833 ± 0.222
5	Lantian, Sh	9	34.15N	109.33E	510	T3(2), T17(1), T18(1), T25(4), T35(1)	0.00266 ± 0.00056	0.806 ± 0.120
6	Chang'an, Sh	21	34.15N	108.90E	435	T3(3), T4(3), T18(4), T23(1), T25(10)	0.00158 ± 0.00030	0.729 ± 0.077
7	Baishui, Sh	6	35.18N	109.70E	495	T3(2), T18(2), T19(1), T25(1)	0.00268 ± 0.00050	0.867 ± 0.129
8	Lushi, He	5	34.25N	111.01E	569	T3(4), T25(1)	0.00091 ± 0.00054	0.400 ± 0.237
9	Luanchuan, He	12	33.83N	111.46E	866	T3(4), T6(2), T9(5), T11(1)	0.00330 ± 0.00039	0.742 ± 0.084
10	Nanzhao, He	3	33.57N	112.11E	363	T4(1), T5(1), T18(1)	0.00354 ± 0.00128	1.000 ± 0.272
11	Xixia, He	17	33.29N	111.44E	222	T3(7), T8(1), T12(1), T13(1), T14(1), T16(1), T24(1), T29(1), T30(1), T31(1), T32(1)	0.00273 ± 0.00036	0.846 ± 0.089
12	Nanyang, He	11	32.93N	112.41E	115	T3(2), T6(1), T7(1), T10(2), T15(1), T20(1), T21(1), T26(1), T34(1)	0.00406 ± 0.00036	0.964 ± 0.051
13	Tanghe, He	5	32.68N	112.69E	113	T3(3), T6(2)	0.00228 ± 0.00066	0.600 ± 0.175
14	Yunxi, Hu	3	33.10N	110.27E	605	T3(3)	0	0
15	Zhuxi,Hu	14	32.32N	109.72E	445	T1(1), T2(1), T3(8), T6(1), T27(1), T38(1), T41(1)	0.00151 ± 0.00046	0.692 ± 0.137
16	Wushan, Ch	5	31.12N	109.97E	494	T3(1), T15(1), T28(2), T36(1)	0.00288 ± 0.00123	0.900 ± 0.161
	Total	144					0.0026 ± 0.00017	0.798 ± 0.033

LPN = local population number; Sh = Shaanxi; Si = Sichuan; Hu = Hubei; He = Henan; Ch = Chongqing; SS = sample size; P_i = nucleotide diversity; H_d = haplotype diversity.

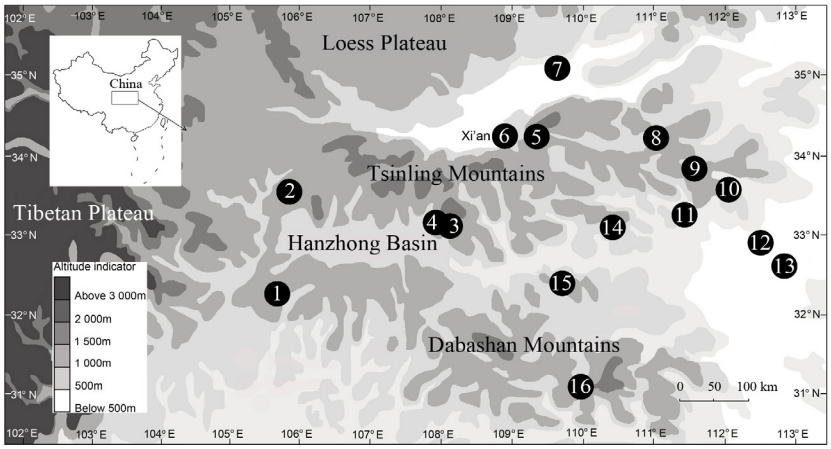

Figure 1. Sampling locations.

The development of the functional flight muscles was observed and the flight-wing configuration was estimated using the parameter $(F_L \times F_W)/(E_L \times E_W)$ where F is the flight wing, E is the elytron, L is the length, and W is the width) (Liebherr, 1988). Some specimens were ensnared by lighting to make sure that this species can fly.

