國立臺灣大學生命科學院生態學與演化生物學研究所

碩士論文

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Master Thesis

臺灣中部山區地景因子對台灣高山田鼠(*Microtus kikuchii*)和台灣森鼠(*Apodemus semotus*)族群遺傳結構之影響

The influence of landscape features on population genetic structure of Taiwan vole (*Microtus kikuchii*) and Formosan mouse (*Apodemus semotus*) in central Taiwan

李徵葳

Cheng-Wei Lee

指導教授：林雨德 博士

Advisor: Yu-Teh Kirk Lin, Ph.D.

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研究生：李徵葳 Cheng-Wei Lee　學號：R95B44002

指導教授：林雨德 博士 Yu-Teh Kirk Lin, Ph.D.

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中文摘要

瞭解地景因子對目標物種的族群遺傳結構之影響，是族群生態學中很重要的一環。地景因子包括目標物種分布範圍內的植被類型、海拔高度、地形變化等，它們會影響棲地品質，進而影響個體的分散狀態和播遷，塑造出族群在空間上的遺傳結構變異。根據傳統的距離隔離效應(isolation by distance, IBD) 理論，族群間的遺傳距離會與地理距離成正相關，然而傳統的「直線距離」(Euclidean distance)往往缺乏生物意義而與遺傳距離相關性不高；而以地景因子所建構出的「最小成本路徑距離」(least cost distance) 可直觀地提供族群遺傳變異更佳的解釋。本研究以台灣高山田鼠 (*Microtus kikuchii*)和台灣森鼠(*Apodemus semotus*)兩種小型哺乳動物為目標物種，探討地景因子對族群遺傳結構的影響。研究地點位於雪山山脈與中央山脈北段的思源啞口及兩側，武陵山區及南湖山區一帶。我在此三個樣區採集兩物種組織，以分析粒線體 DNA 中的 D-loop 片段得到各族群間的遺傳距離 F_{ST} 值；再利用地理資訊系統(GIS)建立三種不同的地理距離：直線距離、由地形變化或植被類型建構出的最小成本路徑距離，並與遺傳距離做相關分析。結果顯示，台灣森鼠不具族群遺傳結構，而台灣高山田鼠具高度族群遺傳結構，而其遺傳結構與植被類型有顯著正相關。

關鍵字：族群遺傳結構、地景遺傳學、距離隔離、最小成本模式、台灣高山田鼠、台灣森鼠

Abstract

Landscape features, including vegetation type, elevation, and topography, can influence the dispersal and distribution of animals, and lead to variation in spatial population genetic structure. According to the isolation-by-distance (IBD) model, the genetic distances among populations can be positively correlated with geographic distances. Recently, researchers have started to use the least-cost-path distance instead of Euclidean distance in examining IBD because of the unrealistic meaning of the latter to organisms. In this thesis, I studied the influences of landscape features on population genetic structure of two rodent species, the Taiwan field vole (*Microtus kikuchii*) and the Formosan field mouse (*Apodemus semotus* Thomas) in central Taiwan. Through amplifying the D-loop sequence in mtDNA from animal tissues from 6~8 populations of each species, I computed the genetic distance among populations, the F_{ST} value. I calculated the geographic distances under three models: Euclidean distance and two least-cost-path distances based on topography or vegetation type, using geographic information system (GIS). I then analyzed the correlation between genetic distance and the geographic distances conducted from the three models. The results showed that there was no population genetic structure among *Apodemus semotus*, yet a strong genetic structure was present among *Microtus kikuchii* populations, and significantly positively correlated with vegetation type.

Key words: Population genetic structure, Landscape genetics, Isolation by distance, least cost model, *Microtus kikuchii*, *Apodemus semotus*

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Introduction

The dispersal of animals across habitat boundaries is an important process influencing population (Wright, 1940; Macdonald and Johnson, 2001; Lin and Batzli 2001a, 2004) and community (MacArthur and Wilson, 1967; Palmer et al., 1996; Lin and Batzli 2001b) dynamics. Often, dispersal results in gene flow as dispersers breed successfully in the colonizing populations, a process that compensate for genetic drift (Bohonak, 1999). The characteristics of habitat mosaics that make up a landscape can interact with species-specific life-history to either restrict or promote dispersal, and consequently alter spatial genetic structure of populations (Taylor et al., 1993; Gauffre et al., 2008). In the past decade, many researches have set out to detect landscape features, including composition, configuration and connectivity of habitat patches that may influence gene flow, hence shape population genetic structures, and provided useful information for management and conservation decisions (Manel et al., 2003; Storfer et al., 2007; Holderegger and Wagner, 2008).

As Manel et al. (2003) remarked, the two key steps of studying landscape genetics are the detection of genetic discontinuities (i.e., population structure) and the correlation of these discontinuities with landscape features. For example, Spear et al. (2005) found that the genetic variability of tiger salamander populations was explained more by

including landscape variables than with distance alone, while Pfenninger (2002) pointed out that gene flow of terrestrial snails was correlated with ridge distance in two populations and with habitat distance in one population.

One of the most widely applied tools in landscape genetics is the isolation by distance (IBD, Wright, 1943) model, which states that there is a positive correlation between pair-wise estimates of genetic distances and geographic distances among individuals (Rousset, 2000). In continuous populations with spatially limited dispersal, levels of gene flow tend to decrease with increasing geographic distances, which results in increasing genetic differentiation among individuals or populations. The model has provided a powerful mean to explain population structure (Rousset, 1997, 2000; Sumner et al., 2001; Rueness et al., 2003). Yet, such a model assumes homogeneous landscapes, and ignores variations in demographic parameters caused by the interactions between species-specific life-history and landscape features within a species' distribution range (Slatkin and Maruyama, 1975). Real landscapes are not homogeneous (Slatkin, 1985), and dispersal between populations can be strongly influenced by landscape features, leading to a departure from the expectation of IBD model (Coulon et al., 2004).

Replacing the traditional Euclidean distance used in IBD model, ecologists have devised a more realistic least-cost-path (LCP) distance approach. By incorporating the geographic information system (GIS), the "cost" of each landscape feature exists between a pair of populations could be assigned according to its permeability to dispersal, that is, the known or assumed ability of the focal species to successfully traverse through a landscape feature (Graham, 2001; Michels et al., 2001; Chardon et al.,2003; Broquet et al., 2006). The "permeability" is evaluated through gathering information about the life-history of the species, in forms of empirical data or expert opinions (Adriaensen et al., 2003), such as habitat preference and swimming ability, in relation to the characteristics of landscape features, such as forests vs. meadows, and land vs. water. Once the cost of each landscape feature is assigned, the costs of individual features comprising a path connecting a pair of populations can be summed up, and the path with least costs could be determined. The revised IBD model using LCP distance accounts for features of the intervening landscape that facilitate or impede movement along a single, optimal pathway (McRae, 2006). For example, Michels et al. (2001) found that LCP distances calculated based on dispersal rates of the zooplankton correlated much better with genetic distance than did linear distance. Similarly, Coulon et al. (2004) found that LCP distance based on movement through forested patches explained more of the variations in genetic differences between roe

deer individuals than did Euclidean distance. Vignieri (2005) also found that the least-cost-path that maximized riparian corridors and minimized elevation gains had the greatest correlation with genetic distance of pacific jumping mouse. As Finn et al. (2006) noted, the more biologically realistic the measurement of physical distances, the tighter the expected fit between the geographic and genetic distances.

Taiwan is geologically young, with a very sharp elevation gradient, rising from sea level to nearly 4000m within a horizontal distance of less than 100km (Yu, 1995). Besides, the deep drainages etch through mountains often isolate mountain ridges. As a result, the low elevations and deep river valleys could act as dispersal barriers for those species live in high mountains, such as Taiwan vole (*Microtus kikuchii*) and Formosan mouse (*Apodemus semotus*). Both species are endemic species in Taiwan. The former is restricted to high elevation, mostly above 3000 m (Yu, 1993), and usually occurs in *Yushania niitakayamensis* meadow, which is also restricted to above 3000m in Taiwan (Lee, 1992). Conversely, the latter species has a wider distribution range, distributed above 1400 m in various types of habitats, especially forests (Lin, 1990, 1991; Lee, 1992; Yu, 1993; Alder, 1996). Based on allozyme analyses, Yu (1995) indicated that populations of *M. kikuchii* on different mountaintops are highly differentiated, and suggested low gene flow between populations. In contrary, *A. semotus* is rather

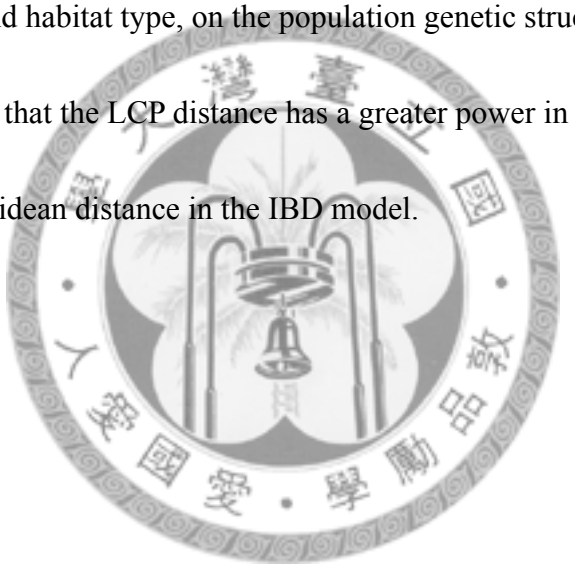
genetically homogeneous, and suggested substantial gene flow among populations (Yu, 1995). However, the mechanism that leads to the differential population genetic structures between the two rodent species was not clear. One possible explanation may be the different life history characteristics among the two species. Besides the different elevational distribution and habitat preference described above, the dispersal ability between the two species was likely different as well, as suggested by their home-range sizes. The home-range size of *A. semotus* (Lin and Shiraishi 1992) was four times larger than that of *M. kikuchii* (Wu 2006), and the average distance of movements and mobility would be greater for the former species. All these life history attributes would interact with landscape features, and contribute to the different patterns in population genetic structure observed for the two species.

