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Phylogeographic Variation in Mitochondrial DNA of Formosan White-bellied Rat *Niviventer culturatus*

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Fu-Hsiung Hsu, Fei-Jann Lin and Yao-Sung Lin (2000) Phylogeographic variation in mitochondrial DNA of Formosan white-bellied rat *Niviventer culturatus*. *Zoological Studies* **39**(1): 38-46. Phylogeographic variation in mtDNA of the Formosan white-bellied rat *Niviventer culturatus* (Muridae) was studied by the PCR-RFLP method, using 128 individuals from 20 locations throughout its distribution range in Taiwan. Restriction fragment polymorphisms represented by the *CYTb/DL* (2800 bp) and *12S/16S* (1900 bp) fragments were assayed by 13 restriction endonucleases. Totally, 25 mtDNA haplotypes were observed. The sequence divergence between all pairs of haplotypes ranged between 0.32% and 2.63%. The amount of within-locality nucleotide diversity was estimated to be from 0% to 0.69%. Pairwise analysis of net nucleotide divergence between locations ranged between -0.08% and 1.71%. There was little phylogeographic patterning among the haplotypes, and most of them were found within a single location or associated with nearby locations, except for some widespread haplotypes. The results suggest that population differentiation in the Formosan white-bellied rat is minor, probably due to historical vicariance events and a high level of gene flow.

Key words: Phylogeographic variation, Restriction endonuclease, Haplotype, *Niviventer culturatus*.

Historical vicariance events and geographical structure have been shown to affect phylogeographic patterns of several species of animals in Taiwan (Tzeng 1986, Yang et al. 1994, Chang and Liu 1997, Toda et al. 1997 1998, Yeh 1997). According to the distribution of freshwater fishes, Tzeng (1986) divided Taiwan into 3 zoogeographical regions: the eastern, the southern, and the north central. Yang et al. (1994) indicated that based on mtDNA variation of the Taipei treefrog, *Rhacophorus taipeianus*, there are northern and central lineages within this species. Chang and Liu (1997) showed that in the Swinhoe's tree lizard, *Japalura swinhonis*, the primary divergence of RFLP data occurred between populations east and west of the Central Mountain Range. Toda et al. (1998) showed the presence of substantial differences in predominant alleles at a few presumptive loci of allozyme data between the eastern and the remaining populations of the Indian rice frog, *Rana limnocharis*. Yeh (1997) also found the mtDNA of 2 well-differentiated lineages (eastern and western) of the Moltrechti's treefrog, *Rhacophorus moltrechti* isolated by the Central Mountain Range. The above evidence suggests a possible occurrence of in situ divergence with a biogeographic event and/or the Central Mountain Range acting as a barrier against gene flows. However, most of the species that have been examined are distributed in lowlands of Taiwan.

The topography of Taiwan is generally mountainous with 2/3 of its area at elevations above 1000 m. The steep Central Mountain Range largely runs along the northeast-southwest longitudinal axis of the island with the highest peak at nearly 4000 m above sea level. As a consequence, many drainages have etched deeply through the mountainous terrains isolating the ridges. Such complex geomorphology causes drastic changes in vegetation along mountain slopes (Yu 1992 1993), resulting in the formation of diverse habitats for terrestrial animals. On the other hand, the high mountain range and a num-

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ber of deep valleys may have interrupted gene flows among conspecific geographical populations.

In this study, we used PCR-RFLP assay to examine phylogeographic variation of mtDNA haplotypes in *Niviventer culturatus* Thomas. This rat is endemic to Taiwan and lives in broadleaf and coniferous forests and subalpine shrubs at elevations between 1500 and 3600 m. It commonly occurs in habitats with dense ground cover, fallen logs, or rocky areas. It breeds year-round with a bimodal or prolonged pattern (Yu 1992 1993 1995). The objectives of this study were (1) to determine phylogeographic variation of *N. culturatus* and (2) to examine whether the topography of the Central Mountain Range constitutes a geographical barrier against gene flow of this highland species.

