Molecular Ecology (2006) **15**, 2119–2130 doi: 10.1111/j.1365-294X.2006.02875.x

Phylogeography of the mole-shrew (*Anourosorex yamashinai*) in Taiwan: implications of interglacial refugia in a high-elevation small mammal

SHOU-LI YUAN,* LIANG-KONG LIN* and TATSUO OSHIDA†‡
**Laboratory of Wildlife Ecology, Department of Life Science, Tunghai University, Taichung, Taiwan 407, ROC,*
†Laboratory of Molecular Ecology, Department of Life Science, Tunghai University, Taichung, Taiwan 407, ROC

Abstract

To test the Pleistocene interglacial refugia hypothesis with a high-elevation mammal, we studied the phylogeography of the mole-shrew (*Anourosorex yamashinai*) using partial mitochondrial cytochrome *b* gene sequences (737 bases). This shrew is endemic to Taiwan. It is mainly distributed in the highlands from 1000 to 2500 m in elevation. We examined 103 specimens from 24 localities in three mountain ranges of Taiwan and found 36 haplotypes. These haplotypes separated into two major phylogroups (Northern and Southern) plus a minor phylogroup (Houhuan) of only one haplotype. This demonstrated strong association with geography. The formation of these three phylogroups may be the result of interglacial refugia during the middle Pleistocene. Distinct sublineages were not found within each major phylogroup, suggesting that the populations (phylogroups) explosively expanded from the interglacial refugia of ancestral founder haplotypes. The present distribution pattern of haplotypes suggests that Mount Houhuan is an effective refugium in central Taiwan. It was not possible to specify the refugia for the Northern and Southern phylogroups.

Keywords: *Anourosorex yamashinai*, cytochrome *b*, geographical isolation, mole-shrew, phylogeography, Taiwan

Received 26 May 2005; revision received 20 October 2005; accepted 9 November 2005

Introduction

The refugia theory proposed by Haffer (1969) has been extensively used to explain the movement of floral and faunal distributions in response to the global climatic changes during Pleistocene glaciations (e.g. Crespi *et al*. 2003; Weider & Hobæk 2003). This theory suggests that populations were isolated when their habitats were disjoined during cyclic expansions and contractions of forests during alternating warm and cold episodes of the Pleistocene (Haffer 1969; Cracraft & Prum 1988). For high-elevation species adapted to colder environments, the Pleistocene glaciations are thought to have provided opportunities for range expansions from high elevations to lower elevations, while during the Pleistocene interglacials, in contrast, ranges of high-elevation species were thought to be strongly restricted to reduced and fragmented refugia in alpine regions (Brown & Gibson 1983; Delcourt & Delcourt 1991; Britten *et al*. 1995; Sullivan *et al*. 2000). Indeed, in the present interglacial period (Holocene), many climatically relict species exist at high elevations (Cox & Moore 2000). For instance, several pika (*Ochotona*) species adapted to arid and cold environments are distributed in the fragmented alpine rocky regions (e.g. Smith 1988; Yu *et al.* 1997).

To test the interglacial refugia hypothesis, we investigated the phylogeography of the mole-shrew (*Anourosorex yamashinai*) in Taiwan, using partial mitochondrial cytochrome *b* gene sequences. This semifossorial shrew (Alexander *et al*. 1987; Corbet & Hill 1992) occurs in mountainous areas of Taiwan from 500 to 3200 m in elevation, but is most common in moist forests or grasslands at 1000–2500 m (Yu 1994). Identification of any distinct phylogeographical structure may permit examination of historical population isolations occurring before the present interglacial.

Correspondence: Liang-Kong Lin, Fax: +866-4-23595845; E-mail: lklin@mail.thu.edu.tw
‡Present address: Laboratory of Wildlife Ecology, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan

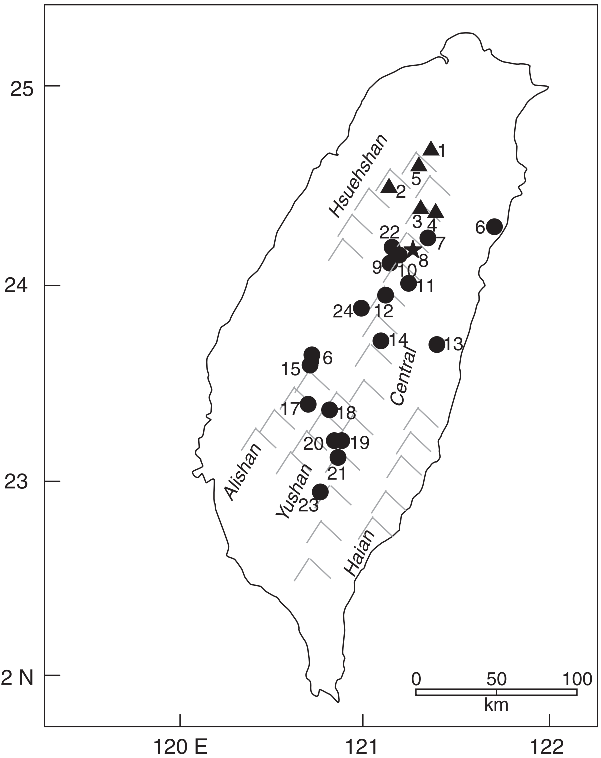

Fig. 1 Sampling localities of *Anourosorex yamashinai* in Taiwan. Numbers correspond to localities identified in Table 1. The Northern mtDNA lineage is indicated with triangles and the Southern one with circles. A star indicates the Houhuan lineage and the locality where all three lineages coexisted. Successive triangle motifs in grey indicate mountain ranges (named).

Anourosorex yamashinai was formerly classified as a Taiwanese form of *Anourosorex squamipes* (Kuroda 1935; Motokawa & Lin 2002). Recently, on the basis of chromosomal characteristics, Motokawa *et al*. (2004) classified this form as a distinct species endemic to Taiwan. They demonstrated that the diploid and fundamental autosomal arm numbers of *A. squamipes* from southern China are 48 and 92, respectively, while those of *A. yamashinai* are 50 and 96, respectively. Although multiple colonizations by this species from the Asian mainland to Taiwan might have occurred in the past, at present phylogeographical information based on molecular data is available only from the Taiwan population.

In Taiwan, mountainous areas account for about 70% of the island. There are several long mountain ranges with elevations > 2000 m (Fig. 1). This makes Taiwan an appropriate locale to test refugia hypotheses for high-elevation species. For instance, Huang *et al*. (2002) inferred that southeastern Taiwan might be a refugium for *Cyclobalanopsis glauca*, a tree occurring from 400 to 2500 m in elevation. Thus, a Pleistocene glacial refugium has already been proposed for plants in Taiwan. Pleistocene interglacial refugia, however, have not yet been examined in any of Taiwan's fauna.

We chose mitochondrial cytochrome *b* sequences to test for Pleistocene interglacial refugia in the phylogeography of *A. yamashinai*. These sequences were successfully used in studies of phylogeographical structures of other shrew populations (Demboski & Cook 2001; Maldonado *et al*. 2001; Ohdachi *et al*. 2001; Stewart *et al*. 2002).

Deep divergences among regional populations would provide evidence for the existence of isolated or fragmented refugia during the Pleistocene interglacial periods. In contrast, absence of distinct phylogeographical groups would suggest that *A. yamashinai* populations were not isolated in the past. This would indicate that either there were no refugia or that the populations expanded extremely rapidly during the last glaciation and thereby erased evidence of geographical structuring throughout Taiwan.

