Patterns of diversification and genetic population structure of small mammals in Taiwan

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Received 22 March 1994; accepted for publication 21 September 1994

Taiwan is a mountainous island off the coast of the Asian continent. The island is located on the continental shelf and rises to an elevation of nearly 4000 m. It became an island approximately 4 million years ago and has been connected to the continent more than once since its emergence. Therefore, the elevational zonation of two Taiwanese pairs of congeners in the rodent genera *Apodemus* and *Niviventer* can be explained by one of two competing hypotheses. One hypothesis assumes that speciation of the two congeners occurred *in situ* after an ancestral species migrated from the continent. In contrast, the second hypothesis argues the zonation resulted from separate incursion events during the connections. The phylogenetic analyses, which are based on electrophoretic allozyme data, reject the first of these hypotheses. Furthermore, genetic population structure and gene flow of three species of Taiwanese rodents (*Apodemus semotus*, *Niviventer culturatus* and *Microtus kikuchii*) are studied in relation to their patterns of elevational distribution. The genetic structure of *A. semotus* and *N. culturatus*, which have extensive elevational distribution, is rather homogeneous and no genic discontinuity or cline in allele frequency was detected. Gene flow among subpopulations for these two species is rather substantial; therefore, the potential isolating effect imposed by deep river valleys is minimal. Conversely, isolation of populations of *M. kikuchii* on different mountaintops is quite complete as mirrored by the high level of genic differentiation and low gene flow.

ADDITIONAL KEY WORDS:—biogeography – Asian mammals – allozyme variation – gene flow – *F*-statistics – elevational cline.

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INTRODUCTION

The island of Taiwan rose from the sea floor on the Asian continental shelf about 4 million years ago (Hsu, 1990). The island is not only geologically young but also has a sharp elevational gradient, its elevation rising from sea level to nearly 4000 m within a horizontal distance of less than 100 km. As a consequence, many drainages etch deeply through the mountainous terrain and isolate the ridges that are divided by these drainages. These geographic barriers might affect the genetic structure of various populations of small mammals (as, for example, in the North American pocket gopher *Thomomys bottae*, Patton & Smith, 1990; Smith & Patton, 1980; Smith *et al.*, 1983) that inhabit different drainages in Taiwan. Moreover, climate and vegetation change drastically along the vertical slopes (Yu, 1992, 1994). These changes create heterogeneous environments that might also affect genetic differentiation in natural populations (Hedrick *et al.*, 1976; Hedrick, 1986; Schnell & Selander, 1981).

The geological history of Taiwan has also likely had importance in shaping the modern distribution of small mammals on the island. The island lies just 120 km offshore east of the Asian continent, separated by the shallow Strait of Taiwan (mostly <100 m deep). Both faunistic analysis (Kano, 1940) and geological evidence (Huang, 1984; Kano, 1940; Liu & Ding, 1984) indicate that Taiwan was connected to the Asian continent at least twice, initially during the Pliocene and again during the Pleistocene of receding sea level in the peak of glaciation. Moreover, during the Pleistocene there might have been more than one connection due to sea-level lowering (Fairbanks, 1989; Gascoyne *et al.*, 1979). These connections potentially allowed multiple faunistic exchanges between the island and continent. Although this scenario is appealing, it has not been tested.

Two aspects of the modern distribution of small mammals in Taiwan (Yu, 1992, 1994; also see Kano, 1940) are of interest in relation to its geography and geological history. First, some species such as *Apodemus semotus* and *Niviventer culturatus* are widely distributed across the elevational gradient (1400–3670 m), whereas an endemic species of vole (*Microtus kikuchii*) is narrowly restricted to tops of high mountains, mostly from 3000 to 3670 m (Yu, 1992, 1994). Genetic variation within species has been shown to be related to their ecological, demographic, biogeographic, and life history correlates (Nevo *et al.*, 1984; Busack, 1986; Bowen, 1982). For Taiwanese small mammals, it is interesting to see if differences in patterns of microgeographic distribution may have influenced genetic characteristics after geological events had isolated these taxa from their continental relatives.

Second, a pair of species in each of the genera *Apodemus* and *Niviventer* occurs in Taiwan, and members of each pair have parapatric elevational distributions. *Apodemus agrarius* lives in lowlands (<1000 m) and *A. semotus* (endemic) in highlands (1400–3670 m); *N. coxingi* occurs no higher than 2000 m and *N. culturatus* from about 2000 to 3600 m on forested slopes (Yu, 1992, 1994). The two species of *Niviventer* are endemic to Taiwan. Furthermore, both genera have several congeners distributed in the Eurasian continent. The genus *Apodemus* has 21 species widely spread in the Palearctic region (Wilson & Reeder, 1993) and 15 species of *Niviventer* occur throughout Southeast Asia (Musser, 1981; Wilson & Reeder, 1993).

At least two alternative evolutionary hypotheses can explain the zonal distribution of two congeners in Taiwan, considering its geological history. One hypothesis is that there was only one incursion of each genus from the continent since the island emerged, followed by parapatric speciation (Endler, 1977), resulting in today's zonal distribution (see Cadle & Patton, 1988; Endler, 1982a,b; Patton *et al.* 1990; Patton & Smith, 1992). However, the zonal distribution also can be interpreted by separate introductions from the mainland at different times following continental allopatric divergence. Assuming the multiple-incursion hypothesis, one can postulate many scenarios of speciation events and subsequent incursions, none of which can be tested easily. However, the hypothesis of a single incursion can be explicitly tested by a robust phylogenetic analysis. As a result of parapatric speciation within the island, this hypothesis demands that the two congeners in Taiwan be sister taxa to each other rather than to any other congeners from the continent (Fig. 1).

I address two questions in this paper. First, I use allozyme electrophoresis to examine the geographic patterns of genetic differentiation in three species of rodents (*A. semotus*, *N. culturatus* and *M. kikuchii*). This examination focuses on: (1) determining intraspecific differentiation existing within each species; and (2) comparing the patterns of differentiation among species, particularly in relation to the two distinct types of elevational distribution and to gene flow. Second, genetic information, which was gathered through allozyme analyses, was used to reconstruct phylogenetic relationships among congeners in *Apodemus* and *Niviventer*, both from Taiwan and the continent, to test the hypothesis that there was a single incursion of small mammals from the Asian mainland to Taiwan.