MATERIALS AND METHODS

Liver samples were obtained from 128 individuals of *N. culturatus* collected from 20 locations in the Central Mountain Range at elevations above 1500 m in Taiwan (Fig. 1; Table 1). Locations are consecutively numbered from north to south. They were frozen in liquid nitrogen in the field and kept at -70 °C in the laboratory, or preserved in 95% ethyl alcohol and kept at ambient temperature. In the initial stages of this study, mtDNA was isolated from 0.1 to 0.5 g of liver tissue according to the protocol established by Hoelzel (1992). Later, an adequately pure mtDNA sample was obtained by using a mtDNA Extraction Kit (GeneLabs Life Science Corp., Natick, Main, USA). The precipitated and dried mtDNA was finally resuspended in 30-50 µl of sterile distilled water and stored at -70 °C for later PCR treatment.

Two distinct fragments of the mitochondrial genome, the cytochrome b/D-loop (*CYTb/DL*) fragment of about 2800 bp and the 12S/16S ribosomal gene (*12S/16S*) fragment of about 1900 bp, were amplified using PCR. The primers used for the *CYTb/DL* fragment were CL1 (5'-CGAAGCTTGATATGAAAAACCATCGTTG-3') (DeWalt et al. 1993) and PB (5'-AGTGGGGTATCTAATCCCAG-3') (supplied by Dr. Yi-Ju Yang), and for the *12S/16S* fragment were 12S5 (5'-TGCCAGCCACCGCGGTTATACG-3') and 16S3 (5'-TATTCTCCGTGGTCGCCCCTTCC-3') (Roe et al. 1985, Nagae et al. 1988).

Thermal cycling was used with a Perkin Elmer-Cetus cycler (Foster City, California, USA). The *CYTb/DL* fragment was amplified with Super *Taq* polymerase (HT Biotechnology, Cambridge, UK). It began with a 'hot start' at 94 °C for 1 min, followed by 38 cycles of primer denaturing for 40 s at 94 °C, annealing for 40 s at 50-55 °C, and a 4-min extension step at 72 °C. A final extension step of 72 °C for 10 min was added to ensure complete polymerization of the product. The *12S/16S* fragment was amplified by 35 PCR cycles (94 °C, 40 s; 55 °C, 1 min; 72 °C, 2.5 min) with Super *Taq* polymerase. A 3-µl sample from each reaction was assayed by electrophoresis on 1.2% agarose minigels with visualization under UV light after ethidium bromide staining.

In the pilot study, 16 restriction enzymes were tested for the 2 amplified fragments of 44 individuals (1 to 4 individuals for each location). Of them, 8 enzymes (*Aci* I, *Dde* I, *Hinf* I, *Hpa* II, *Mbo* I, *Msp* I, *Sau*96 I, and *Xba* I) were identified to cut all *CYTb/DL* fragment products at least once, and 5 enzymes (*Ase* I, *EcoR* V, *Hinf* I, *Mbo* I, and *Ssp* I) were identified to cut all *12S/16S* fragment products. For restriction digests, 3-5 µl aliquots of each amplified product were digested according to the manufacturer's instructions. A final volume of each reaction was 15 µl. Restriction fragments were separated on 2%~3.5% agarose gels (Nusieve agarose: agarose = 3: 1) and 1x TBE buffer at 60 V. The restriction-fragment patterns were visualized and photographed

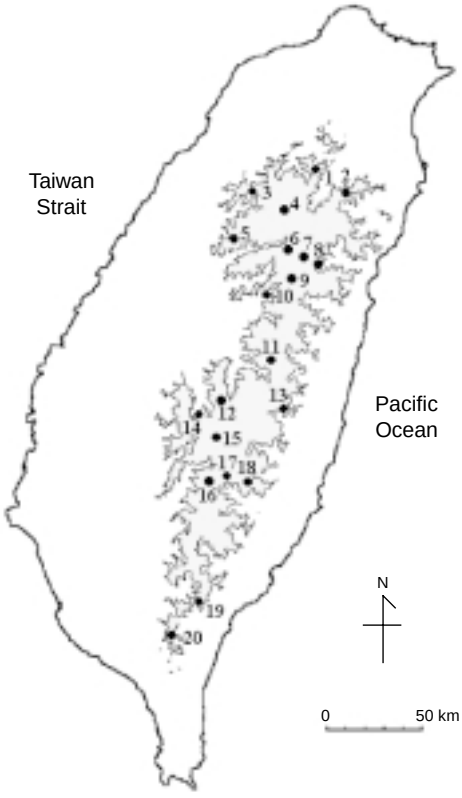

Fig. 1. Map of Taiwan showing sampling locations for *Niviventer culturatus* (shaded area represents elevations above 1500 m; the numbers 1-20 refer to code numbers in table 1).

under UV light. Fragment size was estimated by comparison to a 100-bp DNA ladder (Bethesda Reasearch Laboratories, Rockville, Maryland, USA).