In central Taiwan, the neighboring Taroko and Shei-Pa National Parks protected areas that accommodate many highland species in central Taiwan, including *M. kikuchii* and *A. semotus*. While Taroko National Park is in the northern part of the Central Mountain Range, Shei-Pa National Park is along the southern Snow Mountain Range. A mountain saddle, Si-Yuan connects the two national parks at 1950 m, the highest point between the two parks. It divides the watershed Lan-Yan River basin to the north and Da-Jia River basin to the south (Fig. 1). Because of the deep drainage valley to the

north and south, the Si-Yuan area stands out as the most likely corridor that connect populations distributed in Central Mountain Range and Snow Mountain Range, especially for highland species, such as *M. kikuchii* and *A. semotus* (Wu, 2002). Also, the Si-Yuan area is where the lowest elevation of *M. kikuchii* was recorded (Wu, 2002).

Although the Si-Yuan area could potentially be used by both species as a dispersal corridor, I expect the efficiency for the two species differ. First of all, Si-Yuan area was 1950 m in elevation. For *M. kikuchii* that distribute mainly in mountain meadows above 3000m, it may not be easy for them to go down to the Si-Yuan area due to physiological constraint. While as, for *A. semotus* that distribute widely in forests above 1400 m, the movement would be less restricted. Secondly, the timberlines of mountain ranges in central Taiwan are largely located at around 3000 m altitude. Thus, the area between 3000 m and the Si-Yuan area are composed mainly of forested habitats, providing greater continuous habitats for *A. semotus* than *M. kikuchii*. Together, I expect that the gene flows between the east and west sides of the Si-Yuan (Taroko and Shei-Pa National Parks, respectively), would be greater for *A. semotus* than *M. kikuchi*.populations. In addition, within each species, I expect that the population genetic pattern could be explained by landscape features, such as vegetation type and topography (Fig. 2).

The research goals for this thesis are two folds. First I will investigate the population genetic structures of *M. kikuchii* and *A. semotus* populations inhabiting the two sides of Si-Yua. I hypothesize that because of the distinct habitat preferences, elevational distribution range, and dispersal ability that influence gene flows, the genetic structure of *M. kikuchii* populations will be more heterogeneous (i.e., more structured) than that of *A. semotus* populations. Second, I will examine the effects of landscape features, such as topography and habitat type, on the population genetic structures of the two species. I hypothesize that the LCP distance has a greater power in explaining genetic distance than the Euclidean distance in the IBD model.



Material and Methods

1. Sample Collection

The study site was located at the conjunction of eastern Shei-Pa NP, Si-Yuan saddle, and western Taroko NP in central Taiwan. The Si-Yuan saddle connected the two national parks at 1950 m (Fig. 1). The elevation went up to 3000 m at either side of Si-Yuan within a few kilometers. Populations of the two species were sampled in three regions: the west side of Si-Yuan (WS, hereafter), the Si-Yuan area (SY, hereafter), and the east side of Si-Yuan (ES, hereafter). In region WS, populations were sampled on several mountaintops of the Snow Mountain Range at four locations: Taoxuan1, Taoxuan2, Xinda, and Ziyo. In region SY, I sampled two populations at location Siyuan1 and Siyuan2. In region ES, populations were sampled on several mountaintops of northern Central Mountain Range at three locations: Senmatzen, Nanhu1, and Nanhu2 (Table 1, Fig. 1). The distances among populations within a region were > 500 meters. During 2007~2008, I visited each region three times, live-trapped *M. kikuchii* and *A. semotus* with 100 Sherman live traps (2x2.5x9"), and obtained 6~20 individuals of each species at each location, except that *M. kikuchii* was missing at Siyuan1, and *A. semotus* was missing at Nanhu2, Taoxuan1, and Ziyo (Table 1). I used rolled oats mixed with peanut butter as baits to trap small mammals. I recorded species, sex, and weight of captured voles or mice. Animals were toe-clipped

with a unique combination for future identification, and released immediately at the point of capture. The clipped toes were kept in 70% alcohol and later stored at -20℃ before DNA extraction.

2. Molecular Methods

DNA extraction and amplification

Genomic DNA was extracted from toes using the EasyPure Genomic DNA Spin Kit (Bioman), and used as template to amplify the mitochondrial DNA (mtDNA) control region, which had been used in numerous studies examining intraspecific genetic variations (Vigilant et al., 1991; Wilkinson and Fleming, 1996). Genotyping of partial sequences of mtDNA control region was carried out by polymerase chain reaction using two sets of primers for each species. For *M. kikuchii*, I used MK01 (5' CTATCATTGTGATTCTCATAC) that edited from Micro3 (Kocher et al., 1993) and MK02 (5' TAGGCAAGGCGTCTTTAGC) to amplify the whole sequence of the control region, and used MK01 as primer to sequence the 5' part of mtDNA control region. I used MK03 (5' GACTCAGCATAGCCGTCAAG) and MK04 (5' ATCCATCTAAGCATTTTCAGTG) to amplify the 3' part, and used MK03 as sequencing primer. For *A. semotus*, I used primer 1 (5' ATAAACATTACTCTGGTCT-TGTAAAC) (Bellinvia, 2004) and primer 4 (5' TAATTATAAGGCCAGGACCA)

(Bellinvia, 2004) to amplify the whole sequence of the control region, and primer 1 was used as sequencing primer to get the 5' part of mtDNA control region. Again, I used MK03 and MK04 to amplify the 3' part, and used MK03 as sequencing primer. I used software Primer 3 (Rozen and Skaletsky, 2000) to design MK02, MK03, and MK04. PCR reactions were carried out in a 50ul reagent, including 6ul of DNA, 10ul of each 1uM primer, 5ul of 10X PCR buffer with $MgCl_2$ (Bioman), 0.8ul of 10mM dNTP, 2ul of 5U/ml Taq DNA polymerase (Bioman), and 16.2ul of H_2O. The PCR amplification protocol consisted of pre-denaturation at 95℃ for 5min, followed by 30 cycles of denaturation at 95℃ for 30s, annealing at 50℃ for 30s, extension at 72℃ for 1min, and a 10 min elongation step at 72℃ followed the final cycle.

Post-PCR protocol and sequencing

PCR products were confirmed by running gel electrophoreses on 0.8% gel, 90V in 1X TBE buffer for 30min, dyed in EtBr solution for 15min, and photographed under UV light. The sizes and concentrations of PCR products were compared to a 1Kb DNA ladder (Bioman). PCR products with correct size were then purified by Gel/PCR DNA Fragments Extraction Kit (Geneaid). DNA sequencing was performed on an ABI 3730 DNA Analyser by the Department of Medical Research in College of Medicine, National Taiwan University, using BigDye v3.1 as reagent.

Sequence property

Sequence chromatograms were manually edited and aligned by BioEdit ver. 7.0.5.3 (Hall 1999). The overall transition/transversion bias of substitution rate (R=Ts/Tv) was analyzed by MEGA 4 (Kimura et al. 2001). When Tv≧Ts (R≦1), the sequence is regarded as oversaturated and is not genetically informative. Tajima's test (D) was conducted by DnaSP ver. 4.0.0.6 (Rozas et al., 2003) to make sure the sequence fulfill the predictions of neutral theory (Kimura, 1983; Tajima, 1989).

3. Population Genetic Analyses

Genetic diversity

Genetic diversity was measured for all populations based on nucleotide diversity (π) as well as haplotype diversity (h). Nucleotide diversity was defined as the average number of nucleotide differences per site between two randomly chosen sequences, and haplotype diversity the probability that two randomly chosen sequences were different (Nei, 1987), both computed by DnaSP ver. 4.0.0.6 (Rozas et al., 2003).

Analyses of population structure

A table of uncorrected pair-wise distances between haplotypes was constructed by MEGA 4 (Kimura et al. 2001) in order to investigate difference among haplotypes.

Besides, genetic distances between population pairs were measured using Arlequin ver. 3.11 (Excoffier, 1992) by calculating pair-wise F_{ST} values, which estimate differentiation among populations relative to the total samples of a species (Weir and Cockerham, 1984), and the Fisher's exac test was used to test whether the F_{ST} values were significantly different from 0. Gene flow was represented by the effective migration per generation (N_m) that was estimated from F_{ST} value ($N_m = (1- F_{ST}) /2F_{ST}$). Moreover, geographic pattern of genetic differentiation was evaluated by an analysis of molecular variance (AMOVA, calculated with Arlequin ver. 3.11, Excoffier, 1992), which assessed the extent to which genetic variation was attributable to three hierarchical levels of subdivision: among regions, among populations within regions, and within populations. In this analysis, Φ-statistics (an *F*-statistics analogue) expressed genetic distances among haplotypes. The degree of differentiation among the three regions was expressed as Φ_{CT}, the degree of differentiation among populations within regions Φ_{SC}, and the degree of differentiation among all populations Φ_{ST}. Whether a Φ value given above was significantly different from zero was tested using a nonparametric permutation method (10000 permutations) in Arlequin ver. 3.11 (Excoffier, 1992).

Genealogical analyses

A neighbor-joining (NJ) tree with 1000 bootstrap replications was constructed by MEGA 4 (Kimura et al. 2001) to examine the relationship between haplotypes. The evolutionary model of Tamura-Nei plus gamma was suggested by FindModel (Tao et al. 2009) in building the NJ tree. I also constructed a Median Joining Network to show the frequency and relationship between haplotypes using Network ver. 4.5.1.0 (Bandelt et al., 1999), because a network approach could address genealogical relationships at the population level, such as the existence of ancestral haplotypes and multiple descendant haplotypes, and the often low levels of sequence variation (Posada and Crandall, 2001).