Materials and methods

Study regions and specimens

The profile of *Anourosorex yamashinai* examined in this study is shown in the Appendix. From January 2001 to April 2003, specimens of *A. yamashinai* were collected principally from three mountain ranges: the Hsuehshan, Central, and Alishan ranges (Fig. 1). A total of 103 individuals were collected from 24 localities in these three mountain ranges at elevations of 800–3580 m. Fourteen individuals from two sampling localities (numbers 6 and 13) came from elsewhere (Fig. 1, Table 1, Appendix). Specimens except for 17 in our personal collection were stored in the National Museum of Natural Science (NMNS), Taichung, Taiwan. At present, there are two species (*squamipes* and *yamashinai*) in the genus *Anourosorex* (Motokawa *et al*. 2004). Therefore, three cytochrome *b* sequences (haplotypes MC1, MC2, and MC3; see Appendix) from 10 specimens of *Anourosorex squamipes* were used to root the phylogenetic tree. These specimens were captured in Szechuan in southern China.

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from 99% ethanol-preserved liver or muscle tissue using the phenol–chloroform method (Sambrook *et al*. 1989). The central portion of the cytochrome *b* gene was amplified using polymerase chain reaction (PCR), with newly designed primers: L14183 5′-CATCGTTGTTATTCAACTATAGGAAC-3′ and H15443 5′-GAATATCAGCTTTGGGTGTTGATA-3′. Primer names correspond to the light (L) or heavy (H) strand and the 3′end-position in the *Sorex unguiculatus* mtDNA sequence (Ohdachi *et al*. 2001; Lin *et al*. 2002). The 50-μL reaction mixture contained 50 ng of genomic DNA, 7.5 picomoles of each primer, 0.2 mM dNTPs in reaction buffer including 2.0 mM Tris-HCl (pH 8.0), 0.01 mM EDTA, 0.1 mM DTT, 0.1% Triton X-100, 5% glycerol, and

Table 1 Sampling localities, phylogroups, and mtDNA haplotypes of *Anourosorex yamashinai* examined in this study. N, S, and H indicate the Northern, Southern, and Houhuan phylogroups, respectively

No. in Fig. 1	Locality (county)	Phylogroup	Haplotype (no. of specimens)
1	Li-dungshan (Hsinchu)	N	T1 (1), T4 (4), T5 (1), T6 (3), T9 (1)
2	Guanwu (Miaoli)	N	T4 (1)
3	Wulin farm (Taichung)	N	T3 (1), T4 (1), T7 (1), T8 (2)
4	Suyuanyakou (Yilan)	N	T4 (2)
5	Jhenshibao (Hsinchu)	N	T4 (2)
6	Heping (Hualian)	S	T22 (1)
7	Guanyuan (Hualian)	S	T19 (1), T20 (1), T25 (2)
8	Mount Houhuan (Hualian)	N	T2 (1), T4 (1)
		S	T11 (1), T12 (1), T25 (2)
		H	T10 (1)
9	Meifung (Nantou)	S	T13 (1), T25 (4)
10	Tsufung (Nantou)	S	T16 (1), T25 (1), T36 (1)
11	Nenggau (Nantou)	S	T25 (1), T26 (1)
12	Aowanda (Nantou)	S	T25 (1)
13	Wanrong (Hualian)	S	T13 (13)
14	Danda (Nantou)	S	T13 (1), T14 (1), T15 (3)
15	Xitou (Nantou)	S	T27 (2), T32 (1)
16	Shalinxi (Nantou)	S	T28 (2)
17	Alishan (Jiayi)	S	T29 (5), T30 (1), T31 (1), T32 (1), T35 (10)
18	Tataka (Nantou)	S	T29 (1), T32 (2), T33 (1), T34 (1), T35 (3)
19	Mount Taguan (Kaohsiung)	S	T21 (1)
20	Tianchi (Kaohsiung)	S	T24 (1), T25 (1)
21	Mount Guan (Kaohsiung)	S	T24 (1)
22	Rueiyanshi (Nantou)	S	T25 (1)
23	TengJhih (Kaohsiung)	S	T17 (1), T18 (1), T25 (3)
24	Wujie Dam (Nantou)	S	T23 (1)

1.0 U of r*Taq* DNA polymerase (Viogene). Amplification was carried out for 35 cycles using the following programme: 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 2 min. The extension reaction was completed by incubating at 72 °C for 10 min. The PCR products were purified with PCR Clean Up-M (Viogene) and directly sequenced with an automated DNA sequencer (ABI PRISM 377–96 Sequencer with ABI PRISM 3100 Genetic Analyser, Applied Biosystems). The PCR primer L14183 and one newly designed internal primer (L14577 5′-GTAATAGCCACCGCCTTTATAGG-3′) were used for sequencing. Purification of PCR products and sequencing were carried out by Mission Biotech Co. Ltd.

Sequence and phylogeographical analyses

Sequence alignment was used to identify haplotypes with CLUSTAL W in BIOEDIT 5.0.9 (Hall 1999). MODELTEST version 3.06 (Posada & Crandall 1998) was employed to find the best nucleotide substitution model in PAUP version 4.0 beta10 (Swofford 2001). The hierarchical likelihood-ratio test (hLRT) with an outgroup, implemented in MODELTEST, selected the Tamura–Nei (TN93) model (Tamura & Nei 1993). The best model included the gamma shape parameter (G). Neighbour-joining (NJ) analysis (Saitou & Nei 1987) was employed with the TN93 model following a gamma (G) distribution for variable sites in PAUP version 4.0 beta10. The maximum-parsimony (MP) tree was constructed with the heuristic search algorithm with tree-bisection–reconnection in PAUP. To assess nodal support, bootstrapping (Felsenstein 1985) was performed with 5000 replicates in the NJ analysis and 500 replicates in the MP analysis. To select suitable outgroups for the phylogenetic analysis, in addition to *A. squamipes*, sequences of four species in the subfamily Soricinae (*Soriculus fumidus*, accession number AF348081; *Soriculus sodalis*, accession number AB127978; *Sorex isodon*, accession number AB028500; *Sorex unguiculatus*, accession number AB028501) were tested. Haplotype diversity (h) and nucleotide diversity (π, the average number of nucleotide differences per site between two sequences) (Nei 1987) within phylogroups were calculated using DNASP 3.53 (Rozas & Rozas 1999).

To calculate divergence time between phylogroups, we estimated raw and net nucleotide divergences (Da and Dxy) with standard deviations (SD) between phylogroups (Nei 1987) with the less sophisticated Jukes–Cantor correction (Jukes & Cantor 1969) in DNASP 3.53. Divergence time (T) between phylogroups was estimated as $T = D\text{a}/2\mu$, where 2μ is the divergence rate. The standard divergence rate for mammalian mtDNA is 2%/million years (Myr) (Wilson *et al*. 1985; Avise *et al*. 1998). Following Maldonado *et al*. (2001), we applied this divergence rate (2%/Myr) for estimation of divergence between major phylogroups. Fumagalli *et al*. (1999) reported a 1.36%/Myr cytochrome *b* divergence rate for third position transversions in shrews, based on a 20-million-years-ago split between the shrew subfamilies Crocidurinae and Soricinae as suggested by fossil records (Reumer 1989, 1994). Also, based on third position transversions, Stewart *et al*. (2002) estimated that the divergence between the shrew species *Sorex maritimensis* and *Sorex arcticus* was 2.4 million years ago (Ma). Unfortunately, we could not use these more reliable divergence rates, because there were no diagnostic third position transversions between phylogroups of *A. yamashinai*. To estimate gene flow between phylogroups, the fixation index (F_{ST}) and number of female migrants per generation (*Nm*) were calculated in DNASP 3.51, according to Hudson *et al*. (1992).

To assess whether there was significant geographical differentiation, the partitioning of total genetic variation was hierarchically examined by an analysis of molecular variance (AMOVA) (Excoffier *et al*. 1992) in ARLEQUIN 2.001 (Schneider *et al*. 2000). The significance of fixation indices (Φ_{ST}, Φ_{SC}, and Φ_{CT}) was tested using permutation procedures described by Excoffier *et al*. (1992) with 1000 permutations.