Restriction patterns produced by a given enzyme were identified with the letters A, B, C, and D in descending order according to the pattern's frequency. Each rat was then assigned to an observed composite mtDNA haplotype of multiletter code based on the restriction patterns across all enzymes. From the fragment patterns generated by each enzyme, we inferred the presence or absence of the specific restriction site. Fragments of fewer than 100 base pairs usually were not detected, and some hypothetical fragments were assumed in order to explain all the mutational steps. For each enzyme, a minimum path network of site differences among

Table 1. Sampling locations, sample size, haplotype frequency, and nucleotide and haplotype diversity of *Niviventer culturatus* in Taiwan

Code	Location (latitude-longitude)	Sample size	Haplotypes (frequency)	Nucleotide diversity (%)	Haplotype diversity
1	Yuanyang Lake (24.34N-121.24E)	3	H04 (0.33) H06 (0.33) H21 (0.33)	0.69	1.000
2	Taiping Mt. (24.30N-121.31E)	6	H06 (0.67) H08 (0.33)	0.19	0.533
3	Kuanwu (24.30N-121.06E)	5	H01 (0.20) H06 (0.40) H09 (0.40)	0.41	0.800
4	Chika (24.23N-121.16E)	3	H11 (1.00)	0.00	0.000
5	Anma Mt. (24.17N-121.01E)	6	H01 (1.00)	0.00	0.000
6	Fushou Mt. (24.14N-121.14E)	2	H06 (0.50) H10 (0.50)	0.69	1.000
7	Kuanyuan (24.11N-121.20E)	3	H01 (1.00)	0.00	0.000
8	Bilushenmu (24.11N-121.23E)	7	H01 (0.86) H08 (0.14)	0.21	0.286
9	Hohuan Mt. (24.08N-121.17E)	3	H01 (1.00)	0.00	0.000
10	Juiyenshi (24.07N-121.11E)	3	H06 (1.00)	0.00	0.000
11	Haitientz (23.47N-121.10E)	6	H04 (0.16) H06 (0.67) H17 (0.16)	0.51	0.600
12	Chunta (23.37N-120.56E)	2	H14 (1.00)	0.00	0.000
13	Ruisui (23.33N-121.15E)	17	H01 (0.35) H02 (0.18) H05 (0.24) H13 (0.12) H25 (0.12)	0.56	0.809
14	Tatachia (23.29N-120.53E)	2	H16 (1.00)	0.00	0.000
15	Nantzushienshi (23.27N-120.54E)	6	H22 (0.17) H23 (0.83)	0.64	0.333
16	Tienchih (23.16N-120.54E)	8	H01 (0.13) H13 (0.38) H14 (0.13) H19 (0.38)	0.69	0.786
17	Yakou (23.16N-120.57E)	10	H01 (0.20) H12 (0.10) H13 (0.10) H16 (0.60)	0.54	0.644
18	Hsiangyang (23.15N-120.59E)	14	H01 (0.21) H13 (0.64) H15 (0.07) H16 (0.07)	0.22	0.571
19	Hsiaokuei Lake (22.42N-120.52E)	5	H03 (0.60) H18 (0.40)	0.41	0.600
20	Peitawu Mt. (22.38N-120.43E)	17	H07 (0.23) H20 (0.71) H24 (0.06)	0.51	0.471

fragment profiles was constructed (Avise et al. 1979). Site data for each enzyme were summarized to construct a binary site presence-absence matrix for haplotypes and individuals, respectively. The site data were analyzed with the computer package REAP version 4.0 (McElroy et al. 1991) to generate a sequence divergence matrix for observed haplotypes (Nei and Tajima 1981, Nei and Miller 1990) and to compute estimates of haplotype diversity (Nei 1987), nucleotide diversity, and the nucleotide divergence between locations (Nei and Tajima 1981, Nei 1987).