4. Landscape Genetic Analyses

Geographic distance measurement

In order to look for the relationships between landscape features and genetic structure, I applied three different landscape related models to estimate the pair-wise geographic distance between populations. The first model (DISTANCE, hereafter) used the simple Euclidean distances that were corresponded to the straight-line geographic distances between populations. The model assumed that topography did not exist and all features of landscape were equally permeable to *M. kikuchii* and *A. semotus*. The second model

(PATH, hereafter) incorporated elevation changes in topography, and the length of shortest path between populations was computed. The third model (COVER, hereafter) incorporated effects of habitat types. Each habitat type, such as river and vegetation type, was assigned a cost value (more details below), and the length of the least-cost path that minimized the sum of cost along the path between populations was calculated. All three distance indices were calculated by the module Spatial Analyst implemented in ArcGIS 9.3 (Environmental System Research Institute, Redlands, USA) using two GIS map layers including digital elevation model (DEM, 40-m ×40-m in resolution)), and land use of Taiwan (Lee et al., 2004).

The production of the COVER model involved two steps. First, the landscape was treated as a friction map with a 5-m × 5-m resolution describing the cost to movement through various habitat types based on the map layer of land use of Taiwan. Second, a least-cost algorithm was used to determine the least-cost path between populations, and the accumulated cost along each path was calculated. Based on previous survey reports of *M. kikuchii* and *A. semotus* in highland Taiwan (Lin, 1990, 1991; Lee, 1992; Chen, 1995; Wu, 2008), I defined the cost value of each habitat type in the friction map as the value inversely proportional to the average capture rate in each habitat type. I grouped similar habitat types together, and produced the category displayed in Table 2. By

dividing all values with the smallest value, I adjusted the values such that the smallest value was 1, and then those values higher than 1000 were adjusted as 1000. For the habitat types of fire line, bare ground, and water body, there was no capture record. I assigned fire line the same value as the surrounding conifer-pine forest; bare ground two times the value of the maximum value considering the risk of moving through open area; and water body four times the value of the maximum value considering it an nearly impermeable barrier to dispersal (Table 2).

Isolation by distance

In order to examine the effect of landscape features on population genetic structure, I tested isolation by distance (IBD) by plotting the correlated genetic distance ($F_{ST}/(1-F_{ST})$) among population pairs as a regression function of the geographic distance between those pairs. In both model DISTANCE and PATH, the geographic distances were represented by physical distances measured in kilometer, while in model COVER, the geographic distance was represented by accumulated cost (unit-free) along the least-cost path among populations. The IBD tests were carried out in program IBD, which adopted reduced major axis regressions to calculate IBD slopes and Mantel tests to calculate IBD correlations with 1000 permutations to determine the statistical significance (Bohonak, 2002).

Results

1. Data Description

M. kikuchii

I sampled 6~17 different individuals at each location, a total of 87 individuals, except that *M. kikuchii* were missing at Siyuan1 (M1) (Table 1). PCR amplification of 753-733 partial sequences of mtDNA control region was successful for all samples except 2 samples from Taoxuan2 (W2), 1 sample from Xinda (W3), and 2 samples from Ziyo (W4). The nucleotide frequencies were 28.9% (A), 31.4% (T), 27.2% (C), and 12.5% (G), and the overall transition/transversion bias of substitution rate (R=Ts/Tv) was 5.36. The > 1 value indicated the sequence was not saturated with substitution. On the other hand, Tajima's D was 1.08 and not significantly deviated from 0 (P>0.10), which meant the sequence fulfilled the expectation of neutral theory.

A. semotus

I sampled 7~20 different individuals at each location, a total of 72 individuals, except that *A. semotus* were missing at Nanhu2 (E3), Taoxuan1 (W1), and Ziyo (W4) (Table 1). PCR amplification of 804-806 partial sequence of mtDNA control region was successful for all samples except 1 sample from Senmatzen (E1). The nucleotide frequencies were 34.4% (A), 30.0% (T), 25.5% (C), and 10.1% (G), and the overall transition/transversion bias of substitution rate (R=Ts/Tv) was 7.21, again indicated the

sequence was not saturated with substitution. Similarly, Tajima's D was -0.84, not significantly deviated from 0 (P>0.10), thus satisfied the expectation of neutral theory.

2. Population Genetic Analyses

Genetic diversity

M. kikuchii

There were 67 polymorphic sites with gaps considered as the fifth state, in addition to A, T, C, G nucleotides. A total of 29 haplotypes were identified among the 8 populations (Table 3). Overall nucleotide diversity was 0.023, ranged from 0.002 to 0.018, while overall haplotype diversity was 0.937, ranged from 0.223 to 0.936 among populations (Table 5). Sixteen (55.2%) haplotypes were identified as private haplotypes (those occurred in a single population), which were found in all populations. One haplotype (mk14) was shared between region WS and SY, while no haplotype was shared among all three regions (Table 5). It was worth noted that in population M2, even though only 6 individuals were sampled, 4 haplotypes were identified, a number as high as that identified from 11 samples in population E3. That is, small sample sizes in some populations did not necessarily produce low number of haplotypes.

A. semotus

There were 38 polymorphic sites with gaps considered as the fifth state. A total of 37

haplotypes were identified among the 6 populations (Table 4). Overall nucleotide diversity was 0.007, ranged from 0.001 to 0.008, while overall haplotype diversity was 0.952, ranged from 0.529 to 0.967 (Table 4). Most (75.7%, 28 out of 37) haplotypes were restricted to one population, and were identified as private. Three haplotypes (as5 between WS and SY, as22 and as25 between SY and ES) were shared between two regions, and only 1 haplotype (as3) was shared among all three regions (Table 6).

Analyses of population structure

M. kikuchii

The uncorrected pair-wise distances that estimated the nucleotide divergence between haplotypes ranged from 0.1% to 4.8%, with an average of 2.44% (Table 7). The F_{ST} values ranged from 0.147 to 0.881, with an average value of 0.497 (Table 9). Only two population pairs (M2 vs. W3 and W1 vs. W4) did not show significant genetic differentiation (Fisher's exact tests). Gene flow between populations as represented by the *N*m values ranged from 0.067 to 2.893. Moreover, results of AMOVA revealed that although differences within populations explained 41.59% of genetic variance (Φ_{ST}=0.584, P<0.001), strong genetic structure was found at among region level (Φ_{CT}=0.315, P<0.05) as well as among populations within regions level (Φ_{SC}=0.393, P<0.001, Table 10).

A. semotus

The uncorrected pair-wise distances that estimated the nucleotide divergence between haplotypes ranged from 0.1% to 1.9%, with an average of 0.86% (Table 8). The genetic differentiation among populations was relatively low compared to *M. kikuchii* with pairwise F_{ST} value ranged from 0.054 to 0.552, with an average value of 0.243 (Table 9). Nevertheless, only two population pairs (E1 vs. M2 and E2 vs. M2) did not show significant genetic differentiation (Fisher's exact tests). Gene flow between populations was relatively high, with *N*m ranged from 0.406 to 8.686. The results of AMOVA showed that differences among regions account for a very small portion of genetic variance (3.67%), and no significant differentiation was found (Φ_{CT}=0.037, *P*=0.20). Whereas population genetic structure was found at the among populations within regions level (Φ_{SC}=0.181, *P*<0.001) and within populations level (Φ_{ST}=0.211, *P*<0.001). The former explained 17.40%, and the latter 78.93% of total genetic variances (Table 10).

Genealogical analyses

M. kikuchii

Using the sequence of the southern vole, *Microtus rossiaemeridionalis*, (downloaded from NCBI) as a outgroup, the 29 *M. kikuchii* haplotypes were clearly structured, as

revealed by the NJ tree (Fig. 3). Three clusters of haplotype groups could be identified after applying a cut-off value of bootstrap probability at 70%, except that mk28 and mk29 were grouped together with a 77% bootstrap probability (Fig. 3 and 4). The haplotype group A contained haplotypes shared by all three regions, although mostly belong to region ES. In contrast, the haplotype group B and C were specific to region WS and ES, respectively (Fig. 4). Mapping the distribution of the three haplotype group on the study site revealed a clear geographic pattern (Fig. 5). The Median Joining Network of haplotypes also showed the same pattern. First, the three main haplotype groups identified by NJ tree were also revealed by the network. Three subgroups within haplotype group B were identified (mk1, mk8. mk3, and mk4; mk7 and mk10; mk5, mk11, mk13, mk15, mk2, and mk6). Second, haplotypes tended to be more similar to those sampled from the same region than those from different regions except those in haplotype group A which was shared by the three regions (Fig. 6).

A. semotus

There was no evident haplotype groups in the NJ tree of the 37 haplotypes of *A. semotus*, using the sequence of the striped field mouse, *A. agrarius*, (downloaded from NCBI) as an outgroup, after applying a cut-off value of bootstrap probability at 70% (Fig. 7). Furthermore, the Median Joining Network did not reveal evident geographical subdivision either (Fig. 8).

3. Landscape Genetic Analyses

Isolation by distance

M. kikuchii

Based on the Pearson correlation analysis, both the geographic distances calculated from model PATH and COVER were highly significantly correlated with Euclidean distances between populations (PATH vs. Euclidean: r=0.999, P<0.001; COVER vs. Euclidean: r=0.971, P<0.001). Mantel test revealed that genetic distance was positively correlated with geographic distance in all three models, although only the LCP derived from model COVER was statistical significant (DISTANCE: r=0.307, P=0.054; PATH: r=0.313, P=0.055; COVER: r=0.351, P<0.05). However, based on the reduced major axis regression, little variations could be explained by any of the three models (DISTANCE: r^2=0.094; PATH: r^2=0.098; COVER: r^2=0.123) (Table 11, Fig. 9).