Data analysis

Sequences were aligned with ClustalX 1.83 (Chenna et al., 2003) and double-checked manually. A matrix of 144 individual sequences was submitted in DnaSP 4.0 (Rozas et al., 2003). The number of variable sites, the number of parsimony informative sites, the haplotype diversity, and nucleotide diversity were determined using DnaSP 4.0 (Rozas et al., 2003). The phylogenetic relationship among mtDNA haplotypes were estimated using maximum likelihood (ML) analyses in PAUP*4.0 b10 (Swofford, 2002) and Bayesian analyses in MrBayes 3.0 (Ronquist and Huelsenbeck, 2003). The ground beetles *C. bengalensis* and *C. pallipes* were used as outgroups. ModelTest (Posada and Crandall, 1998) was used to find the best-fit substitution model for ML analyses, and the GTR + I + G model was selected based on the Akaike information criterion. The heuristic search parameters used for the ML analyses were addition sequence of taxa with the tree bisection-reconnection branch swapping, and the Multrees and Collapse options were switched on. The confidence level of ML trees was accessed by 1000 bootstrap replications. Bayesian analyses were performed with 1,250,000 generations, sampling trees every 100 generations. Likelihood values were observed with Tracer v.1.4 (Rambaut and Drummond, 2005), discarding all the trees before stability in likelihood values as a "burn in" (first 3125 trees).

The partition of genetic diversity within and among populations was analyzed by analysis of molecular variance (AMOVA) (Excoffier et al., 1992) using Arlequin 3.0 (Excoffier et al., 2005) with 1000 permutations. A Mantel test was conducted in Arlequin version 3.0 (Excoffier et al., 2005) to assess the significance of isolation by distance between populations with 10,000 random permutations on matrices of pairwise fixation index (F_{ST}) values between populations and the geographical distances. Geographical distances between populations were calculated with the help of the program at http://www.indo.com/distance/. The effective number of migrants (N_m) was estimated using the island-model equation of Wright: $F_{ST} = 1/(1 + 2N_m)$ (Wright, 1951), and the F_{ST} between locations was calculated using DnaSP version 4.0 (Rozas et al., 2003).

The spatial genetic structure of haplotypes was analyzed using the SAMOVA 1.0 program (Dupanloup et al., 2002; http://web.unife.it/progetti/genetica/Isabelle/samova.html) with 1000 permutations. The number of initial conditions was set to 100 as recommended by Dupanloup et al. (2002). The number K of groups of populations was set from 2 to 15. The K with the highest F_{CT} (genetic variance due to differences between groups of populations) represents the best number of groups and the best population configuration. Furthermore, the parameters of population diversity (H_S and H_T) and differentiation (G_{ST} and N_{ST}) were estimated following the methods described by Pons and Petit (1995, 1996) using PERMUT (http://www.pierroton.inra.fr/genetics/labo/Software/PermutCpSSR/index.html). Two different parameters (G_{ST} and N_{ST}) were compared using a permutation test with 10,000 permutations. If the value of N_{ST} is significantly higher than the value of G_{ST}, then the presence of phylogeographic structure can be assumed (Pons and Petit, 1996).

Neutrality tests were implemented in Arlequin 3.0 (Excoffier et al., 2005), Fu's F_S test (Fu, 1997) and Tajima's D (Tajima, 1989) were used to detect evidence of recent demographic expansion within each inferred clade, under which negative values are expected (Schneider and Excoffier, 1999). Also, mismatch analysis of Cox1-tRNALeu-Cox2 sequences were performed using the program Arlequin to explore the demographic history of the populations studied, and the parameters of demographic expansion were estimated. A recent growth is expected to generate a unimodal distribution of pairwise differences between sequences (Rogers and Harpending, 1992). The validity of the expansion model was tested using the sum of squared deviations and Harpending's raggedness index between observed and expected mismatches. Furthermore, the demographical history of this species was estimated using the Bayesian Skyline Plot (BSP) approach conducted in BEAST v1.4.7 (Drummond and Rambaut, 2007). A standard rate of 2.3% substitutions/site/million years was equivalent to a per-branch rate of 0.0115 substitutions/site/million year (Brower, 1994).