MATERIAL AND METHODS

Animals and frozen tissue were collected along three elevational transects (1400–3670 m) during 1989–1990 from Yushan National Park in central

Figure 1. Cladograms of the predicted phylogenetic relationships among extant congeneric taxa both in Taiwan and the continent resulting from (A) single incursion and (B) multiple incursion hypothesis.

Taiwan. Field sampling methods and maps of the transects are given in detail by Yu (1992, 1994). Transect 1 and 2 run along two drainages flanking a mountain ridge and lead to the same mountaintop (Mt. Yushan, 3995 m). Transect 3 is c. 24 km south of Mt. Yushan on a slope which is pinnacled by Mt. Guanshan (3600 m).

Kidney samples of *A. semotus* ($n = 154$), *N. culturatus* ($n = 71$), and *M. kikuchii* ($n = 79$) collected from the three transects were analysed by horizontal starch-gel electrophoresis. In addition, kidney of *A. agrarius* ($n = 2$) and *N. coxingi* ($n = 6$) collected from lowlands of Taiwan, *M. fortis* ($n = 2$) from Korea, *A. agrarius* ($n = 3$) from Serbia, *N. bukit* ($n = 2$) from China, and *M. californicus* ($n = 2$) from the United States are included in the analysis. Nineteen enzymes and other proteins encoded by 26 presumptive gene loci (values follow E.C. numbers) were examined for all samples: Glycerol-3-phosphate dehydrogenase (*Gpd*, E.C. 1.1.1.8), Sorbitol dehydrogenase (*Sordh*, E.C. 1.1.1.14), Lactase dehydrogenase (*Ldh-1*, *Ldh-2*, E.C. 1.1.1.27), Malate dehydrogenase (*Mdh-1*, *Mdh-2*, E.C. 1.1.1.37), Malic enzyme (*Me-1*, *Me-2*, E.C. 1.1.1.40), Isocitrate dehydrogenase (*Icd-1*, *Icd-2*, E.C. 1.1.1.42), 6-phosphogluconate dehydrogenase (*6-Pgd*, E.C. 1.1.1.44), Glucose-6-phosphate dehydrogenase (*Gd*, E.C. 1.1.1.49), Glyceraldehyde-3-phosphate-dehydrogenase (*Gapdh*, E.C. 1.2.1.12), Superoxide dismutase (*Sod-1*, *Sod-2*, E.C. 1.15.1.1), Purine nucleoside phosphorylase (*Np*, E.C. 2.4.2.1), Glutamate oxalate transaminase (*Got-1*, *Got-2*, E.C. 2.6.1.1), Phosphoglucomutase (*Pgm*, E.C. 2.7.5.1), L-leucyl-alanine (*La-1*, 3.4.11.1), L-leucylglycylglycine (*Lgg*, E.C. 3.4.11.4), L-phenylalanyl-L-proline (*Pap*, E.C. 3.4.11.5), Aconitase (*Acon-1*, *Acon-2*, E.C. 4.2.1.3), Glucose-phosphate-isomerase (*Gpi*, E.C. 5.3.1.9), and Albumin (*Alb*). Tissue was first homogenized and centrifuged at 4°C and 15000 rpm for 45 min. The aqueous protein extracts were then stored at -76°C for later electrophoretic analysis. The gel-running conditions follow Yu (1992) and are available on request. The electrophoretic procedures follow those of Patton & Yang (1977) and Selander *et al.* (1971). Protein assays were prepared according to Harris & Hopkinson (1976) and Selander *et al.* (1971). Alleles (electromorphs) at each locus were designated alphabetically in decreasing order of anodal mobility. Because the alleles of Glycero-3-phosphate dehydrogenase (*Gpd*) in *Microtus* could not be scored reliably, the locus was excluded from the analysis for that genus.

Levels of heterozygosity were derived from the electromorphic genotypes by direct count and averaged across loci. The genetic distances of Rogers (1972) and Nei (1978) were calculated from allelic frequencies. Patterns of phenetic similarity among populations and taxa were obtained by UPGMA clustering (Sneath & Sokal, 1973) of Rogers' genetic distances. Phylogenetic analysis was performed by parsimony methods; each locus was treated as a character and allelic combinations as character states (Buth, 1984). Character states were unordered. Loss or gain of an allele between two character states was counted as one step and a step matrix was constructed as described by Mabee & Humphries (1993). All the above analyses except parsimony were executed using the BIOSYS-1 program (Swofford & Selander, 1981) on an IBM 4341 mainframe computer. Parsimony phylogenetic analysis was done by PAUP (version 3.0s; Swofford, 1991) on an Apple Macintosh Classic II. The exhaustive option of PAUP was chosen to search for all shortest

trees based on the coding method described by Mabee & Humphries (1993).

Wright's F -statistics (F_{IS} and F_{ST}) were calculated to analyse genetic structure of the sampled populations (Wright, 1965, 1978). F_{IS} is the inbreeding coefficient measuring the reduction in heterozygosity of an individual due to non-random mating within its subpopulation; positive values indicate heterozygote deficiencies whereas negative ones signal excesses. F_{ST} , the fixation index, assesses the amount of differentiation among subpopulations; its value ranges from 0 to 1, 0 meaning no differentiation at any locus and 1 absolute differentiation at all loci. To test the null hypothesis that F_{IS} equals 0, $n(F_{IS})^2$, where n is the total number of individuals, was computed, with one degree of freedom in a X^2 distribution (Baker, 1981; Nei, 1977). The significance of F_{ST} was tested by the formulae given in Workman and Niswander (1970), with $(r-1)(k-1)$ degrees of freedom, where r is the number of subpopulations and k the number of alleles at a locus, also in a X^2 distribution.