A. semotus

Based on the Pearson correlation analysis, both the geographic distances calculated from model PATH and COVER were highly significantly correlated with Euclidean distances between populations (PATH vs. Euclidean: r=0.999, P<0.001; COVER vs. Euclidean: r=0.964, P<0.001). Mantel test revealed that there was no significant correlation between genetic distance and geographic distances using any model

(DISTANCE: r=-0.021, P=0.460; PATH: r=-0.007, P=0.467; COVER: r=0.120, P=0.358). Moreover, nearly no variation could be explained by the reduced major axis regression in all three models (DISTANCE: r^2=0.000; PATH: r^2=0.000; COVER: r^2=0.014) (Table 11, Fig. 10).

Discussion

1. Genetic Diversity

The amount of mtDNA diversity I discovered in *M. kikuchii* or *A. semotus* was smaller compared with those of other congeners at larger spatial scales. For example, Francl et al. (2008) sampled 323 meadow voles (*M. pennsylvanicus*) from 15 populations separated by a maximum distance of 80 km, and discovered 16 haplotypes, 19 variable sites among 375 bases of control region mtDNA, with nucleotide diversities of 0.002~0.105. Triant and DeWoody (2006) calculated the within genus nucleotide diversity between Eurasian sibling vole (*M. rossiaemeridionalis*) and Taiwan vole (*M. kikuchii*) of 0.08. On the other hand, in the genus *Apodemus*, Koh et al. (2000) studied two subspecies of the striped field mice, *A. agrarius coreae* and *A. agrarius chejuensis*, and found the nucleotide diversities among 282 bases of mtDNA control region 0.0298 and 0.0186, respectively, and the nucleotide diversity between the two subspecies was 0.035. Overall, the nucleotide diversities of *M. kikuchii* and *A. semotus* I found were under the level of species variation.

2. Population Genetic Structure

According to Hartl and Clark (1997), the level of population differentiation obtained from pairwise F_{ST} values can be classified into four classes: no differentiation (F_{ST}

<0.05), moderate differentiation (0.05<F_{ST}<0.15), highly differentiation (0.15<F_{ST}<0.25), and strong differentiation (F_{ST}>0.25). Accordingly, among the 28 pairs of *M. kikuchii* populations, 6 were highly and the remaining 22 were strong differentiated. Such a level of genetic differentiation was similar to or higher than those found in previous studies. For example, Francl et al. (2008) reported high subdivision in the meadow voles (*M. pennsylvanicus*) with a pairwise F_{ST} value up to 0.74, and Heckel et al. (2005) found the pairwise F_{ST} 0.14~0.96 among the Common vole (*M. arvalis*) around Europe. Both studies used mtDNA control region as molecular marker. However, the spatial scale of this study was much smaller than those two studies, implying the gene flow among *M. kikuchii* populations in central Taiwan was highly restricted. Moreover, the results of AMOVA revealed that populations of *M. kikuchii* were highly structured in all hierarchical levels, including among regions, among populations within regions, and within populations. Thus, geographical subdivision was suggested by NJ tree and Median Joining Network of haplotypes, and haplotypes could be assigned to haplotype groups closely related to regions. Similarly, in a phylogeographic study of *M. kikuchii* spanned the whole Taiwan island, Lin (2005) found that species from the same region were grouped together, which provided strong evidence that a strong population genetic structure was present among *M. kikuchii*. Moreover, the Median Joining Network of haplotypes showed that haplotypes specific

to WS or ES were located at the two ends, and those shared by all three regions were in the center of the Median Joining Network. Such a pattern suggested that historically populations of *M. kikuchii* were originated from SY, and later dispersed to WS and ES separately..

On the other hand, gene flow between populations of *A. semotus* was higher than *M. kikuchii*. First, the overall nucleotide diversity among *M. kikuchii* ($\pi = 0.023$) was much higher than that among *A. semotus* ($\pi = 0.007$). Secondly, although population differentiation was also observed from pairwise F_{ST} values among the 15 pairs of *A. semotus* populations. Four of them exhibit moderate differentiation, 6 of them highly differentiation, and 5 of them strong differentiation. The magnitude in general was lower than that among *M. kikuchii* populations. Thirdly, the AMOVA results indicated no population subdivision occurred between regions as only 3.67% of genetic variation can be explained by difference between regions. Lastly, both the NJ tree and Median Joining Network indicated that haplotypes of *A. semotus* were relatively similar to each other and could not be grouped as it could in *M. kikuchii*.

Yu (1995) also observed a similar pattern of population genetic structure of *M. kikuchii* and *A. semotus*, and suggested that the pattern might be related to the different patterns

of elevational distribution of the two species. Besides, the distinct habitat preference could be an additional mechanism. *M. kikuchii* prefers mountain meadows such as the *Y. niitakayamensis* meadows above timberlines (at ~3000 m in elevation). Below the timberline, the grassland habitats become patchy and sporadic. Whereas, *A. semotus* has a wider elevational distribution across relatively continuous forests below 3000 m. Moreover, higher gene flow between populations of A. semotus than *M. kikuchii* can also be resulted from better dispersal ability of *A. semotus*. As a result, the low elevation (< 3000 m) covered with relatively continuous forests and rough topography might act as physical barriers to dispersal for *M. kikuchii*, and the intrinsic poor dispersal ability of the species further hampered gene flow. In a word, the differential life history characteristics have interacted with landscape features to produce the different patterns in population genetic structure between *A.semotus* and *M. kikuchii*.

Nevertheless, population differentiation among *A. semotus* populations was present although minor than that among *M. kikuchii* populations. Similar level of genetic differentiation were found in other studies on *Apodemus*. For example, Suzuki et al. (2004) computed the pairwise F_{ST} values in two Japanese wood mice, *A. speciosus* and *A. argenteus* to be 0.013~0.579 and 0.039~0.547, respectively, within the whole Japan. Hsu et al. (2001) found a northern and south-central geographical division among *A.*

semotus in Taiwan, and suggested historical geographic events, such as glacial cycles and refugia, could explain the pattern observed today. Based on the combined finding of Yu (1995), Hsu et al. (2001), and current study, a scenario of historic events can be depicted to explain the population genetic structure of the two species as follows. In the beginning, after the introgression of the two species from mainland China (Lin, 1989), the glacial period drove and limited the populations to several mountainous refugia where long-term population separations allowed the accumulation of genetic variations. Postglacial re-colonization of suitable habitats and introgression among existing populations formed the geographic differentiation at the island scale (Hsu et al., 2001). This mechanism was also suggested in support of spatial genetic patterns observed in other high-elevation small mammals in Taiwan, including mole-shrew (*Anourosorex yamashinai*) (Yuan et al., 2006) and Père David's red–backed vole (*Eothenomys melanogaster*) (Chang, 2007). However, multiple invasions could be an alternative hypothesis of the intraspecfic genetic variation at the island scale (Kuo, 2002), which could not be teased out in this research. What could be confirmed in my study is that, at a smaller regional scale, the physical barrier of low elevation and habitat types would reduce gene flows among *M. kikuchii* but not *A. semotus*. In conclusion, the genetic pattern we saw today for the two species was strongly influenced by past historical events, the rough topography, and habitat types on the

Taiwan island (Hsu et al., 2001).

On the other hand, the discrepancy in evolutionary rates between *M. kikuchii* and *A. semotus* could also contribute to the distinctive pattern in population genetic structure. After performing pairwise sequences comparison within the genus *Microtus* and other mammal taxa, Triant and DeWoody (2006) have indicated that microtine mtDNA genomes are evolving more rapidly than any other mammalian lineage they sampled, and the cytochrome b gene evolves fastest in *Microtus* than seven other rodent genera, including *Mus* and *Rattus*. If mtDNA of *M. kikuchii* changed more rapidly than that of *A. semotus*, then the populations of *M. kikuchii* today would be more genetically structured than *A. semotus*, given the same degree of physical isolation. However, one would not expect landscape features affect genetic patterns, if evolution rate was the sole mechanism.

3. Effect of Landscape Features on Population Genetic Structure

Significant isolation by distance was observed among *M. kikuchii* populations regardless of the type of IBD model applied, although by incorporating vegetation type, model Cover gave the best correlation with genetic distance. The results supported the hypothesis that the population genetic structure of *M. kikuchii* can be partly explained

by landscape features. Compared to Eucliden distance, the distance culculated from model COVER was more “biologically realistic” (Finn et al., 2006); in other words, incorporating life histroy characteristics of focal species, which was the habitat preference of in this case.

However, similar to most of other landscape genetics studies (Table 12), the use of least-cost-path distance improved little in explaining genetic distance compared to using Euclidean distance (Funk et al., 2005; Broquet et al., 2006; Finn et al., 2006; Walker et al., 2007; Chen, 2007; Macqueen et al., 2008; Wang et al., 2008; Lee-Yaw et al., 2009; Pease et al., 2009). The little change should be a result of incorporating just one landscape feature --- the vegetation type, into the least-cost model. Several studies (e.g., Wang et al. 2008) have pointed out that patch size and shape are important determinants of habitat quality. Besides, other factors related to habitat connectivity, like distance to other types of habitats, and ecological factors, like predator-prey interaction will affect the dispersal of animals as well. They should also be considered, and could increase the fit of the least-cost model. Nevertheless, the interactions of the focal species with the above –mentioned landscape features were largely unknown, and the assignment of cost values would be arbitrary. Nevertheless, my study was the first to use empirical habitat preference data for assigning habitat permeability values, and

the results clearly indicated that gene flow among *M. kikuchii* may be partially influenced by habitat types.

In contrary to *M. kikuchii*, *A. semotus* did not show a pattern of isolation by distance in any of the three models. Coulon et al. (2004) have mentioned that in a homogeneous landscape, the direction of dispersal would be random, and lead to the decrease of correlation between genetic distance and geographic distance. That might be the case for *A. semotus*, inhabiting the continuous forest below 3000 m Moreover, as a habitat generalist, *A. semotus* could inhabit most types of habitats, except human-made structure, bare area, and water body, which I assigned high cost values. According to Brouat et al. (2003) and Monsen and Blouin (2004), the effect of landscape structure on gene flow and genetic structure may be less obvious to habitat generalist, when compared to habitat specialist, like *M. kikuchii*. As a result, spatial structure among *A. semotus* populations could be expected at a large, like the whole island scale, but not at a scale I study.