RESULTS

mtDNA variation

Thirty-two positions were polymorphic sites composed of 22 parsimony informative sites and 10 singleton variable sites. These polymorphic sites defined 43 haplotypes within the 144 individuals sampled from 16 localities (Table 1 and Figure 1). The haplotype diversity of total or every sampled population was relatively high and was accompanied by a lower nucleotide diversity. Of the haplotypes, 20.93% were common haplotypes and 79.07% were private haplotypes with a relatively low frequency (Table 1). The haplotype diversity for all sampled specimens was 0.798 and the nucleotide diversity was 0.0026. The values for the 2 parameters were 0 in the 2nd and 14th sampling locations because only 1 haplotype was found at both locations (Table 1). Transitions were more frequent than transversions (transitions/transversions = 6.807).

Flight-wing configuration, development of flight muscles, and ensnaring by lighting

The flight-wings fully developed, and the area of the flight-wing was over 1.8 times [1.83 ± 0.28 (N = 20)] the area of the elytron, indicating a high flight potential. The functional flight muscles belong to class 5 according to Tietze's classification, which indicated full-grown flight muscles that enable a beetle to fly (Tietze, 1963). Individuals were successfully ensnared by lighting, indicating that this species possesses a certain flight ability.

Phylogenetic relationships

The ML tree had a topology slightly different from the Bayesian tree (Figure 2). There is no phylogeographic structure in the phylogenetic tree.

Population genetic structure and gene flow

AMOVA with a significant F_{ST} value suggested that most of the variation was attributed to variation within populations (79.26%), while differentiation of among populations only contributed 20.74% of the total (Table 2).