To estimate levels of gene flow (Nm), a formula based on genic frequency of 'private alleles' (Slatkin, 1985) was used. When the sample size (n) was less than 25, correcting factors ($25/n$) were multiplied to yield Nm_c (Slatkin, 1985). A second estimate of gene flow was calculated by the formula $F_{ST} = 1/(4Nm+1)$ (Wright, 1943).

For convenient comparison, I divided samples from different transects into comparable elevations; low (below 2000 m), middle (2300–2600 m) and high (above 3300 m) in *A. semotus*, and low (below 2400 m) and high (above 3000 m) in *N. culturatus*. However, since *M. kikuchii* is restricted to mountaintops which are usually above 3000 m, I grouped its samples according to the elevation at which the voles were captured. Consequently, different samples were abbreviated as, for example, T1-low or T3-3000 m which means the sample was collected from the low elevation on Transect 1 and 3000 m on Transect 3, respectively. Locality data and museum catalog numbers for all individuals examined are given in Appendix 1.

RESULTS

Allozymic variation and heterozygosity

Complete lists of allelic frequencies for each species are given in Appendix 2. The following provides a summary of the results. Levels of genetic variation for all populations are summarized in Table 1.

Apodemus

Of the 26 loci scored, 13 (50%) were variable either within or between populations of *A. semotus* and *A. agrarius*. Of these variable loci, 10 (38% of total) showed at least a single heterozygote in *A. semotus*, the others were monomorphic. *Apodemus agrarius* was fixed at four loci at alleles which did not occur in *A. semotus* and was polymorphic at five loci.

For *A. semotus* (Table 1), the mean numbers of alleles per locus ranged from 1.1 to 1.3, with an average of 1.18; the percentage of polymorphic loci ranged from 0 (T1-high) to 15.4 (T2-middle); the mean heterozygosity per

TABLE 1. Sample sizes (n), mean number of alleles per locus, percentage of loci polymorphic, and mean heterozygosity across loci in species and populations of three rodent genera from Taiwan

Population	n	Mean number of alleles per locus	Percent polymorphic loci ^a	Mean heterozygosity
<i>Apodemus semotus</i>				
1 T1-low	20	1.2	7.7	0.013
2 T1-middle	20	1.2	7.7	0.019
3 T1-high	20	1.1	0.0	0.006
4 T2-low	20	1.1	7.7	0.015
5 T2-middle	20	1.3	15.4	0.021
6 T2-high	13	1.1	3.8	0.012
7 T3-low	13	1.2	11.5	0.024
8 T3-middle	20	1.3	11.5	0.035
9 T3-high	8	1.1	7.7	0.010
Mean	17.1	1.18	8.11	0.017
<i>Niviventer culturatus</i>				
1 T1-low	12	1.1	7.7	0.022
2 T1-high	12	1.0	0.0	0.000
3 T2-low	12	1.0	3.8	0.006
4 T2-high	11	1.1	3.8	0.014
5 T3-low	12	1.0	0.0	0.003
6 T3-high	12	1.2	3.8	0.016
Mean	11.8	1.07	3.18	0.010
<i>Microtus kikuchii</i>				
1 T1-3220 m	20	1.3	16.0	0.026
2 T1-3650 m	20	1.3	20.0	0.062
3 T2-3240 m	14	1.2	12.0	0.051
4 T2-3670 m	5	1.2	16.0	0.064
5 T3-3000 m	10	1.3	24.0	0.052
6 T3-3300 m	10	1.2	16.0	0.056
Mean	13.2	1.25	17.33	0.052

^a Frequency of the most common allele less than 0.95.

individual per population was 0.017, ranging from 0.006 (T1-high) to 0.035 (T3-middle).

Niviventer

Fourteen (54%) of 26 loci scored in the three species of *Niviventer* were variable. However, in *N. culturatus* only seven (27%) of the 26 loci showed at least a single heterozygote. *Niviventer coxingi* showed heterozygotes in just two loci. *Niviventer coxingi* was fixed at nine loci at alleles not shared with *N. culturatus*. *Niviventer bukit* showed fixed differences from *N. culturatus* at 10 loci. Five loci had fixed differences between *N. coxingi* and *N. bukit*.

Levels of genetic variation among populations of *N. culturatus* are summarized in Table 1. The mean number of alleles per locus ranged from 1.0 to 1.2, with an average of 1.07. The percentage of polymorphic loci ranged from 0 to 7.7, and averaged 3.18. The mean heterozygosity per individual per population was 0.01, ranging from 0 to 0.022.

TABLE 2. Summary of genetic distance estimates based on allelic frequencies among populations and species of three rodent genera from Taiwan. Both Nei's (1978) and Roger's (1972; in parentheses) distances are given. *n* is the sample size of pairwise comparisons

Comparison	Mean	SD	Range	<i>n</i>
<i>Apodemus</i>				
within <i>semotus</i>	0.012 (0.0004)	0.004 (0.001)	0.006–0.022 (0–0.003)	36
between <i>semotus</i> and Taiwanese <i>agrarius</i>	0.274 (0.298)	0.005 (0.009)	0.265–0.279 (0.277–0.306)	9
between <i>semotus</i> and continental <i>agrarius</i>	0.272 (0.291)	0.004 (0.007)	0.266–0.278 (0.278–0.298)	9
between Taiwanese and continental <i>agrarius</i>	0.064 (0.014)	– –	– –	1
<i>Niviventer</i>				
within <i>culturatus</i>	0.008 (0.0005)	0.004 (0.001)	0.002–0.016 (0–0.002)	15
between <i>culturatus</i> and <i>coxingi</i>	0.397 (0.491)	0.004 (0.004)	0.391–0.402 (0.485–0.495)	6
between <i>culturatus</i> and <i>bukit</i>	0.427 (0.553)	0.004 (0.004)	0.423–0.432 (0.548–0.557)	6
between <i>coxingi</i> and <i>bukit</i>	0.234 (0.249)	– –	– –	1
<i>Microtus</i>				
within <i>kikuchii</i>	0.054 (0.016)	0.024 (0.014)	0.019–0.090 (0–0.037)	15
between <i>kikuchii</i> and <i>fortis</i>	0.230 (0.207)	0.016 (0.021)	0.201–0.243 (0.169–0.223)	6
between <i>kikuchii</i> and <i>californicus</i>	0.309 (0.318)	0.009 (0.018)	0.299–0.320 (0.294–0.345)	6
between <i>fortis</i> and <i>californicus</i>	0.210 (0.196)	– –	– –	1

Microtus

Fourteen (56%) of 25 loci scored in the three species of *Microtus* were variable. In *M. kikuchii* nine loci were variable and 16 loci were monomorphic. *Microtus fortis* showed heterozygotes at three loci. *Microtus californicus* was heterozygous in two loci. *Microtus fortis* had fixed differences at three loci from *M. kikuchii*. *Microtus californicus* showed fixed differences at six loci from *M. kikuchii*. Four loci had fixed differences between *M. fortis* and *M. californicus*.