4. The Unisex Information Provided by mtDNA Genome

Given the property of maternal inheritance of mtDNA genome, the results observed in this study can only represented the female history. In other words, gene flow was more

restricted among *M. kikuchii* than among *A. semotus* females, and the former can be explained by landscape features. However, according to Wu (2006), there was no sex-biased dispersal in *M. kikuchii*, and both male and female clustered with kins, implying that the genetic pattern I observed would be similar for both sexes. Nevertheless, studies using non-sex-biased markers such as nuclear genome are needed to clarify the overall pattern between sexes.

In conclusion, at the scale of present study, populations of *M. kikuchii* were more genetically structured than of *A. semotus*, and the population structure of *M. kikuchii* can be partially explained by landscape features. It is necessary to consider species specific life history characteristics including elevational distribution, habitat preference and dispersal ability, landscape characteristics including topography and habitat types, as well as past historical events in understanding the genetic structure observed today.

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Table 1 The locations of sampling sites, and sample sizes obtained for each population.

Region	Location	Population	Elevation (m)	Location *		Sample size	
				x-coordinate	y-coordinate	*M. kikuchii*	*A. semotus*
WS	Taoxuan1	W1	3315	280893.0	2702947.5	13	-
	Taoxuan2	W2	3291	280687.3	2703036.3	11	7
	Xinda	W3	3186	277434.3	2702875.7	13	9
	Ziyo	W4	3170	277851.1	2702944.2	9	-
SY	Siyuan1	M1	1964	286201.3	2699040.6	-	20
	Siyuan2	M2	1949	285697.7	2698768.6	6	18
ES	Senmatzen	E1	3217	293258.9	2697294.6	12	12
	Nanhu1	E2	3393	295004.4	2696032.6	17	7
	Nanhu2	E3	3439	295359.7	2695959.8	11	-

* Coordinates of the projected coordonate system of Taiwan, TWD67

Table 2 Cost value of each habitat type for *M. kikuchii* and *A. semotus*.

Vegetation	Area Percentage	Cost		comment
		M. kikuchii	*A. semotus*	
conifer-fir	7.74	1.00	4.70	
Y. niitakayamensis	2.37	1.49	9.95	
bush	0.03	3.60	7.83	
meadow	1.35	6.58	1.00	
conifer-hemlock spruce	10.24	7.79	8.37	
conifer-others	7.89	19.27	1.06	
mix broad-leaved and coniferous forest	18.93	22.23	1.17	
conifer-spruce	3.17	23.98	11.00	
conifer-cryptomeria	0.93	115.61	4.42	
broad-leaved forest	11.51	1000.00	6.39	
fire line	0.11	1000.00	2.67	= conifer-pine
conifer-pine	19.63	1000.00	2.67	
conifer-juniper	9.88	1000.00	5.04	
agriculture land	0.98	1000.00	17.87	
other man-made structure	0.07	1000.00	360.30	
bare ground	4.39	2000.00	720.59	max×2
water body	0.78	4000.00	1441.19	max×4

Table 3 Polymorphic sites of the mtDNA partial sequence of *M. kikuchii*. Dots indicate nucleotides identical to those of mk01 and dashes indicate deletions.

Haplotype	Polymorphic Site
	0000111222222222222222222233333334455555556666666666666666666667777 7
	4555117223333444445555667125688948229999901223333345666777889911344
	9146349391236034693679176758389723343478918390123767012019030249608
mk1	ATGCACATCCCTACA-TACTAACTTTTCCAGACTATTCCACTCGCTTCCTCCCCTCCGAAATTGTTT
mk2	G-AT.T..T.TC.A.AC.....T.....T..GT...C..CACTA......TT.....A...C.A.A.
mk3	...............-.........................C.........................
mk4	...............-..T...T............C......T.........T..............
mk5	.-AT.T..T..C.A.AC..C..T..C..T..GT...C..CAC.A......TT.....A...C.A.A.
mk6	G-AT.T..T.TC.A.AC.....T.....T..GT...C..CAC.A......TT.....A...C.A.A.
mk7	...............-..T...T............CC..CACTA......TT.....A...C.A.A.
mk8	...............-..........................T.........T..............
mk9	.--.GT..TT...A.-....GTT..C......TC.G...............................
mk10	...............-....................C..CAC.A......TT.....A...C.A.A.
mk11	G-AT.T..T....A.AC.....T.....T..GT...C..CAC.A......T......A...C.A.A.
mk12	.--.GT..T....A.-....GTT..C......T..GC..G.CTATCC.TCT..T.T.-.....A...
mk13	G-AT.T..T..C.A.AC.....T.....T..GT...C..CAC.A......T......A...C.A.A.
mk14	.--.GT..TT...A.-....GTT..C......TC.GC....CTATCC.TCT..T...-.....A...
mk15	G-AT.T.CT..C.A.AC.....T.....T..GT...C..CAC.A......TT.....A.....A.A.
mk16	.--.GT..TT..GA.-....GTT..C......T..GC....CTATCC.TCT..T.T.-...C.A...
mk17	.--.GT..TT...A.-....GTT..C......T..GC....CTATCC.TCT..T.T.-.....A...
mk18	.--.GT..TT...A.-....GTT..C......T..GC....CTATCC.TCT..T.T.-...C.A...
mk19	.--.GT..TT...A.-....GTT..C......T..GC....CTATCC.TCT..TCT.-G....A..-
mk20	.--.GT..T....A.-....GTT..C......T..GC....CTATCC.TCT..T...-.G...A...
mk21	.--.GT..TT...AG-....GTT..C......T..GC....CTATCC.TCT..T.T.-.....A...
mk22	.--.GT..T....A.-....GTT..C......T..GC....CTATCC.TCT..T...-.....A...
mk23	.--.GT..T....A.-....GTT..C......T..GC....CTATCC.TCT..TCT.-G....A...
mk24	.--.GT..TT...A.-....GTT..C......TC.GC....CTATCC.TCT..T...-.G...A...
mk25	.--.GT..TT...A.-....GTT..C......T..GC....CTAT.C.TCT..T.T.-..G..A...
mk26	.--.GT..TT...A.-....GTT.CCC.....TC.GC....CTATCC.TCT..T.T.-...C.A...
mk27	.--.GT..TT...A.-....GTT.CCC.....TC.GC....CTATCC.TCT..T.T.-.....A...
mk28	G-AT.TG.T.T..A.-CGT...TC...T.GAGT.G.C....CTATCC.TCT..T.T.-.....A...
mk29	G-AT.TG.T.T..A.-CGT...TC...T.GAGT.G.CTT..CT...CTTCTTT..TTA....C.-..

Table 4 Polymorphic sites of the mtDNA partial sequence of *A. semotus*. Dots indicate nucleotides identical to those of mk01 and dashes indicate deletions.

	Polymorphic Site																																					
	0	0	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3	4	4	5	5	5	5	5	5	5	6	6	6	6	6	6	6	7	7	7
Haplotype	7	8	0	2	2	3	3	5	7	8	9	9	0	0	2	6	7	9	4	4	9	3	4	6	6	7	9	9	0	0	0	1	2	2	4	2	3	6
	5	2	2	5	6	5	8	5	7	0	5	9	1	8	9	4	2	4	6	6	0	9	1	7	9	8	5	8	4	6	7	0	0	4	4	7	8	5
as1	G	C	T	A	A	T	T	A	C	C	A	A	T	C	T	T	T	C	C	C	C	T	A	T	C	A	A	C	A	T	C	G	A	G	A	T	T	C
as2	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.
as3	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.
as4	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	-	.	G	.	.	.	C	.	.	.	.	.	C	.	.
as5	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	G	.	.	.	C	.	.	.	.	.	.	.	.
as6	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	-	.	G	.	.	.	C	.	.	.	.	.	.	.	.
as7	.	T	.	.	.	C	.	.	.	T	.	.	C	T	.	C	C	.	.	T	T	.	.	-	.	.	.	.	.	C	T	.	G	.	.	.	C	T
as8	.	T	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	G	-	.	G	.	.	.	C	.	.	G	.	.	.	.	.
as9	.	T	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	G	.	.	.	C	.	.	G	.	.	.	.	.
as10	.	T	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	C	T	.	G	.	.	.	.	T
as11	.	T	C	.	.	C	.	.	.	T	.	.	.	.	.	C	.	T	.	.	T	.	.	-	.	G	.	.	.	C	.	.	G	.	.	.	.	.
as12	.	T	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	G	.	.	.	C	.	.	.	.	.	.	.	.
as13	.	T	.	.	G	C	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	G	-	.	G	.	.	.	C	.	.	G	A	.	.	.	.
as14	.	T	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	.	T	.	.	.	G	-	.	G	.	.	.	C	.	.	G	.	.	.	.	.
as15	.	T	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	C	T	A	G	.	.	.	C	T
as16	.	T	.	.	.	C	.	.	.	T	.	.	C	T	.	C	C	.	.	.	T	.	G	-	.	G	.	.	.	C	.	.	G	.	.	.	.	.
as17	.	T	.	.	.	C	.	.	A	.	.	G	.	.	.	.	.	.	.	.	.	.	.	-	.	G	.	.	.	C	.	.	.	.	.	.	.	.
as18	.	T	.	.	G	C	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	-	.	.	.	.	.	C	T	.	G	.	.	.	.	T
as19	.	T	C	.	.	C	.	.	.	T	.	.	.	.	.	C	.	T	.	.	T	.	G	-	.	G	.	.	.	C	.	.	G	.	.	.	.	.
as20	.	T	.	.	.	C	.	.	A	.	.	G	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	C	T	A	G	.	.	.	C	T
as21	.	T	.	.	.	C	.	.	.	T	.	.	C	T	.	C	C	.	.	.	T	.	.	-	.	G	.	.	.	C	.	.	.	.	.	.	.	.
as22	.	T	.	.	.	C	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	-	.	G	.	.	.	C	.	.	.	.	.	.	.	.
as23	.	T	.	.	.	C	C	.	.	T	.	.	.	.	.	.	.	.	.	T	T	.	.	-	.	G	.	.	.	C	T	.	G	.	.	.	.	T
as24	.	T	.	G	.	C	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	G	-	.	G	.	.	.	C	.	.	G	.	.	.	.	.
as25	.	T	.	.	G	C	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	-	.	G	.	.	.	C	.	.	.	.	.	.	.	.
as26	.	T	.	.	.	C	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	-	.	G	.	.	.	C	.	.	.	.	.	.	.	.
as27	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	G	-	.	G	.	.	.	C	.	.	G	.	.	.	.	.
as28	.	T	.	.	G	C	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	-	.	.	G	.	G	.	.	.	.	.	.	.	.	.
as29	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	-	.	G	.	.	.	C	.	.	.	.	.	.	.	.
as30	.	T	.	.	.	C	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	G	-	T	G	.	.	.	C	.	.	G	.	G	.	.	.
as31	.	T	.	.	.	C	C	.	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.	-	.	G	.	.	.	C	.	.	.	.	.	.	.	.
as32	A	T	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	G	.	T	.	C	.	.	G	.	.	.	.	.
as33	.	T	.	.	.	C	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	G	.	.	.	C	.	.	G	.	.	.	.	.
as34	.	T	.	.	G	C	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.
as35	.	T	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	G	.	.	.	C	.	.	.	.	.	.	.	.
as36	.	T	.	.	G	C	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	-	.	G	.	.	.	C	.	.	G	.	.	.	.	.
as37	.	T	.	.	.	C	.	.	.	T	.	.	C	.	.	C	C	.	T	.	T	.	.	-	.	.	.	.	.	C	T	.	G	.	G	.	C	T