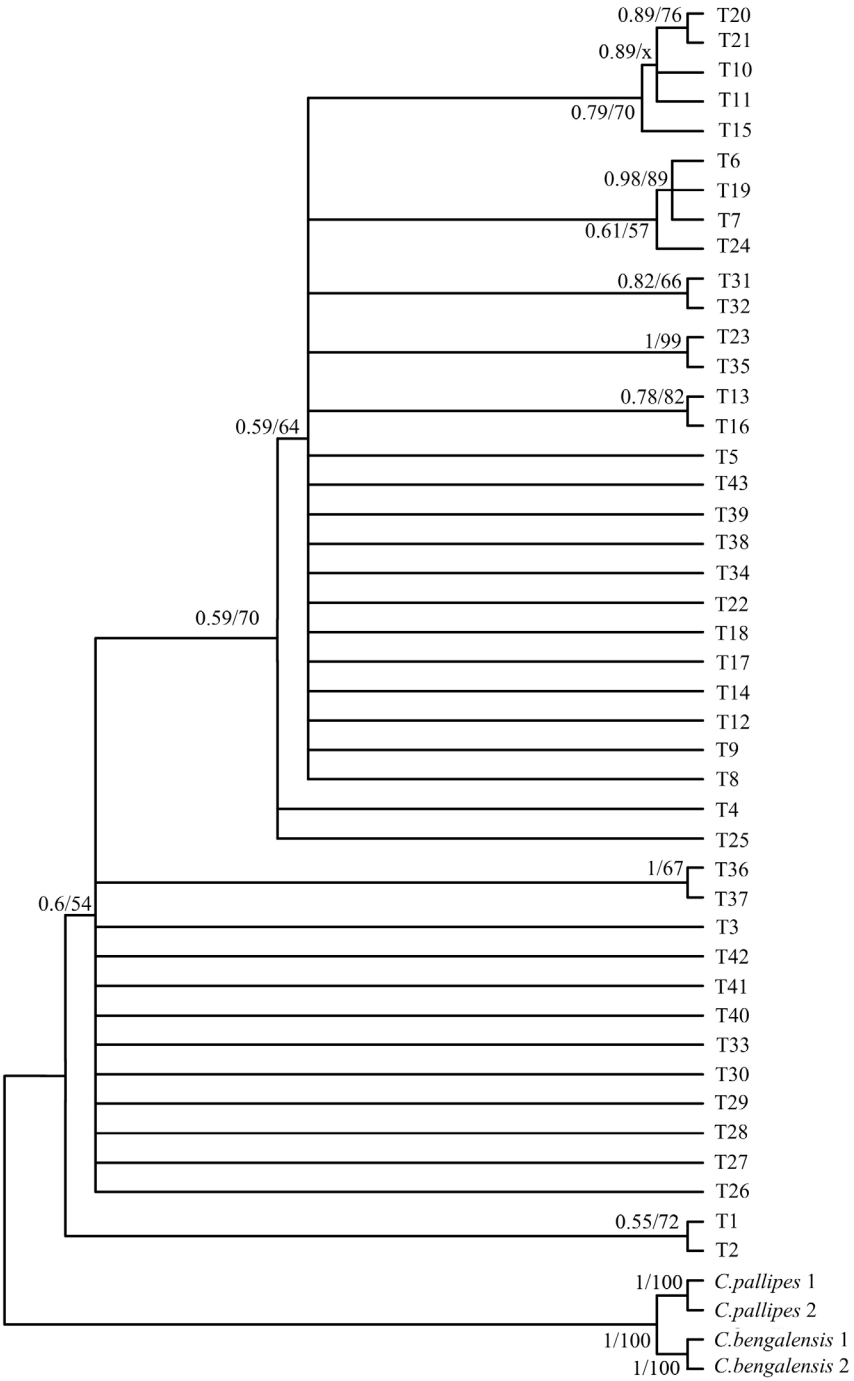

Figure 2. Bayesian tree. Numbers beside the nodes represent the Bayesian posterior probability/maximum likelihood bootstrap; "x" indicates a very low bootstrap value.

Table 2. Analysis of molecular variance (AMOVA) of *Chlaenius costiger*.

Source of variation	df	Sum squares	Variance components	Percentage of variation	Fixation index
Among population	15	68.431	0.36174^{va}	20.74	F_{ST} = 0.2074**
Within population	128	176.958	1.38248^{vb}	79.26	
Total	143	245.389	1.74422		

**P < 0.01; F_{ST}, correlation within populations relative to total. ^{va} represents the covariance component due to differences among the G populations (σ_a^2), while ^{vb} represents the covariance component due to differences among haplotypes in different populations within a group (σ_b^2) (http://cmpg.unibe.ch/software/arlequin35/man/Arlequin35.pdf).

The total genetic diversity H_T (0.767) among all sampled populations was higher than the average within-population diversity H_S (0.656); consequently, the population differentiation across the entire range of the species was considerably high. For the spatial AMOVA (SAMOVA), increasing K values from 2 to 15 were selected, and the F_{CT} values were fairly low and steadily fluctuated from F_{CT} = 0.2165 (when K = 2) to F_{CT} = 0.3887 (when K = 14). Thus, SAMOVA failed to reveal any meaningful phylogeographic groups. Additionally, the results of PERMUT analyses detected no phylogeographic structure of the sampled populations based on the mtDNA haplotype data because the total N_{ST} (0.210) was not significantly higher than G_{ST} (0.145; P = 0.0526).

Mantel test results showed a significant correlation between the genetic distance and geographical distance of the populations with a correlation coefficient equal to 0.216964 (P = 0.0471 < 0.05), indicating the presence of isolation by distance.

Gene flow calculated through the N_m was high between many pairs of populations (Table 3), which suggests a high level of gene flow in the population of this species.

Table 3. Gene flow (migrants per generation, N_m) and fixation index (F_{ST}) values.