Levels of genetic variation among populations of *M. kikuchii* are greater than *A. semotus* and *N. culturatus*. The mean number of alleles ranged from 1.2 to 1.3, with an average of 1.25. The percentage of polymorphic loci ranged from 12.0 to 24.0, and averaged 17.33. The mean heterozygosity per individual per population was 0.052, ranging from 0.026 to 0.064.

Genetic distances

Genetic distances (Nei's and Rogers') are presented as pairwise comparisons among populations (Table 2).

Apodemus

For *A. semotus*, Nei's genetic distances between pairs of populations from different transects and elevations were low, ranging from 0.006 to 0.022, average 0.012. For *A. agrarius*, Nei's distance between populations on the island and on the continent was 0.064 or about five times greater than the average intraspecific Nei's distance within *A. semotus* (0.012). *Apodemus semotus*, however, is equally distant to Taiwanese and continental *A. agrarius*, with Nei's distance of 0.274 and 0.272, respectively.

Niviventer

Similar to *A. semotus*, intraspecific genetic distances among *N. culturatus* were low. Nei's distances ranged from 0.002 to 0.016, and averaged 0.008. The average Nei's distances between *N. culturatus* and *N. coxingi* was 0.397 and that between *N. culturatus* and *N. bukit* was 0.427. However, *N. coxingi* and *N. bukit* were genetically more similar, with a Nei's distance of 0.234.

Microtus

Unlike *A. semotus* and *N. culturatus*, Nei's genetic distances were greater among populations in *M. kikuchii*, varying from 0.019 to 0.090, with an average of 0.054 or about 4–6 times greater than those among *A. semotus* and *N. culturatus*. The average Nei's distance between *M. kikuchii* and *M. fortis* was 0.230, and that between *M. kikuchii* and *M. californicus* was 0.309. Nei's distance between *M. fortis* and *M. californicus* was 0.210.

Genetic population structure

F -statistics (F_{IS} and F_{ST}) were calculated to analyse genetic structure of the sampled populations for *A. semotus*, *N. culturatus* and *M. kikuchii* (Table 3) on the transects.

Apodemus semotus

Mean F_{IS} is low (-0.005) and not significantly different from 0, suggesting random mating. F_{ST} ranged from 0.017 to 0.094, and averaged 0.054. Even though the F_{ST} differs significantly from 0, it is relatively small and therefore the species is genetically more homogeneous across its subpopulations than *M. kikuchii*.

TABLE 3. F -statistics for three species of rodents from Taiwan. n equals number of subpopulations. Values are for the weighted average across all variable loci in each species

Species	n	F_{IS}	F_{ST}^a
<i>Apodemus semotus</i>	9	-0.005 ns	0.054***
<i>Niviventer coxingi</i>	6	0.050 ns	0.065*
<i>Microtus kikuchii</i>	6	0.142 ns	0.203***

^a Equivalent to Nei's (1977) G_{ST} .

* $P < 0.05$; *** $P < 0.001$; ns $P > 0.05$, testing null hypothesis that each value equals to zero.

Niviventer culturatus

Mean F_{IS} is 0.050 and not significantly different from 0, also suggesting random mating. Similar to *A. semotus*, mean F_{ST} (0.065) is significantly different from 0 but small. Therefore, little genetic differentiation exists among subpopulations.

Microtus kikuchii

In contrast to the other two species, *M. kikuchii* showed some evidence of nonrandom mating (mean F_{IS} 0.142); however, this mean value was not significantly different from zero ($P > 0.05$). Moreover, its subpopulations exhibited much higher genetic differentiation (mean F_{ST} 0.203; $P < 0.001$) compared to the other two widely distributed species.

Gene flow

The levels of gene flow for the three species of rodents are given in Table 4. Gene flow estimated by 'private alleles' among populations from different transects and elevations was rather high in *A. semotus* and *N. culturatus*, 3.867 and 5.604, respectively. This result indicates that gene exchange occurs frequently not only among the populations across elevations within one transect but also among those from different drainages, for both species which showed extensive elevational distribution. In contrast, the gene flow among populations of *M. kikuchii* was low, 0.308 estimated by 'private allele' methods, reflecting its physical isolation on tops of mountains. Gene flow estimated by F_{ST} also points to the same trend even though this method indicates greater gene flow in *A. semotus* (4.380) than *N. culturatus* (3.596). Still, gene flow in both species are roughly 3–5 fold of that in *M. kikuchii* (0.982).

Relationships among congeneric species and populations

For each genus of rodent, the phenetic affinity among species and populations suggested by allozyme data are illustrated in UPGMA dendrograms

TABLE 4. Estimates of gene flow (Nm) in three species of rodents from Taiwan. Number (n) and average frequency ($p(1)$) of private alleles, number of demes (d), and average sample size (N) used for Slatkin's method are also given

Species	Nm	n	Slakin (1985) 'private' alleles		N	Wright (1943) Nm^*
			$p(1)$	d		
<i>Apodemus semotus</i>	3.867 ^b	3	0.0533	9	17.1	4.380
<i>Niviventer culturatus</i>	5.604 ^b	6	0.0533	6	11.8	3.596
<i>Microtus kikuchii</i>	0.308	5	0.1580	3 ^c	26.3	0.982

* Calculated by $F_{ST} = 1/(4Nm+1)$.