A nested clade analysis (Templeton *et al*. 1995; Templeton 1998) was also carried out. A haplotype cladogram was constructed using statistical parsimony with a 95% probability limit between haplotypes (Templeton *et al*. 1992) in TCS version 1.13 (Clement *et al*. 2001). For this cladogram, GEODIS version 2.0 (Posada *et al*. 2000) was used to test associations between haplotypes and geography. This program calculates clade distance D_c(X) (average distance of individuals in clade X from the geographical centre of clade X) and the nested clade distance D_n(X) (average distance of individuals in clade X from the geographical centre of the higher-level clade in which clade X is nested). To test whether these distances were significantly small or large at the 5% level, we used 1000 permutations with the Monte Carlo technique (Roff & Bentzen 1989). Output from the GEODIS program was interpreted using the inference key in Templeton (1998, 2004).

Results

Nucleotide sequences

Partial cytochrome *b* sequences (737 bp) from 103 individuals of *Anourosorex yamashinai* yielded 36 distinct haplotypes (Table 2). Sequencing the same cytochrome *b* segment from 10 *Anourosorex squamipes* individuals yielded three different haplotypes. All sequences (Appendix) were deposited in the DNA Data Bank of Japan (DDBJ). Among the *A. yamashinai* haplotypes, there were 53 polymorphic sites of which 22 had parsimony-informative characters (Table 2). In these sites, 13, 6, and 34 occurred first, second, and third codon positions, respectively. The average number of nucleotide differences between two haplotype sequences (Tajima 1983) was 6.48. The ratio of transitional substitutions to transversional substitutions, calculated from all pairwise comparisons among 36 haplotypes, was about 7.1, showing little influence of saturation by multiple substitutions. The pairwise nucleotide differences among the three *A. squamipes* sequences were 1–2. In pairwise sequence comparisons between *A. yamashinai* and *A. squamipes*, the nucleotide differences were 39–47. The number of distinct diagnostic sites separating the two species was 31.

Phylogenetic analysis

Sequence differences between *Anourosorex* and *Soriculus* were approximately 17.5–19.1%. Those between *Anourosorex* and *Sorex* were 17.0–19.8%. Both *Anourosorex* species were clustered together in the NJ tree under the TN93 + G model, showing significant monophyly with a 100% bootstrap value (tree not shown). In addition, distinct clusters of *A. yamashinai* and *A. squamipes* were supported with 100% and 83% bootstrap values, respectively. The relationships of *A. yamashinai* haplotypes to one another with *A. squamipes*, *Soriculus*, and *Sorex* as outgroups were identical to those with only *A. squamipes* as an ourgroup. Therefore, only *A. squamipes* was used to root the phylogenetic trees of *A. yamashinai*.

An NJ tree constructed with the TN93 + G model showed two major mtDNA phylogroups (Northern and Southern) of *A. yamashinai* (Fig. 2). One haplotype (T10 of the Houhuan phylogroup) was separated from both major phylogroups (Fig. 2). The Northern and Southern phylogroups were well supported, with nodal bootstrapping values of 98% and 85%, respectively. The phylogenetic position of the Houhuan phylogroup was not clear, with low nodal support (60%). Therefore, polytomic divergence of three lineages was implied. Interestingly, members of all three phylogroups coexisted only at Mount Houhuan (locality 8) (Fig. 1, Table 1). Locality 8 and the surrounding localities (7, 9–11, and 22) were occupied by haplotypes from the Southern lineage (Fig. 1, Table 1). Within the Northern phylogroup, phylogenetic relationships among haplotypes were not resolved, consistent with the small pairwise differences found in this segment of the mitochondrial genome. The overall phylogenetic relationships of haplotypes in the Southern phylogroup were also unclear. Most nodes received little support, although two high bootstrap values supported clustering of haplotypes T11 and T12 (85%) and haplotypes T16 and T17 (91%). The MP analysis produced a single most-parsimonious tree, with a length of 105, consistency index of 0.838, and retention index of 0.921. This tree (not shown) had the same

Table 2 Variable nucleotide positions within a 737-bp sequence of cytochrome *b* in *Anourosorex yamashinai* populations in Taiwan. The sequenced portion corresponds to positions 106-842 in light strand of *Sorex unguiculatus* cytochrome *b*. Sequences of the outgroup (*A. squamipes*) at these sites are shown. N, H, and S in parentheses indicate the Northern, Houhuan, and Southern phylogrougs, respectively. Dots indicate the same nucleotide as in haplotype T1