A UPGMA phenogram (Sneath and Sokal 1973) and neighbor-joining tree (Saitou and Nei 1987) were constructed among haplotypes from sequence divergence data using the MEGA computer package (Kumar et al. 1993). In addition, maximum-parsimony analysis of the restriction-site data was performed using the computer program PAUP v3.1.1 (Swofford 1993) to treat site presence or absence as discrete binary character states. Analyses were conducted using the heuristic search procedure with the tree bisection and reconnection (TBR) branch-swapping algorithm. Minimum-length trees were saved and all zero-length branches were removed. A single, unrooted consensus tree was obtained by taking the 50% majority rule consensus.

The extent of geographic heterogeneity in the frequency distribution of haplotypes was analyzed with the approach developed by Roff and Bentzen (1989). It uses the Monte Carlo simulation to assess the probability of obtaining a X^2-value from the original matrix that is larger than that obtained from a set of randomization of that matrix. Data were analyzed among locations. The significance level was obtained by 10 000 Monte Carlo randomizations with the computer package REAP v4.0 (McElroy et al. 1991).

RESULTS

Gel electrophoresis of the PCR products of *CYTb/DL* and *12S/16S* fragments revealed no variation in size among individuals. The extensive frag-

Table 2. Mitochondrial DNA haplotypes observed in *Niviventer culturatus* (A, B, C and D, descending order of the restriction pattern frequency; location numbers refer to code numbers in table 1)

	Haplotype			
Code	*CYTb/DL*[a]	*12S/16S*[b]	*n*	Location
H01	A A A A A A A A	A A A A A	31	3,5,7,8,9,13,16,17,18
H02	A A A A A A A A	A A A A C	3	13
H03	A A A A A A A A	A A A A B	3	19
H04	A A A A A A A A	A B A A A	2	1,11
H05	A A A A A A A A	B A A B A	4	13
H06	A A A A A A A B	A A A A A	15	1,2,3,6,10,11
H07	A A A A A A A B	A A A A B	4	20
H08	A A A A A A A B	A B A A A	3	2,8
H09	A A A A A A B A	A A A A A	2	3
H10	A A A A B A A A	A A A A A	1	6
H11	A A A B A B A A	A A A A A	3	4
H12	A A A B A B A A	C A A A A	1	17
H13	A A B A A A A A	A A A A A	15	13,16,17,18
H14	A A B A A A A A	A A B A A	3	12,16
H15	A A B A A A A A	A B A A A	1	18
H16	A A B A A A A B	A A A A A	9	14,17,18
H17	A A C A A A A A	A B A A A	1	11
H18	A D A A A A A A	A A A A A	2	19
H19	A E B B A B A A	A A A A A	3	16
H20	A B A A A A A A	A A A A A	12	20
H21	A B A A A A A B	A B A A A	1	1
H22	A C A B A B A A	C A A A A	1	15
H23	A C D A A A A A	B A A B A	5	15
H24	B A A A A A A A	A A A A B	1	20
H25	B A B A A A A A	A A A A A	2	13

[a]Letters depict profiles from left to right as *Aci* I, *Dde* I, *Hae* III, *Hinf* I, *Hpa* II, *Mbo* I, *Msp* I, *Sau96* I, and *Xba* I.
[b]Letters depict profiles from left to right as *Ase* I, *EcoR* V, *Hinf* I, *Mbo* I, and *Ssp* I.

ment variation generated by each enzyme could be explained by site losses or gains relative to 1 or 2 'central' restriction profiles. Most of the enzymes had 2 variants, except for *Dde* I and *Hinf* I of the *CYTb/DL* fragment, and *Ase* I and *Ssp* I of the *12S/16S* fragment. Restriction digests of the *CYTb/DL* and *12S/16S* fragment combinations produced 48 inferred restriction sites and 33 unique digestion profiles, from which 25 haplotypes were resolved among the 128 rats (Tables 1, 2). Twenty-eight sites (58.3%) were invariant in all individuals examined, and the presence or absence of 8 sites (16.6%) was unique to the haplotypes in which each was found to be autapomorphic.

Haplotypes differed in restriction sites from 1 to 8. Table 3 gives the estimates of sequence divergences between all pairs of haplotypes. The unweighted mean sequence divergence was 1.15% with a range from 0.32% to 2.63%. Within-location haplotype diversity ranged between 0 and 1.00, and nucleotide diversity ranged between 0% and 0.69% (Table 1). Pairwise analysis of net nucleotide divergence between locations ranged between -0.08% and 1.71%, with a mean average of 0.4%.