^b $Nm = Nm_c$, corrected for sample size.

^c Populations from the same transect were pooled.

based on Rogers' genetic distances (Fig. 2). For *Apodemus* and *Niviventer*, the phylogenetic trees based on parsimony are presented in Figure 3. The trees are rooted at mid-point based on the assumption that rates of evolution are equal in all lineages.

Apodemus

Based on UPGMA clustering (Fig. 2A), the population of *A. agrarius* from the lowlands of Taiwan is much closer to that from the Eurasian continent (Serbia) than to *A. semotus* from the highlands of Taiwan. Populations of *A. semotus* are shallowly branched and do not cluster consistently either by one elevation or by one transect. In particular, in *A. semotus* there are no elevational clines defined by allozyme variations. When polymorphic loci are examined individually (Appendix 2), no alleles at any locus appear to correlate with elevation.

Phylogenetic analysis of *Apodemus* by exhaustive search of PAUP reveals three shortest trees each with 31 steps and a consistency index (CI) 0.808. The 50% majority-rule consensus tree is shown in Figure 3A in which two nodes that were identified by more than 90% of bootstrap replicates are indicated. One includes all nine populations of *A. semotus* while the other contains *A. agrarius* from both Taiwan and the continent. The results refute that the two species of *Apodemus* in Taiwan are sister taxa.

Niviventer

Phenetically, *N. coxingi* from Taiwan is more similar to *N. bukit* from China than to the parapatric *N. culturatus* (Fig. 2B). As in *A. semotus*, no clusters are grouped consistently either by elevation or by transect among six populations of *N. culturatus*. The genetic differentiation among populations of *N. culturatus* is also low (the deepest node occur at *c.* 0.01 Rogers' genetic distance) and no elevational clines in polymorphic allozyme frequencies are detected (see Appendix 2).

Phylogenetic analyses of *Niviventer* by PAUP generate 105 equally parsimonious trees 25 steps in length (CI = 0.909). Figure 3B illustrates the 50% majority-rule consensus tree in which two nodes with support of 90% or more bootstrap replicates are identified. The lowland species from Taiwan, *N. coxingi*, forms a sister group with *N. bukit* from China rather than with *N. culturatus* the highland species from Taiwan. All six populations of *N. culturatus* constitute a well-defined group.

Microtus

The UPGMA phenogram in Figure 2C illustrates that populations of *M. kikuchii* form two major clusters. One includes the populations from Transects 1 and 2, and the other consists of those from Transect 3, indicating that populations in Transect 3 (Mt. Guanshan) are somewhat genetically distinct from the others (Mt. Yushan). Besides, Korean *M. fortis* is phenetically more similar to North American *M. californicus* than to Taiwanese *M. kikuchii*.

DISCUSSION

Phylogeny and biogeography

Much light has been shed on the evolutionary history of the mammals in southeastern Asia by examining their patterns of endemism, species diversity,

Figure 2. UPGMA dendrogram of affinity among species and populations in three genera of rodents from Taiwan as measured by Rogers' (1972) genetic distance: (A) *Apodemus*, (B) *Niviventer*, and (C) *Microtus*. The coefficient of cophenetic correlation (r_{cc}) are 1.00, 0.99 and 0.97 for each genus.

Figure 3. Fifty percent majority-rule consensus tree showing phylogenetic relationships among species and populations in two genera of rodents from Taiwan based on allozyme data: (A) *Apodemus* and (B) *Niviventer*. Trees are drawn in proportion to branch length and rooted at the mid-point assuming that rates of evolution in all congeneric lineages are equal (also see Discussion). The solid circles signify internal nodes that were supported by at least 90% of 100 bootstrap replicates (Felsenstein, 1985).

and faunistic affinities (e.g. Kano, 1940; Heaney, 1984, 1986; Heaney & Rickart, 1990). However, by employing allozyme data to analyse two genera of rodents (*Apodemus* and *Niviventer*), this study offers another perspective which emphasizes the patterns of phylogenetic relationship among species and populations to infer their evolutionary history. The approach does not presume either parapatric or allopatric divergence but, instead, acknowledges both as possible processes. The results of the phylogenetic analysis can be used to critically test specific hypotheses concerning evolutionary history of rodents in Taiwan (Fig. 1).

The phylogenetic analyses of the congeners in *Apodemus* and *Niviventer* falsify the hypothesis of a single incursion of each genus from the Asian continent to Taiwan with subsequent divergence. A multiple-incursion colonizing hypothesis better explains the modern distribution of these genera in Taiwan. However, the results of parsimony analysis are contingent on the assumption that rates of evolution in all lineages within a genus are equal. While this assumption may be valid for congeneric species, in the future it is imperative to test whether or not the rates of evolution among all congeneric species are indeed equal, preferably by analysing some DNA sequences. Kano (1940), studying faunistic affinities, argued that the vertebrate fauna in Taiwan consists of—disregarding cosmopolitan species—two major components from two separate zoogeographical regions, the Oriental and Palearctic (Cox & Moore, 1985). The Oriental component is predominant

in the lowlands whereas the Palaearctic component occurs mostly in the highlands. It was therefore postulated (Kano, 1940) that the Palaearctic (cold-adapted) group came to Taiwan during the climax of glaciation and only found suitable habitats in the highlands when the glaciers receded. Meanwhile the lowlands had been recolonized by the Oriental (warm-adapted) group following the retreating glaciers before the land bridge was submerged. Although the argument may still be appropriate for the origin of some Taiwanese mammals, it has shortcomings. On one hand, the taxonomy by which Kano derived faunistic affinities was rudimentary and is now considered obsolete. Many discoveries about the fauna in this general region can be profound compared to what was known, when precise and modern approaches are employed, as shown in the Philippines (Musser & Heaney, 1992). A thorough taxonomic revision of mammals of Taiwan and Southeast Asia is needed in order to reassess faunistic affinities. On the other hand, for some groups of animals (e.g. *Apodemus* and *Niviventer*) the demarcation of zoogeographic boundaries is potentially obscure and difficult. Mice of the genus *Apodemus* are non-commensal murine rodents which occur almost exclusively in the Palaearctic region (Corbet, 1978; Wilson & Reeder, 1993), and yet two Taiwanese species occupy the lowlands and highlands. In contrast, rats of the genus *Niviventer* occur widely across both the Palaearctic and Oriental regions (Musser, 1981; Wilson & Reeder, 1993) and at present it is impossible to determine the zoogeographic origins of individual species. Circumventing such shortcomings, phylogenetic analyses as I have done here for Taiwanese rodents can be implemented on specific groups of animals which are widely distributed in southeast Asia. Such analyses will provide insight to the evolutionary history of animals in this area.