Haplotype (phylogroup)	Positions of variable nucleotides																																																				
	2	3	30	60	64	81	89	123	127	135	147	171	175	177	180	187	201	207	217	285	321	343	360	363	372	373	384	393	423	426	435	450	460	469	513	526	552	564	571	589	590	591	600	604	627	644	651	659	684	693	716	729	735
Codon positions	2	3	3	3	1	3	2	3	1	3	3	3	1	3	3	1	3	3	1	3	3	1	3	3	3	1	3	3	3	3	3	3	1	1	3	1	3	3	1	1	2	3	3	1	3	2	3	2	3	3	2	3	3
T1(N)	T	A	T	T	T	T	C	T	T	T	A	C	T	A	T	G	A	T	T	G	C	C	T	C	T	T	A	C	C	A	T	A	G	C	C	T	C	C	A	G	T	C	G	T	G	T	C	A	T	T	T	T	T
T2(N)	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
T3(N)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•
T4(N)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
T5(N)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	C	•
T6(N)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
T7(N)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	C	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
T8(N)	•	•	•	•	•	•	•	•	•	•	•	•	C	•	•	•	•	•	•	A	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
T9(N)	•	•	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
T10(H)	•	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	C	•	A	•	T	•	T	•	•	•	•	•	•	•	•	•	•	T	C	•	T	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•
T11(S)	•	•	•	C	C	C	•	•	•	•	•	•	•	•	•	•	•	C	•	A	•	•	•	T	•	•	G	•	•	•	•	•	•	T	T	C	•	T	•	A	•	•	A	•	•	•	T	•	•	•	•	•	•
T12(S)	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	C	•	A	T	•	•	T	•	•	G	•	•	•	•	•	•	T	T	C	•	T	•	A	•	•	A	•	•	•	T	•	•	•	•	•	•
T13(S)	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	C	•	A	•	•	•	T	•	•	•	•	•	•	•	•	•	T	T	C	•	T	•	•	C	•	•	•	•	•	T	•	•	•	•	•	•
T14(S)	C	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	C	•	A	•	•	•	T	•	•	•	•	•	•	•	•	•	T	T	C	•	T	•	•	C	•	•	•	•	•	T	•	•	•	•	•	•
T15(S)	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	C	•	A	•	•	C	T	•	•	•	•	•	•	•	•	•	T	T	C	•	T	•	•	C	•	•	•	•	•	T	•	•	•	•	•	•
T16(S)	•	C	•	C	•	C	•	A	•	C	•	•	•	•	•	•	G	C	•	A	A	•	•	T	•	•	•	•	•	•	•	G	•	T	T	C	•	T	•	•	C	•	•	•	•	•	T	•	•	•	•	•	•
T17(S)	•	C	•	C	•	C	•	A	•	•	•	•	•	•	•	•	•	C	•	A	A	•	•	T	•	•	•	•	•	•	•	G	•	T	T	C	•	T	•	•	C	•	•	•	•	•	T	•	•	•	•	•	•
T18(S)	•	C	•	C	•	C	•	•	•	•	•	•	•	G	•	•	•	C	•	A	•	•	•	T	•	•	•	•	•	•	•	•	•	T	T	C	•	T	•	•	C	•	•	•	•	•	T	•	•	•	•	•	•
T19(S)	•	•	•	C	•	C	•	A	•	•	•	•	•	•	•	•	•	C	•	A	•	•	•	T	•	•	•	•	•	•	•	•	•	T	T	C	•	T	•	•	C	•	•	•	•	•	T	•	•	•	•	•	•
T20(S)	•	•	•	C	•	C	•	•	•	•	•	•	•	•	•	•	•	C	•	A	•	•	•	T	•	•	•	•	•	•	•	•	•	T	T	C	•	T	•	•	C	•	•	•	•	•	T	•	•	C	•	•	•
T21(S)	•	•	•	C	•	C	•	•	•	•	•	•	•	•	•	•	•	C	•	A	•	•	•	T	•	C	•	T	•	•	•	•	•	T	T	C	•	T	•	•	C	•	•	•	•	•	T	•	•	•	•	•	•
T22(S)	•	•	•	C	•	C	•	•	•	•	•	•	•	•	•	•	•	C	•	A	•	•	•	T	•	•	•	•	•	•	•	•	•	T	T	C	•	T	C	•	C	•	•	•	•	•	T	•	•	•	•	•	•
T23(S)	•	•	•	C	•	C	•	•	•	•	•	•	•	•	•	•	•	C	•	A	•	•	•	T	C	•	•	•	•	•	•	•	•	T	T	C	•	T	•	•	C	•	•	•	•	•	T	•	•	•	•	•	•
T24(S)	•	•	•	C	•	C	•	•	•	•	•	•	•	•	•	A	•	C	•	A	•	•	•	T	•	•	•	•	•	•	•	•	•	T	T	C	•	T	•	•	C	•	•	•	•	•	T	•	•	•	•	•	•
T25(S)	•	•	•	C	•	C	•	•	•	•	•	•	•	•	•	•	•	C	•	A	•	•	•	T	•	•	•	•	•	•	•	•	•	T	T	C	•	T	•	•	C	•	•	•	•	•	T	•	•	•	•	•	•
T26(S)	•	•	•	C	•	C	•	•	•	•	•	•	•	•	•	•	•	C	•	A	•	•	•	T	•	•	•	•	•	•	•	•	•	T	T	C	•	T	•	•	C	•	•	•	•	G	T	C	•	•	•	•	G
T27(S)	•	•	C	C	•	C	•	•	•	•	•	•	•	•	•	•	•	C	•	A	•	•	•	T	•	•	•	•	•	T	•	•	•	T	T	C	T	T	•	•	C	•	•	•	•	•	T	•	C	•	•	•	•
T28(S)	•	•	•	C	•	C	•	•	•	•	•	•	•	•	•	•	•	C	•	A	•	•	•	T	•	•	•	•	•	T	•	•	•	T	T	C	T	T	•	•	C	•	•	•	•	•	T	•	C	•	•	•	•
T29(S)	•	•	•	C	•	C	•	•	•	•	•	•	•	•	•	•	•	C	•	A	•	•	•	T	•	•	•	•	•	•	•	•	•	T	T	C	T	T	•	•	C	•	•	•	•	•	T	•	C	•	•	•	•
T30(S)	•	•	•	C	•	C	•	•	•	C	•	•	•	•	•	•	•	C	C	A	•	•	•	T	•	•	•	•	•	•	•	•	•	T	T	C	T	T	•	•	C	•	•	•	•	•	T	•	C	•	•	•	•
T31(S)	•	•	•	C	•	C	T	G	•	•	C	•	•	•	•	•	•	C	•	A	T	•	•	T	•	•	•	•	•	•	•	•	•	T	T	C	T	T	•	•	C	•	•	•	•	•	T	•	C	•	•	•	•
T32(S)	•	•	•	C	•	C	•	•	•	•	•	•	•	•	•	•	•	C	•	A	T	•	•	T	•	•	•	•	•	•	•	•	•	T	T	C	T	T	•	•	C	•	•	•	•	•	T	•	C	•	•	•	•
T33(S)	•	•	•	C	•	C	•	•	•	•	•	•	•	•	•	•	•	C	•	A	T	•	•	T	•	•	•	•	T	•	•	•	•	T	T	C	T	T	•	•	C	•	•	•	•	•	T	•	C	•	•	•	•
T34(S)	•	•	•	C	•	C	•	•	•	•	•	•	•	•	•	•	•	C	•	A	•	•	•	T	•	•	•	•	•	•	•	•	•	T	T	C	T	T	•	•	C	•	•	•	•	•	•	•	C	•	•	•	•
T35(S)	•	•	•	C	•	C	•	•	•	•	•	A	•	•	•	•	•	C	•	A	•	•	•	T	•	•	•	•	•	•	C	•	•	T	T	C	•	T	•	•	C	•	•	C	A	•	T	•	C	•	•	•	•
T36(S)	•	•	•	C	•	C	•	•	•	•	•	•	•	•	•	•	•	C	•	A	•	•	•	T	•	•	•	•	•	•	•	•	•	T	T	C	•	T	•	•	C	•	•	•	•	•	T	•	•	•	•	•	•
MC1	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	C	•	A	A	•	C	T	•	•	•	•	•	•	•	•	•	•	•	C	A	T	•	•	•	•	•	•	•	•	•	•	•	C	•	•	•
MC2	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	C	•	A	A	•	C	T	•	•	•	•	•	•	•	•	•	•	•	C	A	T	•	•	•	•	•	•	•	•	•	•	•	C	•	•	•
MC3	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	C	•	A	A	•	C	T	•	•	•	•	•	•	•	•	•	•	•	C	A	T	•	•	•	•	•	•	•	•	•	•	•	C	•	•	•

topology as the NJ tree and bootstrap values that were essentially same (Fig. 2).

Phylogroups and genetic variation

The observed sequence differences among haplotypes within the Northern phylogroup, within the Southern phylogroup, and between the two phylogroups were 1–3, 1–11, and 9–18, respectively. The uncorrected genetic distances (*p*-distances) among haplotypes within the Northern phylogroup, within the Southern phylogroup, and between two phylogroups were 0.14–0.41% (0.27% on average), 0.14–1.49% (0.67% on average), and 1.22–2.44% (1.78% on average), respectively. The sequence differences and *p*-distances among haplotypes between Northern and Houhuan phylogroups were 8–10 and 1.09–1.36% (1.22% on average), respectively. Those between the Southern and Houhuan phylogroups were 7–14 and 0.95–1.90% (1.38% on average), respectively.