The extensive polymorphism detected in mtDNA was characterized by many localized haplotypes. Eighteen of the 25 haplotypes were restricted to a single location (Table 2). The result of the Monte Carlo simulation suggested a significantly heterogenous geographic distribution of haplotypes among locations ($X^2 = 1200$, $p < 0.0001$). Five of the 25 haplotypes (01, 06, 13, 16, and 20) represented 64.1% of all individuals examined, whereas the other 20 haplotypes were observed in less than 5 individuals. Haplotype 01 was the most common and widespread being found in 31 individuals at 9 locations from Kuanwu to Hsiangyang. Haplotype 06 was represented by 15 individuals at 6 locations from Yuangyang Lake to Haitientz. Haplotype 13 was represented by 15 individuals at 4 locations from Ruisui to Hsiangyang.

Within a maximum parsimony framework, we treated restriction sites from the *CYTb/DL* and *12S/16S* fragments as having discrete presence or absence, with each weighted equally as to restriction site gains and losses. A heuristic search strategy was used to determine the relationship among haplotypes in 1422 trees of equal shortest length. All trees were 28 steps long. The consensus tree showed that all 25 haplotypes were closely related with no distinctly differentiated groups and no obvious association with geographical locations (Fig. 2A;

Table 3. Estimates of percentage sequence divergence (below diagonal) and numbers of restriction site differences (above diagonal) between the 25 haplotypes inferred from restriction-site data for *Niviventer culturatus*

	H01	H02	H03	H04	H05	H06	H07	H08	H09	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21	H22	H23	H24	H25
H01		1	1	1	2	1	2	2	1	1	2	3	1	2	2	2	2	1	4	1	3	4	4	2	2
H02	0.34		2	2	3	2	3	3	2	2	3	4	2	3	3	3	3	2	5	2	4	5	5	3	3
H03	0.36	0.73		2	3	2	1	3	2	2	3	4	2	3	3	3	3	2	5	2	4	5	5	1	3
H04	0.34	0.69	0.73		3	2	3	1	2	2	3	4	2	3	1	3	1	2	5	2	2	5	5	3	3
H05	0.68	1.03	1.08	1.03		3	4	4	3	3	4	5	3	4	4	4	4	3	6	3	5	6	2	4	4
H06	0.36	0.73	0.79	0.73	1.08		1	1	2	2	3	4	2	3	3	1	3	2	5	2	2	5	5	3	3
H07	0.79	1.22	0.38	1.22	1.59	0.38		2	3	3	4	5	3	4	4	2	4	3	6	3	3	6	6	2	4
H08	0.73	1.13	1.22	0.34	1.48	0.36	0.79		3	3	4	5	3	4	2	2	2	3	6	3	1	6	6	4	4
H09	0.33	0.66	0.69	0.66	0.99	0.69	1.12	1.05		2	3	4	2	3	3	3	3	2	5	2	4	5	5	3	3
H10	0.33	0.66	0.69	0.66	1.01	0.69	1.12	1.06	0.65		3	4	2	3	3	3	3	2	5	2	4	5	5	3	3
H11	0.67	0.98	1.02	0.98	1.35	1.02	1.46	1.38	0.98	1.00		1	3	4	3	3	3	2	5	2	4	5	5	3	3
H12	0.98	1.32	1.38	1.32	1.68	1.38	1.87	1.75	1.28	1.31	0.32		4	5	5	5	5	4	3	4	6	1	7	5	5
H13	0.33	0.66	0.69	0.66	0.99	0.69	1.12	1.05	0.66	0.65	0.98	1.28		1	1	1	3	2	3	2	4	5	5	3	1
H14	0.66	0.98	1.02	0.98	1.31	1.02	1.45	1.37	0.99	0.97	1.29	1.59	0.32		2	2	4	3	4	3	5	6	6	4	2
H15	0.66	1.00	1.05	0.32	1.33	1.05	1.54	0.66	0.98	0.97	1.28	1.61	0.33	0.64		2	2	3	4	3	3	6	6	4	2
H16	0.69	1.05	1.12	1.05	1.40	0.34	0.72	0.69	1.02	1.01	1.34	1.68	0.34	0.67	0.71		4	3	4	3	3	6	6	4	2
H17	0.68	1.03	1.09	0.33	1.38	1.09	1.59	0.68	1.01	1.00	1.32	1.65	1.01	1.34	0.66	1.41		3	6	3	3	6	6	4	4
H18	0.33	0.66	0.69	0.66	1.00	0.69	1.12	1.05	0.66	0.65	0.98	1.28	0.66	0.99	0.98	1.02	1.01		5	2	4	5	5	3	3
H19	1.33	1.63	1.70	1.63	2.01	1.70	2.15	2.04	1.65	1.66	0.65	0.96	0.98	1.29	1.28	1.34	2.01	1.65		5	7	4	8	6	4
H20	0.33	0.66	0.69	0.66	0.99	0.69	1.12	1.05	0.66	0.65	0.98	1.28	0.66	0.99	0.98	1.02	1.01	0.66	1.65		2	5	5	3	3
H21	1.05	1.44	1.54	0.66	1.78	0.69	1.12	0.33	1.37	1.36	1.68	2.04	1.37	1.69	0.98	1.02	1.01	1.37	2.36	0.71		7	7	5	5
H22	1.28	1.61	1.68	1.61	1.97	1.68	2.17	2.04	1.59	1.60	0.62	0.30	1.59	1.89	1.90	1.98	1.95	1.59	1.27	1.59	2.34		6	6	6
H23	1.31	1.64	1.72	1.64	0.65	1.72	2.22	2.09	1.63	1.63	1.95	2.26	1.63	1.95	1.95	2.04	2.01	1.63	2.63	1.63	2.40	1.91		6	6
H24	0.69	1.06	0.34	1.06	1.42	1.12	0.72	1.55	1.01	1.02	1.36	1.71	1.01	1.33	1.36	1.44	1.41	1.01	2.03	1.01	1.85	2.00	2.04		2
H25	0.65	0.97	1.01	0.97	1.32	1.01	1.44	1.36	0.97	0.98	1.30	1.60	0.32	0.63	0.64	0.67	1.32	0.97	1.30	0.97	1.68	1.90	1.94	0.67	