In particular, rats of *Niviventer* occupy all major continental islands in Southeast Asia (notably Hainan, Borneo, Java and Sumatra) and the Asian continent (Musser, 1981; Wilson & Reeder, 1993). There are at least two species of *Niviventer* occurring on each of those islands and they have parapatric elevational distributions similar to those of their Taiwanese counterparts. Moreover, these islands share a similar recent geological history to Taiwan's, having been repeatedly connected to the continent during the Pleistocene. It will therefore be worthwhile to include all the species of *Niviventer* in rigorous phylogenetic analyses to elucidate their evolutionary history in Southeast Asia and to determine whether similar biogeographic patterns occur throughout the range of the genus.

Genetic variation and environmental heterogeneity

The levels of genetic differentiation in the three species of small mammals analysed here are related to two distinct patterns of their elevational distribution. The murines *A. semotus* and *N. culturatus* have wider elevational distribution and relatively low intraspecific genic variability compared to *M. kikuchii* (Table 1), which is restricted to mountaintops normally above 3000 m (Yu, 1992, 1994). For the two murine rodents, moreover, the genetic distances among samples representing populations from different elevations and drainages were low (Table 2) and these samples therefore clustered shallowly at the tips of their UPGMA dendrograms (Fig. 2A,B). Conversely,

for *M. kikuchii*, its average interpopulational Nei's genetic distances (0.054) was approximately 4–6 fold greater than those of *A. semotus* (0.012) and *N. culturatus* (0.008). Furthermore, genetic differentiation as measured by F_{ST} among populations of *M. kikuchii* was about 3.8 and 3.1 times greater than that of *A. semotus* and *N. culturatus*, respectively (Table 3). The UPGMA dendrogram (Fig. 2C) on *M. kikuchii* shows that samples from the adjacent Transect 1 and Transect 2, constitute a distinct cluster whereas those from the more distant Transect 3 form another (see maps in Yu 1992 & 1994). In other words, while populations of *A. semotus* and *N. culturatus* do not show much differentiation, populations of *M. kikuchii* from two mountaintops (Mt. Yushan and Mt. Guanshan) are well differentiated genetically, probably because of physical isolation.

For the two elevationally widespread species, it is noteworthy that allozyme variation as measured by mean heterozygosity, *A. semotus* (0.017) and *N. culturatus* (0.010), is relatively low compared to other mammals (Gillespie, 1991; Nevo *et al.*, 1984) and no genetic discontinuity or cline in allele frequency were associated with elevation (Appendix 2). Yet, the habitat heterogeneity represented by over 2200 m of elevational change in Taiwan and the potential selection forces associated with this change remain impressive. For instance, along this gradient, mean annual temperature drops from 19 to 4°C and atmospheric pressure decreases from 89 to 64% of that at sea level (Yu, 1992, 1994). Few cases of protein polymorphism have been demonstrated to have differential effects on the level of physiology in such a way as to influence Darwinian fitness of the whole organism (see review by Gillespie, 1991). A study of polymorphism in haemoglobin in *A. semotus* and *N. culturatus* may well prove worthwhile in light of the protein's adaptive significance for animal's capability to coping with hypoxia at high elevation as shown in *Peromyscus maniculatus* of North America (Chappell & Snyder, 1984).

Although *M. kikuchii* is confined to small areas on mountaintops, no strong evidence of inbreeding is found (F_{IS} no different from 0) and its genic variation is higher than that of *A. semotus* and *N. culturatus*. Perhaps the genic variation in *M. kikuchii* is maintained by some selection forces associated with the environmental fluctuations (e.g. monthly temperature) at high elevation. Besides, the mating system in this vole may play a role in promoting the variation.

Gene flow (Table 4) among subpopulations for *A. semotus* and *N. culturatus* is substantial, 3.867 and 5.604 by the 'private' alleles method, respectively. By the same estimating method, the highest gene flow in mammals listed by Slatkin (1985) was 2.20 for *Peromyscus californicus* and that listed by Patton & Smith (1990) was 2.94 for *Thomomys bottae*. Therefore, the potential isolating effect imposed by the deep river valleys separating the transects appears minimal for these two species. The high gene flow in *A. semotus* and *N. culturatus* might be enough to swamp out local differentiation or cline (if any) within the sharp elevational gradient that is occupied by them. Conversely, gene flow between populations of *M. kikuchii* on different mountaintops is quite low (<1) but not completely terminated by the physical and/or habitat isolation. Perhaps downward migration of *M. kikuchii* during the dry (also cold) season to lower elevation (Yu, 1992, 1994) has facilitated

gene exchange between populations from various mountaintops through the lower portion of the valleys. The low gene flow in *M. kikuchii* appears to promote genetic differentiation among its subpopulations. Likewise in the Philippines (Peterson & Heaney, 1993), bats of the genus *Haplonycteris* having low gene flow ($Nm = 0.05$) show more population differentiation than the genus of *Cynopterus*, which have higher gene flow ($Nm = 7.5$).