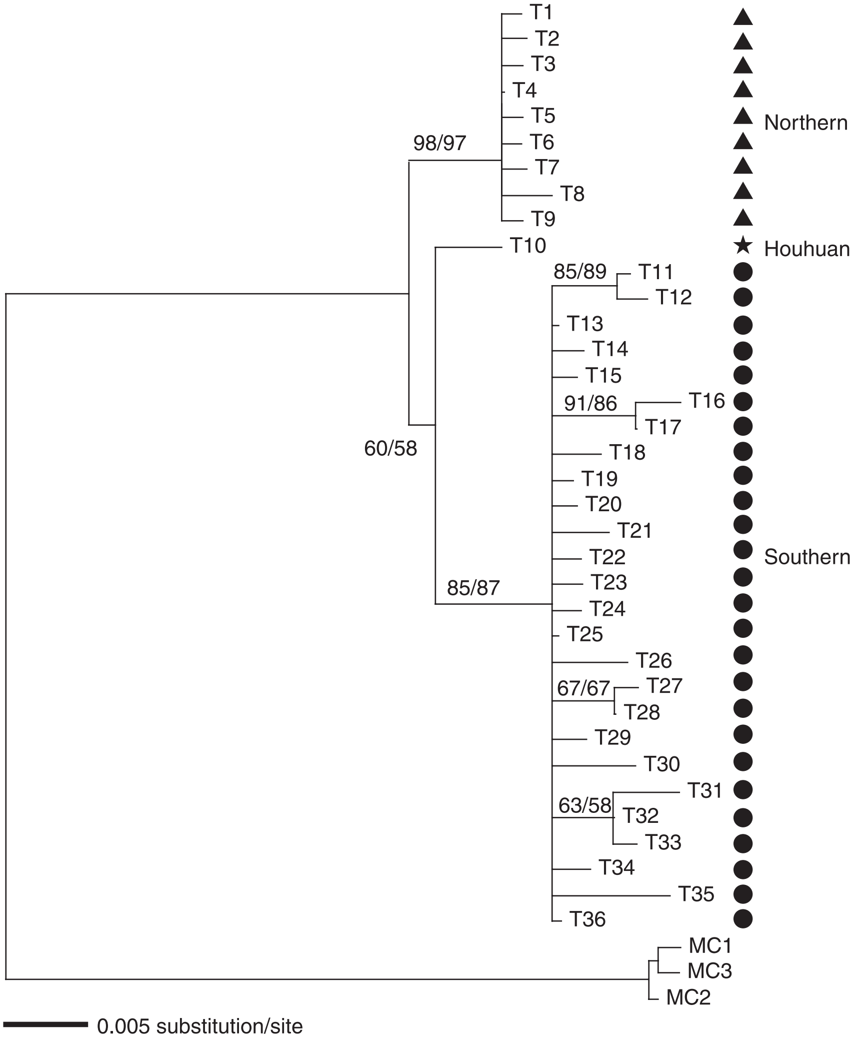

Fig. 2 Neighbour-joining tree for the 36 cytochrome *b* gene haplotypes identified from *Anourosorex yamashinai* of Taiwan and three *Anourosorex squamipes* outgroup haplotypes (MC1, MC2, and MC3). The tree was based on the TN93 + gamma distribution model. Bootstrap support values of > 50% in the NJ (before slash) and MP (after slash) analyses are given on branches. Symbols indicate mtDNA lineages: Northern (triangles), Southern (circles), and Houhuan (star).

Table 3 Intrapopulation variability for mtDNA phylogroups of *Anourosorex yamashinai* in Taiwan. Total contains the Northern, Southern, and Houhuan phylogroups. Standard deviations are in parentheses

Group	Sample size	No. of haplotypes	No. of polymorphic sites	Haplotype diversity	Nucleotide diversity in %
Northern	22	9	9	0.745 (0.093)	0.154 (0.032)
Southern	80	26	36	0.896 (0.018)	0.500 (0.040)
Total	103	36	53	0.926 (0.012)	0.887 (0.059)

Haplotype diversity within the Southern lineage was slightly higher than that within the Northern lineage (Table 3). Nucleotide diversity within the Southern lineage, however, was three times as high as that within the Northern lineage. Further, there are four times as many polymorphic sites in the Southern lineage as in the Northern one. For the genetic differentiation between the mtDNA phylogroups, the Jukes–Cantor corrected raw and net divergences were 1.64 ± 0.08% and 1.33 ± 0.08%, respectively. Divergence time between Northern and Southern phylogroups was estimated to be 0.63–0.71 Ma based on the net divergences, assuming an evolutionary rate of 2%/Myr (Wilson *et al*. 1985; Avise *et al*. 1998). The F_{ST} between the two phylogroups was 0.80, and the *Nm* was 0.13.

Phylogeographical analysis

The statistical parsimony procedure yielded three divergent haplotype clades: 3-1, 3-2, and 3-3 (Fig. 3). Within clade 3-1, haplotypes corresponding to the Northern lineage

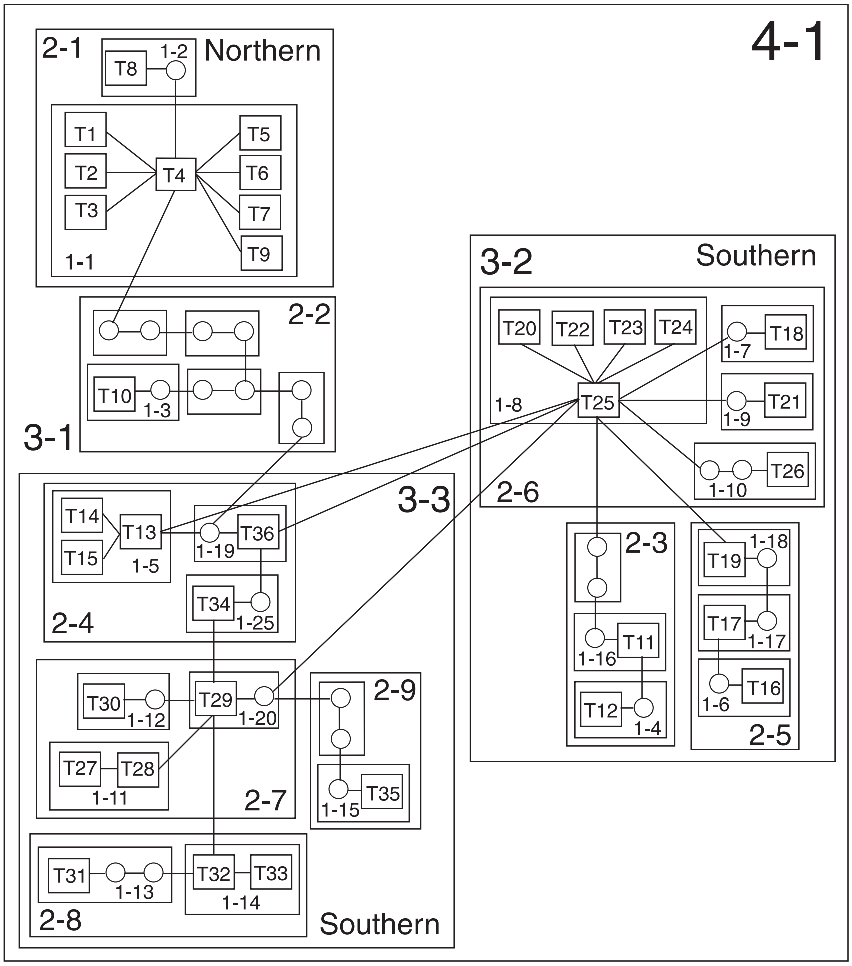

Fig. 3 Network of the 36 cytochrome *b* gene haplotypes of *Anourosorex yamashinai* in Taiwan, based on statistical parsimony and associated with the nested clade design. Haplotype names correspond to Table 1. Missing hypothetical haplotypes are indicated by open circles. Lines connecting haplotypes indicate relationship. Nested boxes represent clades of increasing numbers of steps; clade numbers are given in each box.

(clade 2-1) and the Houhuan lineage (clade 2-2) were found. The haplotypes of clades 3-2 and 3-3 belonged to the Southern lineage. Haplotype T4 is implied to be the ancestral form of the Northern lineages. The null hypothesis of no geographical association of clades was significantly rejected ($P < 0.0001$) by clade 3-3 and the total cladogram (Table 4) in a nested contingency analysis. Biogeographical interpretation of clade 3-3 and the total cladogram using the inference keys in Templeton (1998, 2004) found the structure of both higher-level clades consistent with a past occurrence of fragmentation or long-distance colonization.

We tested for partitioning of the total genetic variation among the three mountain ranges, among populations within these mountain ranges, and among individuals within populations. There was significant genetic variation among mountain ranges ($P < 0.0005$), populations ($P < 0.0001$), and individuals ($P < 0.0001$) (Table 5).

Discussion

The sequence difference between *Anourosorex yamashinai* and *Anourosorex squamipes* was 5.3–6.4%. Motokawa *et al*. (2004) reported the differences between both *Anourosorex* species in diploid and fundamental autosomal arm numbers. However, there are other shrew species with appreciable intraspecific chromosomal variation (e.g. King 1993; Bystrakova *et al*. 2003; Fredga 2003). Therefore, chromosomal difference is not sufficient evidence for distinctness among shrew species. Fumagalli *et al*. (1999) reported that intraspecific cytochrome *b* sequence differences of *Sorex* species varied from 0 to 5.21 ± 0.75%. Our interspecific result (5.3–6.4%) was similar to the highest of these values. A complete study of the systematics of the genus *Anourosorex* lies outside the scope of this study. Judging from the sequence differences, however, *A. squamipes* was a suitable outgroup for phylogeographical analysis of *A. yamashinai*.