Table 2). The parsimony consensus tree presented here (Fig. 2A) is not a true phylogeny, but an effective illustration of the general relationships among mtDNA haplotypes. The neighbor-joining and UPGMA trees generated by the matrix of sequence divergence values from restriction-site data (Table 3) provide a depiction of relationships similar to the maximum-parsimony tree in most important details (Fig. 2B), e.g., grouping of haplotypes ([08+21], [04+17], and 15), ([{12+22}+11], and 19), and grouping of haplotypes ([{03+24}+07] and [06+16]). The UPGMA tree showed the same pattern as the neighbor-joining tree.

By using the approach of Avise et al. (1979), a composite parsimony network was constructed for the 25 haplotypes (Fig. 3). It evoked 28 character state changes, retained branches of unitary length, and required 4 hypothetical haplotypes that were not actually observed. The network corroborates the neighbor-joining and UPGMA analyses. This network presents in detail the complexity of the evolutionary relationships among mitochondrial haplotypes within *N. culturatus*. Starlike genealogies represent those networks which were shallow and dominated by a single, or a few, haplotypes, and where rare haplotypes differed from the most common haplotypes by only a few restriction sites.

DISCUSSION

The PCR-RFLP assay resolved 25 mtDNA haplotypes of *N. culturatus*. This assay offered 4 advantages: (1) facilitation of 'hands-on' appraisal of phylogeographic patterns, (2) avoidance of potential complication changes among adjacent nucleotides (Walker et al. 1995), (3) low cost as compared to direct sequencing, and (4) an efficacious alternative method to conventional restriction approaches (Osentoski and Lamb 1995).