ACKNOWLEDGEMENTS

I owe thanks to J. L. Patton, C. Patton, O. P. Pearson, W. P. Sousa, J. G. Groth, M. F. Smith, D. A. Good, H. Carson, M. Long and B. D. Patterson for critically reviewing various drafts of this manuscript. D. A. Good and G. Roderick helped in phylogenetic analysis. Two reviewers' comments and suggestions much improved the manuscript. Y. S. Lin, C. M. Kuo and L. L. Lee kindly assisted in obtaining financial support for my field work. I am obliged to Y. S. Lin who provided a home base while I was doing field work in Taiwan. I also thank all the people who assisted me with the field work. I particularly appreciate their undampened spirit to slog with me along those steep muddy slopes in heavy rain. The field work was funded by the Yushan National Park of Taiwan, Republic of China. I was supported by the Museum of Vertebrate Zoology and the Department of Integrative Biology, University of California at Berkeley during my stay in Berkeley. This study served as partial fulfillment for a PhD degree in Zoology from the University of California at Berkeley. A special thanks is given to J. L. Patton for tremendous support. Finally, I want to thank Yu-Lan Chien; I could not have finished my study without her help.

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APPENDIX 1

Specimens examined

Taxa, localities, and museum numbers of vouchers for all specimens from which tissue was taken for electrophoretic analysis are as follows (all the specimens are deposited in the Museum of Vertebrate Zoology (MVZ)):

Apodemus semotus

(1) Transect 1. Taiwan: Nantou Co.; Sha-Li-Xian trail, Xin-Yi Township, 2000 m (MVZ180183, 180184, 180159-180176), Sha-Li-Xian trail, Xin-Yi Township, 2400 m (MVZ180208-180215, 180217-180219, 180223-180226, 180228-180232); Chiayi Co.; 3 km up from Pai-Yun Hostel on trail to Yun-Feng campsite, Alishan Township, 3650 m (MVZ180451, 180458, 180479-180487, 180490-180492).

(2) Transect 2. Taiwan: Nantou Co.; Le-Le hut, 5.5 km Ba-Tong-Guan historical trail, Xin-Yi Township, 1560 m (MVZ180076-180095), 12.8 km Ba-Tong-Guan historical trail, Xin-Yi Township, 2300 m (MVZ180371-180380, 180394-180396, 180398, 180400-180401, 180403, 180405-180407), 1.5 km up from Lao-Nong-Xi campsite, Xin-Yi Township, 3670 m (MVZ180431-180437, 180442-180447).

(3) Transect 3. Taiwan: Kaohsiung Co.; 131.6 km southern cross-island highway, Tao-Yuan Township, 1980 m (MVZ180185-180197), 143.4 km southern cross-island highway, Tao-Yuan Township, 2600 m (MVZ180258-180264, 180287-180298, 180319). On trail to Guan-Shan, Tao-Yuan Township, 3300 m (MVZ180495-180498, 180519-180521, 180529).

Apodemus agrarius

(1) Taiwan: Grassland behind Taichung Veteran Hospital, Taichung city, 150 m (MVZ180533, 180534).

(2) Continent: Yugoslavia; Cornfield, 5 km N. 3 km E. Sremska Mitrovica, Serbia (Frozen-tissue Collection 5213-5215 in MVZ).

Niviventer culturatus

(1) Transect 1. Taiwan: Nantou Co.; Sha-Li-Xian trail, Xin-Yi Township, 2400 m (MVZ180543, 180549, 180550, 180552, 180556, 180557, 180612, 180613, 180617, 180618, 180627, 180630); Chiayi Co.; 2 km down from Pai-Yun Hostel on trail to Ta-Ta-Jia saddle, Alishan Township, 3220 m (MVZ180562-180567, 180702-180707).

(2) Transect 2. Taiwan: Nantou Co.; Dui-Guan, 10.2 km Ba-Tong-Guan historical trail, Xin-Yi Township, 1980 m (MVZ180568, 180571, 180573, 180577, 180580, 180583, 180586, 180589, 180592, 180595, 180598, 180601), 1 km down from Lao-Nong-Xi campsite, Xin-Yi Township, 3240 m (MVZ180692-180695, 180698-180701), 1.5 km up from Lao-Nong-Xi campsite, Xin-Yi Township, 3670 m (180691, 180696, 180697).

(3) Transect 3. Taiwan: Kaohsiung Co.; 135.3 km southern cross-island highway, 2280 m (MVZ180632-180638, 180640, 180650, 180651, 180664-180666). On trail to Guan-Shan, Tao-Yuan Township, 3000 m (MVZ180709, 180711, 180713, 180715, 180717, 180719, 180721). On trail to Guan-Shan, Tao-Yuan Township, 3300 m (MVZ180708, 180722-180725).

Niviventer coxingi

Taiwan: Nantou Co.; Le-Le hut, 5.5 km Ba-Tong-Guan historical trail, 1560 m (MVZ180740); Kaohsiung Co., 131.6 km southern cross-island highway, Tao-Yuan Township, 1980 m (MVZ180735-180739).

Niviventer bukiti

China: Yunnan Prov., vicinity of Menlung, near Laotian border (Frozen-tissue Collection 5459-5460, MVZ).

Microtus kikuchii

(1) Transect 1. Taiwan: Chiayi Co.; 2 km down from Pai-Yun Hostel on trail to Ta-Ta-Jia saddle, Alishan Township, 3220 m (MVZ180746, 180747, 180750, 180751, 180754, 180755, 180722, 180773, 180790, 180791, 180843, 180844, 180847, 180848, 180853, 180854, 180858, 180859, 180862, 180863), 3 km up from Pai-Yun Hostel on trail to Yun-Feng campsite, Alishan Township, 3650 m (MVZ180757, 180758, 180761, 180762, 180766, 180769, 180770, 180776, 180777, 180781, 180782, 180786, 180787, 180793, 180794, 180841, 180842, 180851, 180852, 180857).

(2) Transect 2. Taiwan: Nantou Co.; 1 km down from Lao-Nong-Xi campsite, Xin-Yi Township, 3240 m (MVZ180825-180831, 180834-180840), 1.5 km up from Lao-Nong-Xi campsite, 3670 m (MVZ180822-180824, 180832, 180833).

(3) Transect 3. Taiwan: Kaohsiung Co.; On trail to Guan-Shan, Tao-Yuan Township, 3000 m (MVZ180871-180875, 180877, 180879-180881); On trail to Guan-Shan, 3300 m (MVZ180867-180870, 180882-180887).