We found two major and one minor mtDNA phylogroups of *A. yamashinai* in Taiwan (Figs 1–3). Divergence time between the major phylogroups was estimated to be 0.63–0.71 Ma, suggesting they separated during the middle Pleistocene. Judging from the genetic distances in the NJ analysis (Fig. 2), the Houhuan phylogroup would have diverged from the others at the same time. This suggests the *A. yamashinai* population was probably restricted to

Table 4 Geographical associaition of genetic clades. The chi-squared statistic was performed at the 5% level from a minimum of 1000 random permutations to lower-level categories within the nesting clade against geographical locality (P, probability). Significant small or large values ($P < 0.05$) for clade (D_c), nested (D_n), and interior to tip clade (I-T) distances are indicated by 'S' and 'L', respectively. The biogeographical interpretation is based on Templeton's (1998, 2004) inference keys for nested clade analysis

Nesting level	Phylogroup	Haplotype/ clade no.	Location	D_c	D_n	Chi-squared statistic	P	Biogeographical interpretation
1-1	Northern	T1	Tip	0	3.26	9.8990	0.522	Inconclusive
		T2	Tip	0	76.28			
		T3	Tip	0	3.26			
		T4	Interior	5.67	5.96			
		T5	Tip	0	3.26			
		T6	Tip	0	3.26			
		T7	Tip	0	3.26			
		T9	Tip	0	3.26			
			I-T	5.67	–5.41			
2-1	Northern	1–1	Interior	6.25	5.96	0.2200	1.000	Inconclusive
		1–2	Tip	0	2.95			
			I-T	6.25	3.02			
2-4	Southern	1–5	Tip	0	3.67	19.000	0.099	Restricted gene flow with isolation by distance
		1–19	Interior	0	3.67			
		1–25	Interior	0	39.39 L			
			I-T	0	17.86			
3-1	Northern and Houhuan	2–1	Tip	5.67	6.96	6.9697	0.135	Inconclusive
		2–2	Interior	0	75.20			
			I-T	–5.67				
					68.23			
3-3	Southern	2–4	Interior	6.15 S	29.62 L	47.0046	< 0.0001	Past fragmentation and/or long-distance colonization
		2–7	Interior	0 S	12.00 S			
		2–8	Tip	0 S	12.00 S			
		2–9	Tip	0 S	12.00 S			
			I-T	4.03	11.59 L			
Total cladogram		3–1	Tip	8.20 S	61.54 L	122.9259	< 0.0001	Past fragmentation and/or long-distance colonization
		3–2	Tip	0 S	19.22 S			
		3–3	Interior	17.28 S	42.20			
			I-T	3.65	4.27			

Table 5 Analysis of molecular variance (AMOVA) among *Anourosorex yamashinai* populations from three major mountain ranges. Va: variance components; %: percentage of variation; P: probability of the significance test of Φ-statistics; Φ_{CT}, Φ_{SC}, and Φ_{ST}: fixation indices of Φ-statistics (Excoffier *et al*. 1992)

Among ranges				Among populations within ranges				Among individuals within populations			
Va	%	P	Φ_{CT}	Va	%	P	Φ_{SC}	Va	%	P	Φ_{ST}
2.948	66.99	< 0.0005	0.670	0.289	6.57	< 0.0001	0.199	1.163	26.44	< 0.0001	0.736

fragmented refugia around the Cromerian Interglacial of the middle Pleistocene, but not during the late interglacial periods. The Cromerian Interglacial was from 0.63 to 0.56 Ma (e.g. Sibrava 1986). During this period, the *A. yamashinai* population could have been intensely affected by warmer environments. There could have been at least three refugia in Taiwan: the Northern, Southern, and Houhuan refugia (Fig. 4a). Although it is difficult to specify the range of the Northern and Southern refugia, the area around the peak of Mount Houhuan could have been a refugium for *A. yamashinai*. The elevation (3210 m) of locality number 8 at Mount Houhuan is one of the highest sampling localities in this study (Fig. 1). During the interglacial periods, it would have been an effective refugium from warmer temperatures at lower elevations.

Judging from the coexistence of three distinct phylogroups in locality 8, after the Cromerian Interglacial, these three phylogroups are postulated to have mixed, probably in central Taiwan (Fig. 4b). In the next interglacial (probably the Holsteinian Interglacial), populations of *A. yamashinai* could again have been isolated in a few fragmented refugia (Fig. 4c). Unlike the refugia of the previous (the Cromerian)

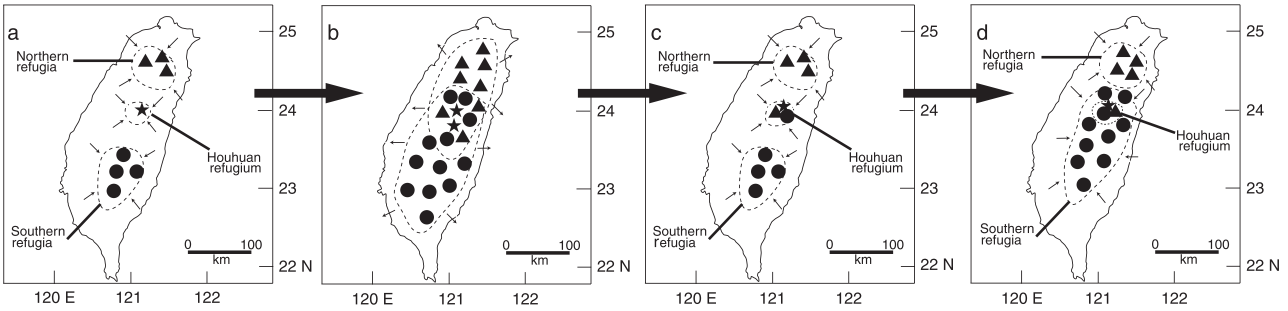

Fig. 4 Interglacial refugia hypothesis for *Anourosorex yamashinai* in Taiwan: (a) extreme population bottleneck during the Cromerian Interglacial; (b) population expanded throughout Taiwan during glaciation; (c) reduced population during the interglacial period; (d) reduced population after re-expansion during the glaciation. Current phylogroups indicated by symbols: Northern (triangles), Southern (circles), and Houhuan (star). Large arrows indicate sequence of events. Small arrows indicate directions of population movements. Dotted areas indicate the possible refugia.

interglacial, the Houhuan refugium is suggested to have retained haplotypes derived from all three of the founder phylogroups. Subsequently, the *A. yamashinai* populations would have expanded again, giving the three phylogroups another opportunity to mix. During the present Holocene Interglacial, which began about 10 000 years ago (e.g. Lowe & Walker 1984), the Northern phylogroup may have separated from the others, leaving the Houhuan refugium inside the range of the Southern phylogroup (Fig. 4d).

The F_{ST} (0.8) and *Nm* (0.13) calculated from this F_{ST} between the Northern and Southern phylogroups indicate low gene flow. The F_{ST} value indicates that 80% of the variation observed occurs among populations, while the *Nm* value suggests that there has on average been one female migrant between groups only about every eight generations. The results of the nested clade analysis (Table 4) and the AMOVA (Table 5) indicated that these phylogroups were significantly correlated with geography. In the nested clade analysis, Templeton's (1998; 2004) inference keys revealed fragmentation or long-distance colonization. Thus, *A. yamashinai* could have experienced extreme geographical isolation in a few refugia during the Cromerian Interglacial. Therefore, the Taiwanese population probably experienced a bottleneck in which haplotype T4 would have been ancestral to the Northern phylogroup (Fig. 3). After this interglacial, the isolated and reduced populations are suggested to have expanded to come in contact with one another (Fig. 4b). That the nucleotide diversity and number of polymorphic sites within the Southern lineage are higher than those of the Northern lineage (Table 3) suggests that the genetic variability in the refugia producing the Southern lineage may have been higher than that in the refugia producing the Northern lineage.