Sampling sites	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1		-0.069	0.017	0.005	0.11	0.14	0.078	0.03	0.121	0.17	0.019	0.147	0.131	0.064	0.016	-0.03
2	-7.74		-0.098	-0.063	0.14	0.161	0.121	0	0.165	0.242	0.011	0.198	0.182	-0.105	-0.027	-0.088
3	28.54	-5.61		-0.025	0.265	0.307	0.325	0.152	0.346	0.448	0.182	0.359	0.347	0.158	0.063	0.033
4	91.41	-8.5	-20.36		0.158	0.192	0.147	-0.067	0.244	0.31	0.091	0.257	0.185	0	-0.013	-0.04
5	4.05	3.08	1.39	2.67		-0.01	-0	-0.048	0.104	0.034	0.102	0.093	-0.015	0.227	0.102	0.104
6	3.07	2.61	1.13	2.1	-49.38		0.028	-0.05	0.16	0.075	0.153	0.143	0.033	0.285	0.14	0.149
7	5.92	3.64	1.04	2.91	-123.05	17.58		-0.153	0.053	-0.07	0.04	0.042	-0.1	0.278	0.077	0.036
8	16.21	*	2.78	-8	-11	-10.55	-3.77		0.174	0.148	0.056	0.159	0	0	-0.035	-0.077
9	3.62	2.53	0.95	1.55	4.3	2.63	8.87	2.38		0.041	0.06	0.055	0.02	0.356	0.172	0.138
10	2.43	1.56	0.61	0.74	14.42	6.19	-7.69	2.87	11.58		0.119	0.01	-0.067	0.444	0.206	0.188
11	26.51	46.54	2.25	5.02	4.4	2.77	12.13	8.48	7.77	3.7		0.115	0.092	0.218	0.065	-0.002
12	2.91	2.02	0.89	1.45	4.89	2.98	11.38	2.64	8.53	48.52	3.85		0	0.356	0.173	0.135
13	3.33	2.25	0.94	2.2	-3.57	14.45	-5.5	*	24.93	-8	4.94	*		0.333	0.136	0.136
14	7.26	-5.25	2.671	*	1.7	1.25	1.3	*	0.91	0.63	1.79	0.9	1		0.003	-0.047
15	30.75	-19.21	7.39	-37.73	4.4	3.07	5.97	-14.77	2.41	1.93	7.15	2.4	3.17	161.84		-0.029
16	-16.9	-6.21	14.68	-13	4.33	2.87	13.4	-7	3.12	2.16	-325.18	3.21	3.17	-11.1	-17.8	

N_m values are shown below the diagonal, and F_{ST} values are shown above the diagonal; *F_{ST} = 0.0, N_m is considered undefined when N_m is negative or infinity (F_{ST} = 0.0).

Demographic history

The results of the neutrality test of the total population indicated that Fu's F_S was a significant negative value, while Tajima's D (P value) = -1.194 (P = 0.09). Mismatch distribu-

tion analyses showed a unimodal frequency distribution of pairwise differences in the total population, and both the sum of squared deviations and Harpending's raggedness index test cannot reject the hypothesis of a sudden expansion model (Figure 3). The estimated expansion time of the whole sampled population was 0.125 million years, which is consistent with the result of BSP analysis (Figure 4).

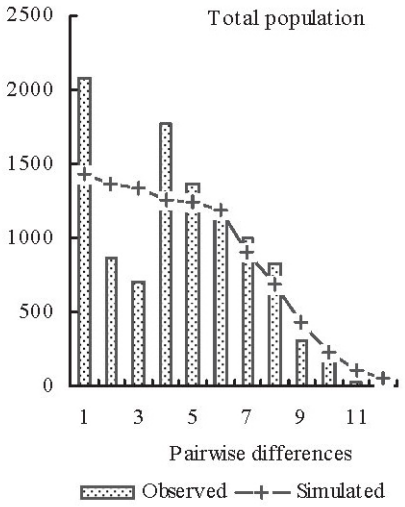

Figure 3. Mismatch distribution analysis. The histograms represent the observed frequencies of pairwise differences among haplotypes, and the line shows the curve expected for a population that has expanded.