The mtDNA data obtained by the PCR-RFLP assay demonstrated that *N. culturatus* exhibits limited geographical variation across the Central Mountain Range of Taiwan. The estimated genetic distances in terms of base substitution per nucleotide site between mtDNA genotypes were small (unweighted mean ≒ 0.012). Moreover, the phylogenetic assemblages of the haplotypes reflected by neighbor-joining analysis were widely distributed geographically, and the single most common mtDNA haplotype was present in nearly half of all locations. Therefore the mtDNA phylogenetic network (Fig. 3) was characterized by few mutational steps between most pairs of genotypes and little geographic orienta-

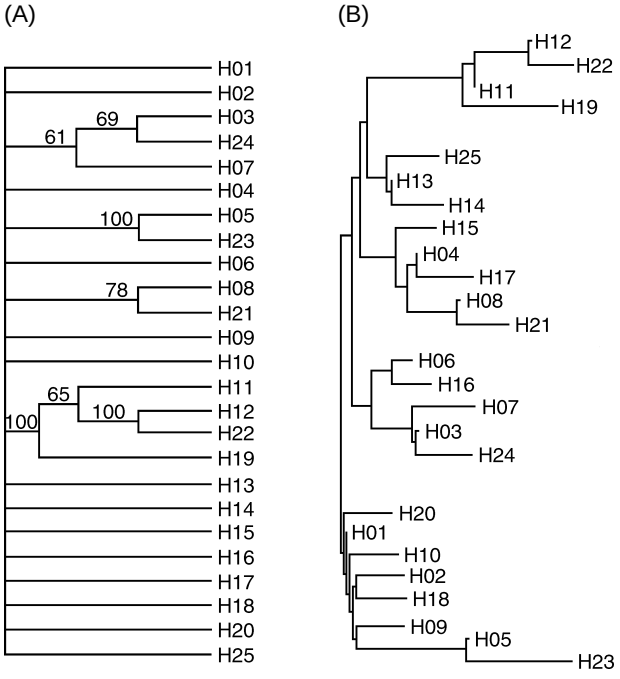

Fig. 2. Majority-rule consensus tree (1422 trees, 28 steps long, CI = 0.71, RI = 0.70) depicting relationships among mtDNA haplotypes (A) and neighbor-joining tree using matrix of distances among haplotypes (B) of *Niviventer culturatus* (haplotypes H01 to H25 refer to haplotype code numbers in table 2).

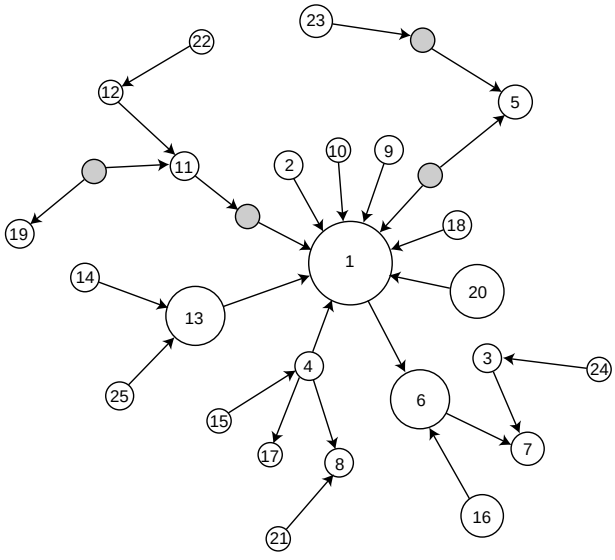

Fig. 3. A possible maximum parsimony network showing the phylogenetic relationship among mtDNA haplotypes of *Niviventer culturatus* (open circles, haplotypes found in this survey; shaded circles, hypothetical haplotypes; circled areas, occurrence frequencies in the total sample; arrow directions, loss of restriction site; numbers of haplotypes refer to code numbers in table 2).

tion to the haplotype branches. Also, most of the haplotypes were found within a single location or associated with nearby locations, except for the common haplotypes 01, 06, and 13. The result of Monte Carlo simulation suggested a significantly heterogenous geographic distribution of the haplotypes. Therefore, some mtDNA haplotypes are geographically widespread, whereas allied haplotypes are localized, so that the overall pattern is one of a nested series of phylogeographic relationships, representing an example of the phylogeographic category V as defined by Avise et al. (1987).