Table 5 Haplotype frequencies and the diversity indices for the 8 populations of *M. kikuchii*. Private haplotypes are marked with *.

Population / Haplotypes	WS				SY	ES			Total
	W1	W2	W3	W4	M2	E1	E2	E3	
mk1	3		1						4
mk2	2		1						3
* mk3	1								1
mk4	1	1		6					8
mk5	2	7							9
* mk6	1								1
* mk7	1								1
* mk8	1								1
* mk9	1								1
* mk10		1							1
* mk11			1						1
* mk12			3						3
* mk13			1						1
mk14			5		1				6
* mk15				1					1
* mk16					2				2
* mk17					1				1
* mk18					2				2
* mk19						1			1
* mk20						6			6
* mk21						4			4
* mk22						1			1
* mk23							15		15
* mk24							1		1
* mk25							1		1
* mk26								1	1
* mk27								6	6
* mk28								1	1
* mk29								3	3
Total	13	9	12	7	6	12	17	11	48
# of haplotypes	9	3	6	2	4	4	3	4	29
H	0.936	0.417	0.803	0.286	0.867	0.682	0.228	0.673	0.937
Π	0.002	0.002	0.018	0.010	0.002	0.003	0.002	0.002	0.023

Table 6 Haplotype frequencies and the diversity indices for the 6 populations of *A. semotus*. Private haplotypes are marked with *.

Population / Haplotypes	WS		SY		ES		Total
	W2	W3	M1	M2	E1	E2	
* as1	1						1
* as2	1						1
as3	5		1	3	2	1	12
* as4		1					1
as5		1		1			2
* as6		6					6
* as7		1					1
as8			5	1			6
as9			2	2			4
* as10			1				1
* as11			1				1
as12			1	1			2
* as13			1				1
* as14			1				1
* as15			1				1
* as16			1				1
* as17			1				1
* as18			1				1
* as19			1				1
* as20			1				1
* as21			1				1
as22				1	2		3
* as23				1			1
* as24				1			1
as25				2		2	4
* as26				1			1
* as27				1			1
* as28				1			1
* as29				1			1
* as30				1			1
* as31					5		5
* as32					1		1
* as33					1		1

Table 6 (continued)

Population / Haplotypes	WS		SY		ES		Total
	W2	W3	M1	M2	E1	E2	
* as34						1	1
* as35						1	1
* as36						1	1
* as37						1	1
Total	7	9	20	18	11	7	72
# of haplotypes	3	4	15	14	5	6	37
H	0.529	0.583	0.942	0.967	0.782	0.952	0.952
π	0.001	0.001	0.007	0.006	0.005	0.008	0.007

Table 7 Table of uncorrected pair-wise distances (%) between haplotypes of *M. kikuchii*.

Haplotype	mk1	mk2	mk3	mk4	mk5	mk6	mk7	mk8	mk9	mk10	mk11	mk12	mk13	mk14	mk15	mk16	mk17	mk18	mk19	mk20	mk21	mk22	mk23	mk24	mk25	mk26	mk27	mk28	mk29
mk1	-																												
mk2	3.32	-																											
mk3	0.13	3.18	-																										
mk4	0.66	3.45	0.79	-																									
mk5	3.18	0.66	3.05	3.58	-																								
mk6	3.18	0.13	3.05	3.58	0.53	-																							
mk7	1.99	1.86	1.85	1.59	1.99	1.99	-																						
mk8	0.26	3.32	0.40	0.40	3.45	3.45	1.99	-																					
mk9	1.59	3.58	1.72	1.86	3.18	3.45	3.18	1.86	-																				
mk10	1.46	1.86	1.32	2.12	1.72	1.72	0.53	1.72	3.05	-																			
mk11	2.79	0.53	2.65	3.18	0.66	0.40	1.86	3.05	3.05	1.59	-																		
mk12	3.19	3.32	3.05	3.19	3.19	3.45	2.92	3.19	2.12	3.05	3.05	-																	
mk13	2.92	0.40	2.79	3.32	0.53	0.26	1.99	3.18	3.18	1.72	0.13	3.19	-																
mk14	3.19	3.45	3.05	3.19	3.32	3.59	3.05	3.19	1.59	3.19	3.19	0.53	3.32	-															
mk15	3.05	0.53	2.92	3.45	0.66	0.40	2.12	3.32	3.32	1.86	0.53	3.32	0.40	3.45	-														
mk16	3.45	3.45	3.32	3.45	3.32	3.59	3.05	3.45	2.12	3.19	3.19	0.53	3.32	0.53	3.72	-													
mk17	3.19	3.45	3.05	3.19	3.32	3.59	3.05	3.19	1.86	3.19	3.19	0.27	3.32	0.27	3.45	0.27	-												
mk18	3.32	3.32	3.19	3.32	3.19	3.45	2.92	3.32	1.99	3.05	3.05	0.40	3.19	0.40	3.59	0.13	0.13	-											
mk19	3.46	3.72	3.32	3.46	3.59	3.86	3.32	3.46	2.13	3.46	3.46	0.53	3.59	0.53	3.72	0.53	0.27	0.40	-										
mk20	3.05	3.32	2.92	3.05	3.19	3.45	2.92	3.05	1.99	3.05	3.05	0.40	3.19	0.40	3.32	0.66	0.40	0.53	0.66	-									
mk21	3.32	3.59	3.19	3.32	3.45	3.72	3.19	3.32	1.99	3.32	3.32	0.40	3.45	0.40	3.59	0.40	0.13	0.27	0.40	0.53	-								
mk22	2.92	3.19	2.79	2.92	3.05	3.32	2.79	2.92	1.86	2.92	2.92	0.27	3.05	0.27	3.19	0.53	0.27	0.40	0.53	0.13	0.40	-							
mk23	3.32	3.59	3.19	3.32	3.45	3.72	3.19	3.32	2.26	3.32	3.32	0.40	3.45	0.66	3.59	0.66	0.40	0.53	0.13	0.53	0.53	0.40	-						
mk24	3.32	3.59	3.19	3.32	3.45	3.72	3.19	3.32	1.73	3.32	3.32	0.66	3.45	0.13	3.59	0.66	0.40	0.53	0.66	0.27	0.53	0.40	0.80	-					
mk25	3.19	3.45	3.05	3.19	3.32	3.59	3.05	3.19	1.86	3.19	3.19	0.53	3.32	0.53	3.45	0.53	0.27	0.40	0.53	0.66	0.40	0.53	0.66	0.66	-				
mk26	3.72	3.72	3.59	3.72	3.59	3.85	3.32	3.72	2.12	3.45	3.45	0.80	3.59	0.53	3.98	0.53	0.53	0.40	0.80	0.93	0.66	0.80	0.93	0.66	0.80	-			
mk27	3.59	3.85	3.45	3.59	3.72	3.98	3.45	3.59	1.99	3.59	3.59	0.66	3.72	0.40	3.85	0.66	0.40	0.53	0.66	0.80	0.53	0.66	0.80	0.53	0.66	0.13	-		
mk28	4.25	2.92	4.12	4.12	3.59	3.05	3.98	4.25	4.52	4.25	2.92	2.66	3.05	2.92	3.19	2.92	2.66	2.79	2.93	2.79	2.79	2.66	2.79	3.05	2.92	3.19	3.05	-	
mk29	4.65	3.45	4.52	4.25	4.12	3.59	4.52	4.38	4.91	4.78	3.72	4.26	3.85	4.52	3.72	4.52	4.26	4.39	4.53	4.39	4.39	4.26	4.39	4.65	4.26	4.79	4.65	1.60	-

Table 8 Table of uncorrected pair-wise distances (%) between haplotypes of *A. semotus.*