Microtus fortis

Korea: Kyung Gi Do, Dong Duchun (MVZ148428, 148429).

Microtus californicus

The United States: California, 2 km NNE Orinda, Contra Costa Co. (MVZ181529, 181530).

Allelic frequencies of rodent populations from Yushan National Park, Taiwan. Sample sizes and related information on each population are available in Table 1.

[illegible]

Appendix 2—continued

(b) Allelic frequencies for 14 polymorphic loci in six populations of *Niviventer culturatus*, and samples of *N. coxingi* (NC) and *N. bukit* (NB)

Locus and allele	Populations of <i>culturatus</i>						NC	NB
		1	2	3	4	5	6	
<i>Me-1</i>	<i>a</i>	0.04						
	<i>b</i>	0.96	1.00	1.00	1.00	1.00	1.00	
	<i>c</i>							1.00
	<i>d</i>							1.00
<i>Me-2</i>	<i>a</i>	1.00	1.00	1.00	1.00	1.00	0.96	
	<i>b</i>							1.00
	<i>c</i>						0.04	
<i>Sordh</i>	<i>a</i>	1.00	1.00	1.00	1.00	1.00	1.00	
	<i>b</i>							1.00
<i>Icd-1</i>	<i>a</i>							1.00
	<i>b</i>							1.00
	<i>c</i>	1.00	1.00	1.00	1.00	1.00	1.00	
<i>Got-2</i>	<i>a</i>							1.00
	<i>b</i>	1.00	1.00	1.00	1.00	1.00	1.00	1.00
<i>Np</i>	<i>a</i>	0.17		0.17	0.23	0.04	0.04	
	<i>b</i>	0.83	1.00	0.83	0.77	0.96	0.96	1.00
<i>Gpi</i>	<i>a</i>	1.00	1.00	1.00	1.00	1.00	1.00	
	<i>b</i>							1.00
	<i>c</i>							1.00
<i>Gpd</i>	<i>a</i>						0.04	0.33
	<i>b</i>	1.00	1.00	1.00	1.00	1.00	0.96	0.67
<i>Sod-1</i>	<i>a</i>	1.00	1.00	1.00	1.00	1.00	1.00	
	<i>b</i>							0.25
	<i>c</i>							0.75
<i>Acon-1</i>	<i>a</i>							1.00
	<i>b</i>							1.00
	<i>c</i>	1.00	1.00	1.00	1.00	1.00	1.00	
<i>Pgm</i>	<i>a</i>	1.00	1.00	1.00	1.00	1.00	1.00	
	<i>b</i>							1.00
<i>6-Pgd</i>	<i>a</i>							1.00
	<i>b</i>						0.08	1.00
	<i>c</i>	1.00	1.00	1.00	1.00	1.00	0.92	
<i>Alb</i>	<i>a</i>	0.92	1.00	1.00	1.00	1.00	1.00	1.00
	<i>b</i>	0.08						
<i>La-1</i>	<i>a</i>				0.04			1.00
	<i>b</i>	1.00	1.00	1.00	0.96	1.00	1.00	1.00

Appendix 2—continued

(c) Allelic frequencies for 14 polymorphic loci in six populations of *Microtus kikuchii*, and in samples of *M. fortis* (MF) and *M. californicus* (MC)

Locus and allele	Population of <i>kikuchii</i>						MF	MC
	1	2	3	4	5	6		
<i>Me-1</i>	<i>a</i>				0.25	0.50	1.00	
	<i>b</i>							1.00
	<i>c</i>	1.00	1.00	1.00	1.00	0.75	0.50	
<i>Me-2</i>	<i>a</i>	1.00	1.00	1.00	1.00	1.00	1.00	
	<i>b</i>						1.00	1.00
<i>Sordh</i>	<i>a</i>							1.00
	<i>b</i>	1.00	1.00	1.00	1.00	1.00	1.00	
<i>Icd-1</i>	<i>a</i>	1.00	1.00	1.00	1.00	1.00		0.50
	<i>b</i>						1.00	0.50
<i>Icd-2</i>	<i>a</i>	0.85	0.90	1.00	1.00	0.80	1.00	1.00
	<i>b</i>	0.15	0.10			0.20		
<i>Got-1</i>	<i>a</i>	0.95	0.92	1.00	1.00	1.00	1.00	1.00
	<i>b</i>	0.05	0.08					
<i>Got-2</i>	<i>a</i>	1.00	1.00	1.00	1.00	1.00	0.50	1.00
	<i>b</i>						0.50	
<i>Np</i>	<i>a</i>				0.05	0.05	0.50	0.50
	<i>b</i>	0.97	0.95	0.86	0.80	0.95	0.50	0.50
	<i>c</i>	0.03	0.05	0.14	0.20			
<i>Gpi</i>	<i>a</i>		0.03	0.04				
	<i>b</i>	1.00	0.97	0.96	1.00	1.00	1.00	1.00
<i>Sod-1</i>	<i>a</i>	0.03		0.20				
	<i>b</i>	0.97	1.00	1.00	0.80	1.00	1.00	1.00
<i>Acon-1</i>	<i>a</i>							1.00
	<i>b</i>	0.20	0.38	0.29	0.60	0.05		
	<i>c</i>	0.80	0.62	0.71	0.40	0.95	1.00	
<i>Pgm</i>	<i>a</i>	0.82	0.52	0.64	0.60		0.25	
	<i>b</i>	0.08	0.33	0.21	0.40	0.35	0.75	1.00
	<i>c</i>	0.08	0.15	0.14		0.15	0.30	
	<i>d</i>	0.02				0.50	0.20	
<i>6-Pgd</i>	<i>a</i>							1.00
	<i>b</i>					0.20	0.15	
	<i>c</i>	1.00	1.00	1.00	1.00	0.65	0.75	1.00
	<i>d</i>					0.15	0.10	
<i>La-1</i>	<i>a</i>	1.00	1.00	1.00	1.00	1.00		
	<i>b</i>						1.00	1.00