Based on pollen records of the last glacial maximum in Taiwan, Liew *et al*. (1998) described the highland forests as semi-open and stated that most of the lowlands were occupied by grasslands. This drastic environmental change would have increased the habitats of *A. yamashinai* in Taiwan. In the NJ tree (Fig. 2), we did not find distinct sublineages in each phylogroup. In general, if isolated populations each underwent recent population expansions, each phylogroup should demonstrate a starlike phylogeny (e.g. Avise 2000). Therefore, this tree suggests that both the Northern and Southern populations explosively expanded from the ancestral founder haplotypes in the refugia. In the network (Fig. 3), haplotype T4 could be a typical ancestral haplotype for the Northern phylogroup. Such founder effects are found in many mammalian species, e.g. the roe deer (*Capreolus capreolus*) in Europe (Vernesi *et al*. 2002) and the Norwegian lemming (*Lemmus lemmus*) in Scandinavia (Fedorov & Stenseth 2001). After the interglacial of the middle Pleistocene, mixing of the two *A. yamashinai* phylogroups would have been incomplete (Fig. 4b). Because of the quick alternation of glacial and interglacial periods during the Pleistocene, there probably was not enough time for the species to expand throughout Taiwan during glaciation. Unlike large terrestrial mammals, the small *A. yamashinai* would need more time to fully expand its distribution ranges. Its semifossorial behaviour would also slow expansion of *A. yamashinai*.

There are a few reports of the phylogeographical structure of high-elevation species in Taiwan. Based on the PCR–RFLP (restriction fragment length polymorphism) of mtDNA, Hsu *et al*. (2000) found that the Formosan white-bellied rat (*Niviventer culturatus*), which inhabits forests at elevations of 1500–3600 m, has no distinct phylogroups. Chan (2004) reported that the mitochondrial control region sequences of the spiny country rat (*Niviventer coxingi*), distributed from sea level to 3000 m, also lacks a clear phylogeographical structure. In the present interglacial period, these rodents are adapted to forests. They do not show distinct population isolation, even though they may have been isolated during glaciation into refugia of fragmented forests. Therefore, the phylogeographical histories of these rodents and *A. yamashinai* appear contradictory.

The differences in history and in ecology between these two rodent species and *A. yamashinai* may explain the differences in phylogeographical structure.

To clarify the interglacial refugia hypothesis in Taiwan and to help identify the locations of the interglacial refugia, the phylogeographical structure of other high-elevation animals should be investigated. Congruent phylogeographical patterns between *A. yamashinai* and other high-elevation animals would strongly support the interglacial refugia hypothesis.

Acknowledgements

We are grateful to Dr Cara Lin Bridgman for her critical reading of the manuscript. We thank Ms Yen-Jean Chen and Mr Ching-Lung Liao of the National Museum of Natural Science, Taichung, for their assistance in making specimens available and providing information about the specimens. We thank Drs Yi Wu and Masashi Harada for supplying specimens. We thank Drs Satoshi Ohdachi and Hisashi Abe for their critical comments. This study was supported by the Council of Agriculture, Taiwan, ROC.

References

Alexander PS, Lin L-K, Huang BM (1987) Ecological notes on two sympatric mountain shrews (*Anourosorex squamipes* and *Soriculus fumidus*) in Taiwan. *Journal of Taiwan Museum*, **40**, 1–7.

Avise JC (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press, London.

Avise JC, Walker D, Johns GC (1998) Speciation durations and Pleistocene effects on vertebrate phylogeography. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **265**, 1707–1712.

Britten HB, Brussard PF, Murphy DD, Ehrlich PR (1995) A test for isolation-by-distance in central Rocky Mountain and Great Basin populations of Edith's checkerspot butterfly (*Euphydryas editha*). *Journal of Heredity*, **86**, 204–210.

Brown JH, Gibson AC (1983) *Biogeography*. CV Mosby Company, St Louis.

Bystrakova N, Bulatova N, Koval'skaya Y *et al.* (2003) Geographical limits of chromosome races of common shrew *Sorex araneus* L. in the Middle Volga (East European Russia). *Mammalia*, **68**, 187–191.

Chan H-T (2004) *Geographic variation of* Niviventer coxingi *in body size and mitochondrial D-loop region*. Master Thesis, National Chungshan University, Kaohsiung, Taiwan.

Clement M, Derington J, Posada D (2001) *TCS: estimating gene genealogies, Version* 1.13. Brigham Young University, Provo.

Corbet GB, Hill JE (1992) *The Mammals of the Indomalayan Region: A Systematic Review*. Oxford University Press, Oxford.

Cox CB, Moore PD (2000) *Biogeography: An Ecological and Evolutionary Approach*, 6th edn. Blackwell Science Ltd, Oxford.

Cracraft J, Prum RO (1988) Patterns and processes of diversification: speciation and historical congruence in some Neotropical birds. *Evolution*, **42**, 603–620.

Crespi EJ, Rissler LJ, Browne RA (2003) Testing Pleistocene refugia theory: phylogeographical analysis of *Desmognathus wrighti*, a high-elevation salamander in the southern Appalachians. *Molecular Ecology*, **12**, 969–984.

Delcourt HR, Delcourt PA (1991) *Quaternary Ecology: A Paleoecological Perspective*. Chapman & Hall, London.

Demboski JR, Cook JA (2001) Phylogeography of the dusky shrew, *Sorex monticolus* (Insectivora, Soricidae): insight into deep and shallow history in northwestern North America. *Molecular Ecology*, **10**, 1227–1240.

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

Fedorov VB, Stenseth NC (2001) Glacial survival of the Norwegian lemming (*Lemmus lemmus*) in Scandinavia: inference from mitochondrial DNA variation. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **268**, 809–814.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783–791.

Fredga K (2003) Chromosome races of *Sorex araneus* in Norway: description of two new races. *Mammalia*, **67**, 179–185.

Fumagalli L, Taberlet P, Stewart DT, Gielly L, Hausser J, Vogel P (1999) Molecular phylogeny and evolution of *Sorex* shrews (Soricidae: Insectivora) inferred from mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution*, **11**, 222–235.

Haffer J (1969) Speciation in Amazonian forest birds. *Science*, **165**, 131–137.

Hall TA (1999) BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Research Symposium Series*, 41, 95–98.

Hsu F-H, Lin F-J, Lin Y-S (2000) Phylogeographic variation in mitochondrial DNA of Formosan white-bellied rat *Niviventer culturatus*. *Zoological Studies*, **39**, 38–46.

Huang SSF, Hwang S-Y, Lin T-P (2002) Spacial pattern of chloroplast DNA variation of *Cyclobalanopsis glauca* in Taiwan and East Asia. *Molecular Ecology*, **11**, 2349–2358.

Hudson RR, Slatkin M, Maddison WP (1992) Estimation of levels of gene flow from DNA sequence data. *Genetics*, **132**, 583–589.

Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: *Mammalian Protein Metabolism* (ed. Munro HN), pp. 21–132. Academic Press, New York.

King M (1993) *Species Evolution: The Role of Chromosome Change*. Cambridge University Press, Cambridge.

Kuroda N (1935) Formosan mammals preserved in the collection of Marquis Yamashina. *Journal of Mammalogy*, **16**, 277–291.

Liew P-M, Kuo C-M, Huang S-Y, Tseng M-H (1998) Vegetation change and terrestrial carbon storage in eastern Asia during last glacial maximum as indicated by a new pollen record from central Taiwan. *Global and Planetary Change*, **16–17**, 85–94.

Lin Y-H, McLenachan PA, Gore AR *et al.* (2002) Four new mitochondrial genomes and the increased stability of evolutionary trees of mammals from improved taxon sampling. *Molecular Biology and Evolution*, **19**, 2060–2070.

Lowe JJ, Walker MJC (1984) *Reconstructing Quaternary Environments*. Longman Scientific and Technical, Chelmsford, UK.

Maldonado JE, Vilà C, Wayne RK (2001) Tripartite genetic subdivisions in the ornate shrew (*Sorex ornatus*). *Molecular Ecology*, **10**, 127–147.

Motokawa M, Lin L-K (2002) Geographic variation in the mole-shrew *Anourosorex squamipes*. *Mammal Study*, **27**, 113–120.

Motokawa M, Harada M, Lin L-K (2004) Geographic differences in karyotypes of the mole-shrew *Anourosorex squamipes* (Insectivora, Soricidae). *Mammalian Biology*, **69**, 197–201.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Ohdachi S, Dokuchaev NE, Hasegawa M, Masuda R (2001) Intraspecific phylogeny and geographical variation of six species of northeastern Asiatic *Sorex* shrews based on the mitochondrial cytochrome *b* sequences. *Molecular Ecology*, **10**, 2199–2213.