Halotype	as1	as2	as3	as4	as5	as6	as7	as8	as9	as10	as11	as12	as13	as14	as15	as16	as17	as18	as19	as20	as21	as22	as23	as24	as25	as26	as27	as28	as29	as30	as31	as32	as33	as34	as35	as36	as37
as1	-																																				
as2	0.37	-																																			
as3	0.12	0.25	-																																		
as4	0.75	0.37	0.62	-																																	
as5	0.37	0.50	0.25	0.37	-																																
as6	0.62	0.25	0.50	0.12	0.25	-																															
as7	1.74	1.37	1.61	1.49	1.61	1.37	-																														
as8	0.87	0.75	0.75	0.62	0.50	0.50	1.61	-																													
as9	0.75	0.62	0.62	0.50	0.37	0.37	1.49	0.12	-																												
as10	0.87	0.75	0.75	0.87	0.75	0.75	1.12	0.50	0.37	-																											
as11	1.24	0.87	1.12	0.75	0.87	0.62	1.24	0.87	0.75	1.12	-																										
as12	0.62	0.50	0.50	0.37	0.25	0.25	1.61	0.25	0.12	0.50	0.87	-																									
as13	1.24	1.12	1.12	0.99	0.87	0.87	1.49	0.62	0.75	1.12	0.75	0.87	-																								
as14	0.99	0.87	0.87	0.75	0.62	0.62	1.74	0.12	0.25	0.62	0.99	0.37	0.75	-																							
as15	1.12	0.99	0.99	1.12	0.99	0.99	1.12	0.75	0.62	0.25	1.37	0.75	1.37	0.87	-																						
as16	1.49	1.12	1.37	0.99	1.12	0.87	0.75	0.87	0.99	1.37	0.75	1.12	0.75	0.99	1.61	-																					
as17	0.75	0.62	0.62	0.50	0.37	0.37	1.74	0.37	0.25	0.62	0.99	0.12	0.99	0.50	0.87	1.24	-																				
as18	1.12	0.99	0.99	1.12	0.99	0.99	0.87	0.99	0.87	0.50	0.87	0.99	0.62	1.12	0.75	1.12	1.12	-																			
as19	1.37	0.99	1.24	0.87	0.99	0.75	1.37	0.75	0.87	1.24	0.12	0.99	0.62	0.87	1.49	0.62	1.12	0.99	-																		
as20	1.24	1.12	1.12	1.24	1.12	1.12	1.24	0.87	0.75	0.37	1.49	0.87	1.49	0.99	0.12	1.74	0.75	0.87	1.61	-																	
as21	1.24	0.87	1.12	0.75	0.87	0.62	0.75	1.12	0.99	1.37	0.75	0.87	0.99	1.24	1.61	0.25	0.99	1.12	0.87	1.74	-																
as22	0.75	0.62	0.62	0.50	0.37	0.37	1.24	0.62	0.50	0.87	0.50	0.37	0.50	0.75	1.12	0.75	0.50	0.62	0.62	1.24	0.50	-															
as23	1.37	0.99	1.24	0.87	0.99	0.75	0.87	0.99	0.87	0.75	0.87	0.99	1.12	1.12	0.99	1.12	1.12	0.75	0.99	1.12	1.12	0.87	-														
as24	0.99	0.87	0.87	0.75	0.62	0.62	1.74	0.12	0.25	0.62	0.99	0.37	0.75	0.25	0.87	0.99	0.50	1.12	0.87	0.99	1.24	0.75	1.12	-													
as25	0.87	0.75	0.75	0.62	0.50	0.50	1.37	0.75	0.62	0.99	0.62	0.50	0.37	0.87	1.24	0.87	0.62	0.50	0.75	1.37	0.62	0.12	0.99	0.87	-												
as26	0.75	0.62	0.62	0.50	0.37	0.37	1.49	0.62	0.50	0.87	0.99	0.37	0.99	0.75	1.12	0.99	0.50	1.12	1.12	1.24	0.75	0.50	1.12	0.75	0.62	-											
as27	0.87	0.75	0.75	0.62	0.50	0.50	1.37	0.25	0.37	0.75	0.62	0.50	0.37	0.37	0.99	0.62	0.62	0.75	0.50	1.12	0.87	0.37	0.99	0.37	0.50	0.62	-										
as28	0.87	0.75	0.75	1.12	0.99	0.99	1.61	1.24	1.12	1.24	1.12	0.99	0.87	1.37	1.49	1.37	1.12	0.75	1.24	1.61	1.12	0.62	1.49	1.37	0.50	1.12	0.99	-									
as29	0.50	0.37	0.37	0.25	0.12	0.12	1.49	0.62	0.50	0.87	0.75	0.37	0.99	0.75	1.12	0.99	0.50	1.12	0.87	1.24	0.75	0.50	0.87	0.75	0.62	0.50	0.62	1.12	-								
as30	1.24	1.12	1.12	0.99	0.87	0.87	1.74	0.62	0.75	1.12	1.24	0.87	0.99	0.75	1.37	0.99	0.99	1.37	1.12	1.49	1.24	0.99	1.37	0.75	1.12	0.50	0.62	1.61	0.99	-							
as31	0.87	0.75	0.75	0.62	0.50	0.50	1.86	0.75	0.62	0.99	1.12	0.50	1.12	0.87	1.24	1.37	0.62	1.24	1.24	1.37	1.12	0.62	0.99	0.87	0.75	0.62	0.75	1.24	0.62	1.12	-						
as32	0.99	0.87	0.87	0.75	0.62	0.62	1.74	0.37	0.25	0.62	0.99	0.37	0.99	0.50	0.87	1.24	0.50	1.12	1.12	0.99	1.24	0.75	1.12	0.50	0.87	0.75	0.62	1.37	0.75	0.99	0.87	-					
as33	0.87	0.75	0.75	0.62	0.50	0.50	1.37	0.25	0.12	0.50	0.62	0.25	0.62	0.37	0.75	0.87	0.37	0.75	0.75	0.87	0.87	0.37	0.75	0.37	0.50	0.62	0.50	0.99	0.62	0.87	0.75	0.37	-				
as34	0.62	0.50	0.50	0.87	0.75	0.75	1.37	0.99	0.87	0.99	0.87	0.75	0.62	1.12	1.24	1.12	0.87	0.50	0.99	1.37	0.87	0.37	1.24	1.12	0.25	0.87	0.75	0.25	0.87	1.37	0.99	1.12	0.75	-			
as35	0.50	0.62	0.37	0.50	0.12	0.37	1.74	0.62	0.50	0.87	0.99	0.37	0.99	0.75	1.12	1.24	0.50	1.12	1.12	1.24	0.99	0.50	1.12	0.75	0.62	0.50	0.62	1.12	0.25	0.99	0.62	0.75	0.62	0.87	-		
as36	0.99	0.87	0.87	0.75	0.62	0.62	1.24	0.62	0.50	0.87	0.50	0.62	0.25	0.75	1.12	0.75	0.75	0.37	0.62	1.24	0.75	0.25	0.87	0.75	0.12	0.75	0.37	0.62	0.75	0.99	0.87	0.75	0.37	0.37	0.75	-	
as37	1.74	1.37	1.61	1.49	1.61	1.37	0.50	1.61	1.49	1.12	1.24	1.61	1.49	1.49	1.12	0.99	1.74	0.87	1.37	1.24	0.99	1.24	1.12	1.74	1.37	1.49	1.37	1.61	1.49	1.49	1.86	1.74	1.37	1.37	1.74	1.24	-

Table 9 Pairwise F_{ST} (below the diagonal) and N_m (above the diagonal) for (A) *M. kikuchii* and (B) *A. semotus*. Significant values of pairwise F_{ST} are indicated by bold type.

(A)

Population	W1	W2	W3	W4	M2	E1	E2	E3
W1	-	2.065	1.234	2.520	0.371	0.303	0.214	0.658
W2	**0.195**	-	0.659	0.315	0.148	0.126	0.084	0.407
W3	**0.288**	**0.431**	-	0.507	2.831	2.037	0.847	2.893
W4	0.166	**0.613**	**0.497**	-	0.115	0.107	0.067	0.408
M2	**0.574**	**0.772**	0.150	**0.813**	-	0.977	0.246	1.763
E1	**0.622**	**0.799**	**0.197**	**0.824**	**0.339**	-	0.489	1.060
E2	**0.700**	**0.856**	**0.371**	**0.881**	**0.670**	**0.506**	-	0.648
E3	**0.432**	**0.551**	**0.147**	**0.551**	**0.221**	**0.320**	**0.436**	-

(B)

Population	W2	W3	M1	M2	E1	E2
W2	-	0.406	0.694	1.232	0.693	0.794
W3	**0.552**	-	1.811	3.285	1.530	1.568
M1	**0.419**	**0.216**	-	8.686	1.970	2.521
M2	**0.289**	**0.132**	**0.054**	-	5.569	8.372
E1	**0.419**	**0.246**	**0.202**	0.082	-	2.165
E2	**0.386**	**0.242**	**0.166**	0.056	**0.188**	-

Table 10 Analyses of molecular variance (AMOVA) of (A) *M. kikuchii* and (B) *A. semotus* populations at three hierarchical levels.

(A)

Source of variation	d. f.	Sum of squares	Variance components	Percentage of variation explained	Fixation indices	*P* value
Among regions	2	232.745	3.223	31.47%	Φ_{CT}=0.315	<0.05
Among populations within regions	5	188.897	2.949	26.95%	Φ_{SC} =0.393	<0.001
Within populations	79	359.462	4.55	41.59%	Φ_{ST} =0.584	<0.001
Total	86	781.103	10.942			

(B)

Source of variation	d. f.	Sum of squares	Variance components	Percentage of variation explained	Fixation indices	*P* value
Among regions	2	21.088	0.109	3.67%	Φ_{CT}=0.037	0.20
Among populations within regions	3	25.359	0.518	17.40%	Φ_{SC} =0.181	<0.001
Within populationss	66	154.998	2.348	78.93%	Φ_{ST} =0.211	<0.001
Total	71	201.444	2.975			

Table 11 The amount of genetic distances explained by and correlated with geographic distances based on the three isolation by distance (IBD) models for *M. kikuchii* and *A. semotus*.