This limited phylogeographic variation of *N. culturatus* in highlands differs from those of conspecific studies of other animals in the lowlands of Taiwan (Yang 1994, Chang and Liu 1997, Toda et al. 1997 1998, Yeh 1997). The first possible explanation of the difference is the later invasion of the ancestor of *N. culturatus* from the Asiatic mainland. Based on the allozyme and karyotypic data, Yu (1995) and Yu et al. (1996) proposed a secondary incursion hypothesis to explain a pair of congeneric endemic rodents *N. culturatus* and *N. coxingi*, because they are not sister taxa. The net nucleotide divergence between locations ranged from -0.08% to 1.71% with a mean average of 0.4% for *N. culturatus*, which was smaller than the 0% to 11.8% for *Japalura swinhonis* (Chang and Liu 1997), 0.12% to 2.54% with a mean average 1.1% for *Rhacophorus taipeianus* (Yang et al. 1994), and 0% to 2.21% for *Rhacophorus moltrechti* (Yeh 1997). Based on geological evidence, Taiwan was connected to and separated from the Asian continent several times in past geological times (Lin 1963 1966). The first separation was in the late Pliocene, and there might have been several times of connection and separation under the interactive effects of tectonic dynamics and sea-level changes in the Pleistocene (Yang et al. 1994). The last connection may have occurred 150 000-10 000 years ago (Lin 1966). Each event of connection and separation would have provided terrestrial animals on the Asian mainland an opportunity to disperse to Taiwan and to evolve into new species. The magnitude of sequence divergence among lineages generally increases with time.

The second possible explanation is that *N. culturatus* is a demographically "young" species, whose extensive range represents a rapid colonization from multiple intermountain refugia after the last glaciation in Taiwan. Certainly, much of the current range of *N. culturatus* in the mountain area has become habitable since the retreat of the last glacier some 10 000 years ago (Lin 1966). Based on pollen records at elevations of 550 and 750 m in central Taiwan (Tsukada 1966 1967), the temperature during the Pleistocene glacier period was 5.0-9.0 °C cooler than today, and thus *N. culturatus* might have taken shelter in lowland refugial areas.

The third possible explanation is closely intertwined with the second explanation and relates to the fact that *N. culturatus* has a greater dispersal capability and gene flow than do reptiles and frogs. This may be inferred from its relatively low sequence divergence over a broad contiguous geographic area (Hayes and Harrison 1992). Although the movements of *N. culturatus* are unknown, it is the most common rat in the mountain range at elevations of 1500 to 3600 m. It is also an agile tree climber (Yu 1993). Recently, Yu (1995) estimated gene flow based on the electrophoretic allozyme data of *N. culturatus* at different transects and elevations in central Taiwan. The gene flow among subpopulations was 5.604, indicating that the gene flow was substantial, and the potential isolating effect imposed by deep river valleys was minimal. We found that many haplotypes had a limited distribution (Table 2) with a small net nucleotide divergence between locations. Avise et al. (1987) assumes that common haplotypes are plesiomorphic (ancestral), while other genotypes are apomorphic (derived) in this phylogeographic pattern. The low nucleotide divergence between locations for *N. culturatus* indicates that each local population has been established by many founders and/or has recently expanded from a polymorphic ancestral population (Nei 1987, Slatkin and Maddison 1989).

It may be concluded that *N. culturatus* exhibits limited geographical variation in mtDNA. The topography of the Central Mountain Range plays a limited role as a barrier against gene flow for this highland species. This supports the hypothesis that the magnitude of mtDNA phylogenetic structure among conspecific vertebrate populations appears to correlate with historical vicariance and dispersal events.

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高山白腹鼠(*Niviventer culturatus*)粒線體 DNA 之地理類緣變異

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本研究利用 PCR-RFLP 的方法分析臺灣地區 20 個族群， 128 隻高山白腹鼠(*Niviventer culturatus*)的地理類緣關係變異。利用聚合酶連鎖反應(PCR)分別增幅含粒線體 DNA 之 *CYTb/DL* (約 2800 bp) 及 *12S/16S* (約 1900 bp)等基因的兩個片段，再以 13 種限制內切酶來分析這兩個增幅的片段，總共得到 25 個粒線體 DNA 的單倍基因型(haplotype)。各單倍基因型的序列差異介於 0.32% 至 2.63% 之間，族群內粒線體 DNA 的分歧度估計值介於為 0% 至 0.69% ，而族群與族群之間的差異介於 -0.08% 至 1.71% 。除了少數幾個單倍基因型的族群地理分布較廣之外，大多數的單倍基因型都只發現在單一族群或相鄰近的族群內，而各單倍基因型之間並沒發現明顯的地理類緣關係組合。這結果可能是受到歷史地質事件及較高程度基因互流(gene flow)的影響。

關鍵詞：地理類緣關係變異，限制內切酶，單倍基因型，高山白腹鼠。

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