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

Posada D, Crandall KA, Templeton AR (2000) GEODIS: a program for the cladistic nested analysis of the geographical distribution of genetic haplotype. *Molecular Ecology*, **9**, 487–488.

Reumer JWF (1989) Speciation and evolution in the Soricidae (Mammalia: Insectivora) in relation with the paleoclimate. *Revue Suisse de Zoologie*, **96**, 81–90.

Reumer JWF (1994) Phylogeny and distribution of the Crocidosoricinae (Mammalia: Soricidae). In: *Advances in the Biology of Shrews* (eds Merritt JF, Kirkland J, Rose GL, Rose RK), pp. 345–356. Carnegie Museum, Pittsburgh.

Roff DA, Bentzen P (1989) The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. *Molecular Biology and Evolution*, **6**, 539–545.

Rozas J, Rozas R (1999) DNASP, version 3.14: an integrated program for molecular population genetics and molecular evolution analysis. *Bioinformatics*, **15**, 174–175.

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**, 406–425.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN version 2.000: A software for population genetics data analysis*. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Geneva, Switzerland.

Sibrava V (1986) Correlation of European glaciations and their relation to the deep-sea record. *Quaternary Science Reviews*, **5**, 433–441.

Smith AT (1988) Patterns of pika (genus *Ochotona*) life history variation. In: *Evolution of Life Histories of Mammals: Theory and Pattern* (ed. Boyce M), pp. 233–256. Yale University Press, New Haven, Connecticut.

Stewart DT, Perry ND, Fumagalli L (2002) The maritime shrew, *Sorex maritimensis* (Insectivora: Soricidae): a newly recognized Canadian endemic. *Canadian Journal of Zoology*, **80**, 94–99.

Sullivan J, Arellano E, Rogers DS (2000) Comparative phylogeography of Mesoamerican highland rodents: concerted versus independent response to past climatic fluctuations. *American Naturalist*, **155**, 755–768.

Swofford DL (2001) *PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0. Beta*. Sinauer Associates, Sunderland, Massachusetts.

Tajima F (1983) Evolutionary relationship of DNA sequences in finite populations. *Genetics*, **105**, 437–460.

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **10**, 512–526.

Templeton AR (1998) Nested clade analysis of phylogeographic data: testing hypotheses about gene flow and population history. *Molecular Ecology*, **7**, 381–397.

Templeton AR (2004) Statistical phylogeography: methods of evaluating and minimizing inference errors. *Molecular Ecology*, **13**, 789–809.

Templeton AR, Crandall KA, Sing CF (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.

Templeton AR, Routman E, Phillips CA (1995) Separating population structure from population history: a caldistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. *Genetics*, **140**, 767–782.

Vernesi C, Pecchioli E, Caramelli D, Tiedemann R, Randi E, Bertorelle G (2002) The genetic structure of natural and reintroduced roe deer (*Capreolus capreolus*) populations in the Alps and central Italy, with reference to the mitochondrial DNA phylogeography of Europe. *Molecular Ecology*, **11**, 1285–1297.

Weider LJ, Hobæk A (2003) Glacial refugia, haplotype distributions, and clonal richness of the *Daphnia pulex* complex in arctic Canada. *Molecular Ecology*, **12**, 463–473.

Wilson AC, Cann RL, Carr SM *et al.* (1985) Mitochondrial DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnean Society*, **26**, 375–400.

Yu H-T (1994) Distribution and abundance of small mammals along a subtropical elevational gradient in central Taiwan. *Journal of Zoology, London*, **234**, 577–600.

Yu N, Zheng C, Shi L (1997) Variation in mitochondrial DNA and phylogeny of six species of pikas (*Ochotona*). *Journal of Mammalogy*, **78**, 387–396.

Shou-Li Yuan is a PhD student investigating phylogeography and ecology of shrews. This research is based on his MSc thesis. Liang-Kong Lin and Tatsuo Oshida are associate professors who are interested in phylogeography, ecology and evolutionary biology of small mammals distributed in Asia.

Appendix

Specimens of ***Anourosorex yamashinai*** and ***Anourosorex squamipes*** examined in this study. Haplotypes and accession numbers are for the 737-bp mtDNA cytochrome *b* sequences. Locality numbers 1–24 match those in Fig. 1 and Table 1. Names of collecting localities for *A. squamipes* are shown. Identity numbers indicate the specimens stored in the National Museum of Natural Science, Taichung, Taiwan (T), and in our personal collection at Tunghai University, Taichung, Taiwan (THU)

Species	Haplotype (individuals)	Accession number	Locality number or name (identity number)
A. yamashinai	T1 (1)	AB190460	1 (T8307)
	T2 (1)	AB190461	8 (T6404)
	T3 (1)	AB190462	3 (T6358)
	T4 (11)	AB190463	1 (T8300, T8306, T8308, T8309), 2 (T6406), 3 (T6360), 4 (T8329, T8330), 5(T5330, T5331), 8 (T6403)
	T5 (1)	AB190464	1 (T8303)
	T6 (3)	AB190465	1 (T8301, T8302, T8304)
	T7 (1)	AB190466	3 (T6359)
	T8 (2)	AB190467	3 (T6356, T6357)
	T9 (1)	AB190468	1 (T8305)
	T10 (1)	AB190469	8 (T4828)
	T11 (1)	AB190470	8 (T8326)
	T12 (1)	AB190471	8 (T8325)
	T13 (15)	AB190472	9 (T6389), 13 (T6382, T6383, T6384, T6385, T6386, T6387, T6388, T8317, T8318, T8319, T8320, T8321, T8331), 14 (T6399)
	T14 (1)	AB190473	14 (T6398)
	T15 (3)	AB190474	14 (T6396, T6397, T8332)
	T16 (1)	AB190475	10 (T8443)
	T17 (1)	AB190476	23 (THU:Te5)
	T18 (1)	AB190477	23 (THU:Te4)
	T19 (1)	AB190478	7 (T6361)
	T20 (1)	AB190479	7 (T8323)
	T21 (1)	AB190480	19 (T8444)
	T22 (1)	AB190481	6 (THU:P1)
	T23 (1)	AB190482	24 (T8316)
	T24 (2)	AB190483	20 (T6377), 21 (T10709)
	T25 (16)	AB190484	7 (T8322, T8324), 8 (T6405, THU:H6), 9 (T6390, T6391, T6392, T6393) 10 (T6394), 11 (THU:N1), 12 (T6400), 20 (T6378), 22 (T4850), 23 (THU:Te1, THU:Te2, THU:Te3)
	T26 (1)	AB190485	11 (T8328)
	T27 (2)	AB190486	15 (THU:Xi1, THU:Xi2)
	T28 (2)	AB190487	16 (T1790, T1791)
	T29 (6)	AB190488	17 (T6363, T6368, T6373, T6374, THU:A11), 18 (THU:Yu4)
	T30 (1)	AB190489	17 (T6366)
	T31 (1)	AB190490	17 (T6375)
	T32 (4)	AB190491	15 (THU:Xi3), 17 (T6372), 18 (T6402,T8327)
	T33 (1)	AB190492	18 (T4858-1)
	T34 (1)	AB190493	18 (T4836)
	T35 (13)	AB190494	17 (T6362, T6364, T6365, T6367, T6369, T6370, T6371, T6376, THU:A9, THU:A10), 18 (T4858-2, THU:Yu2, THU:Yu3)
	T36 (1)	AB190495	10 (T6395)
A. squamipes	MC1 (4)	AB190496	Nachung, Szechuan, China (0103, 0105, 0106, 0109)
	MC2(4)	AB190497	Nachung, Szechuan, China (0102, 0107, 0108, 0111)
	MC3 (2)	AB190498	Mianyang, Szechuan, China (HB403, HB405)