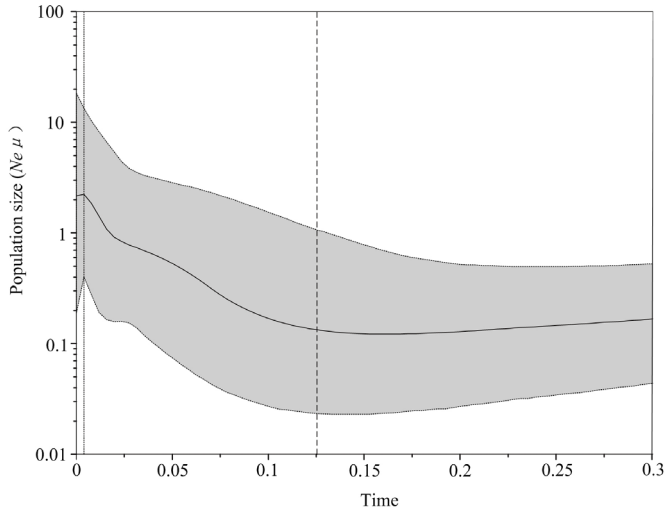

Figure 4. A Bayesian skyline plot. The x-axis is in units of million years in the past, and the y-axis is N_E x μ (effective population size x mutation rate per site per generation). The median estimates are shown as thick solid lines, and the 95% highest posterior density (HPD) limits are shown in gray.

DISCUSSION

It is difficult to determine the origin center or the glacial refugium for the Tsinling-Dabashan Mountains population because most local populations showed high genetic diversity. The high level of habitat diversity on the Tsinling-Dabashan Mountains is probably one of the main factors that led to the high genetic diversity in this species.

AMOVA showed significant genetic differentiation within populations. The low genetic variation among the local populations, on the other hand, affirmed the lack of phylogeographic structure in the Tsinling-Dsbashan Mountains population of this species.

SAMOVA and the PERMUT analyses showed no significant phylogeographic structure.

Mantel test results showed significant correlation between the genetic distance and geographical distance of the populations ($P = 0.0471 < 0.05$), indicating the presence of an isolation-by-distance pattern of genetic divergence, which suggests that the distribution of genetic variation is due to geographical separation rather than natural selection.

The high haplotype diversity and low nucleotide diversity of the total population implied that the population probably underwent expansion after a period of low effective population size. The unimodal mismatch frequency distribution pattern based on the mtDNA sequence was fairly consistent with the predicted distribution under a model of population expansion (Rogers and Harpending, 1992). Tajima's D value and Fu's F_S values are sensitive to bottleneck effects and population expansion, which lead to the more negative values of Tajims's D and Fu's F_S (Tajima, 1993, 1996; Aris-Brosou and Excoffier, 1996; Martel et al., 2004), and the latter statistic is more sensitive to recent population growth (Fu, 1997). Additionally, neither the sum of squared deviations nor Harpending's raggedness index test can reject the hypothesis of a sudden expansion model. A population with a high haplotype diversity value ($H_d > 0.5$) and low nucleotide diversity ($P_i < 0.005$ or 0.5%) indicates that it experienced a bottleneck effect (Grant and Bowen, 1998). On the other hand, high haplotype diversity values and lower nucleotide diversity values indicate that the time after population expansion is long enough to examine the change in haplotypes that resulted from mutation, but it is not long enough to accumulate big differences among sequences (Avise, 2000). The estimated expansion time of the whole sampled population was 0.125 million years.

The parameter $(F_L \times F_W)/(E_L \times E_W)$ is very close to that of species that exhibited flight (Liebherr, 1988). Migratory flight could lead to a high level of gene flow, which results in a lack of phylogeographic structure. Another cause for the lack of phylogeographic structure is the expansion in population size, which lasted 0.125 million years in this species. Considering the slow population expansion and constant migration that occurred over hundreds of kilometers every generation with a large N_m between some sampling sites, the lack of phylogeographic structure is due to the migratory flight rather than to population expansion.

In conclusion, the complex topography in the Tsinling-Dabashan Mountains area led to the high level of genetic diversity, and migratory flight resulted in the high level of gene flow leading to the lack of phylogeographic structure.

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