Model	Description	*M. kikuchii*		*A. semotus*	
		r^2	*r*	r^2	*r*
DISTANCE	Euclidean distance only	0.094	0.307	0.000	-0.021
PATH	Consider topography	0.098	0.313	0.000	-0.007
COVER	Consider habitat type	0.123	0.351*	0.014	0.120

r^2: proportion of variance in genetic distances explained by the variation in geographic distances computed with the reduced major axis (RMA) regression

r: correlation coefficient of Mantel test between genetic distance and geographic distances

***** Significant correlation with P-value < 0.05

Table 12 The correlation coefficient of Mantel test (*r*) or proportion of variance of RMA regression (r^2) between genetic distance and Euclidean or LCP distance reported in previous studies. Significance of *r* was indicated by *: *****p<0.05, ******p<0.01, *****p<0.001

Species	*r*	r^2	LCP variables	Reference
	Euclidean/ LCP distance	Euclidean/ LCP distance		
Pacific jumping mouse *(Zapus trinotatus)*	0.064/ 0.420 *	–	elevation, riparian habitat	Vignieri, 2005
Red-bellied tree squirrel (*Callosciurus erythraeus*)	0.1147 ***/ 0.1496 ***	–	and use	Chen, 2007
Spiny rat (*Niniventer coninga*)	0.123 ***/ 0.161 ***	–	habitat suitability modeling	Wang, 2008
Australian bush rat (*Rattus fuscipes*)	0.601 */ 0.621 *	–	cover	Macqueen et al., 2008
Mountain vizcacha (*Lagidium viscacia*)	0.26/ 0.40 **	–	geology	Walker et al., 2007
American marten *(Martes americana)*	–	0.0032/ 0.0043	land use	Broquet et al., 2006
California mule deer (*Odocoileus hemionus*)	0.064/ 0.096 *	–	climate, vegetation, elevation	Pease et al., 2009
Wood frog *(Rana sylvatica)*	–	0.17/ 0.27	land cover	Lee-Yaw et al., 2009
Andean tree frog (*Hypsiboas andinus*)	0.21/ 0.45 **	0.05/ 0.20	vegetation	Koscinski et al., 2009
Columbia spotted frog (*Rana luteiventris*)	0.719 */ 0.832 *	0.517/ 0.692	mountain ridge	Funk et al., 2005
Black fly *(Prosimulium neomacropyga)*	0.80 */ 0.72 *	–	streams, surface	Finn et al., 2006

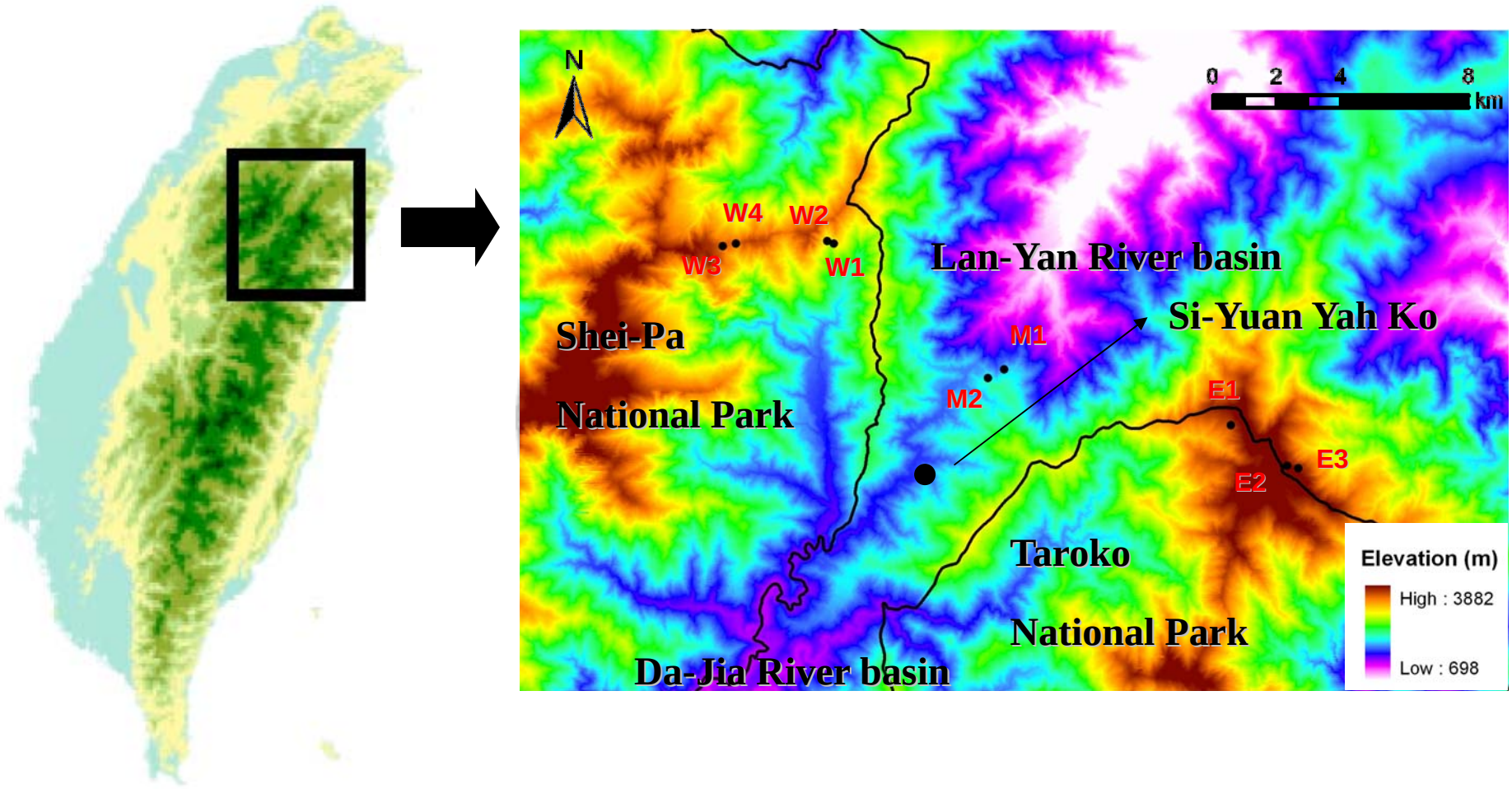


Fig. 1 Si-Yuan Yah Ko connects Shei-Pa NP and Taroko NP (boundary in black line), and divides the Lan-Yan River basin to the north and the Da-Jia River basin to the south. Locations of study sites are represented with small black dots and indicated with red texts.

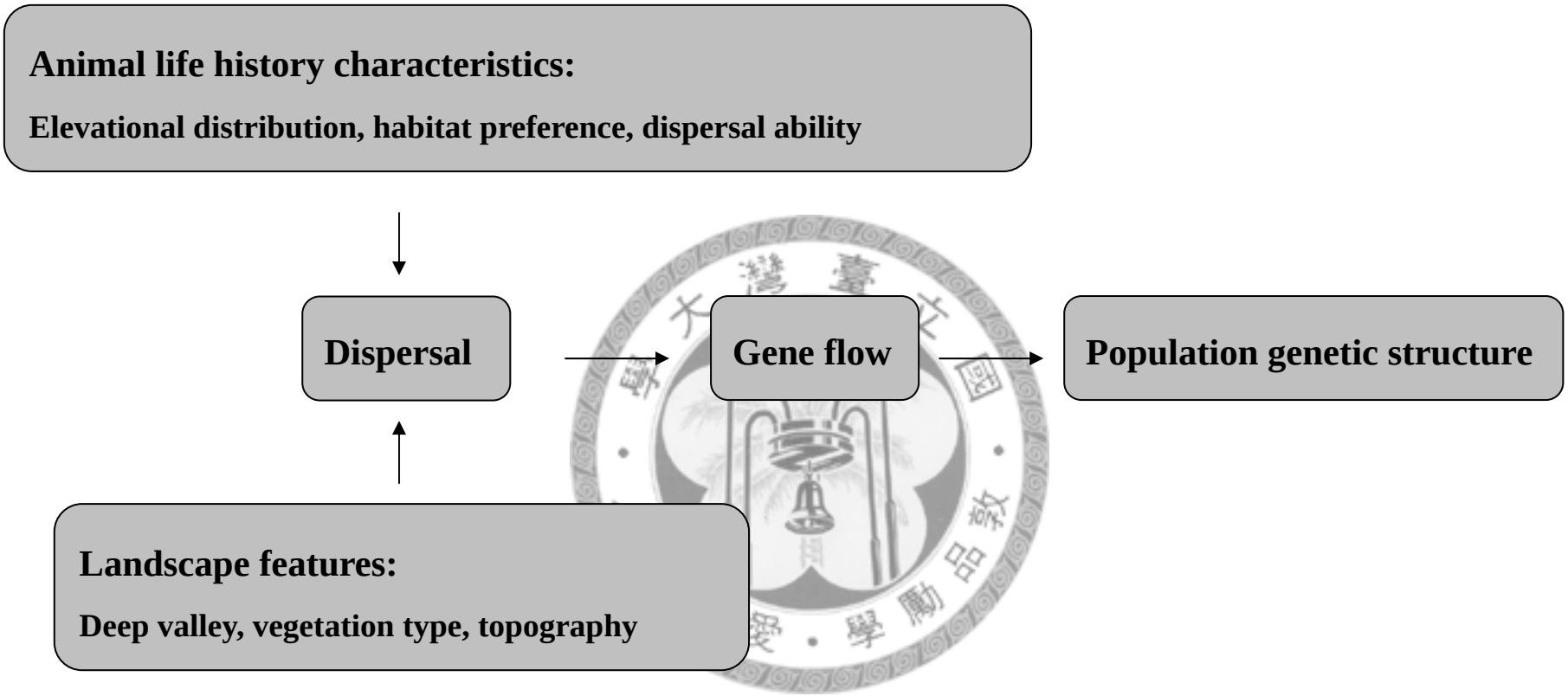


Fig. 2 The concept map of how landscape features influence population genetic structure of *M. kikuchii* and *A. semotus*.

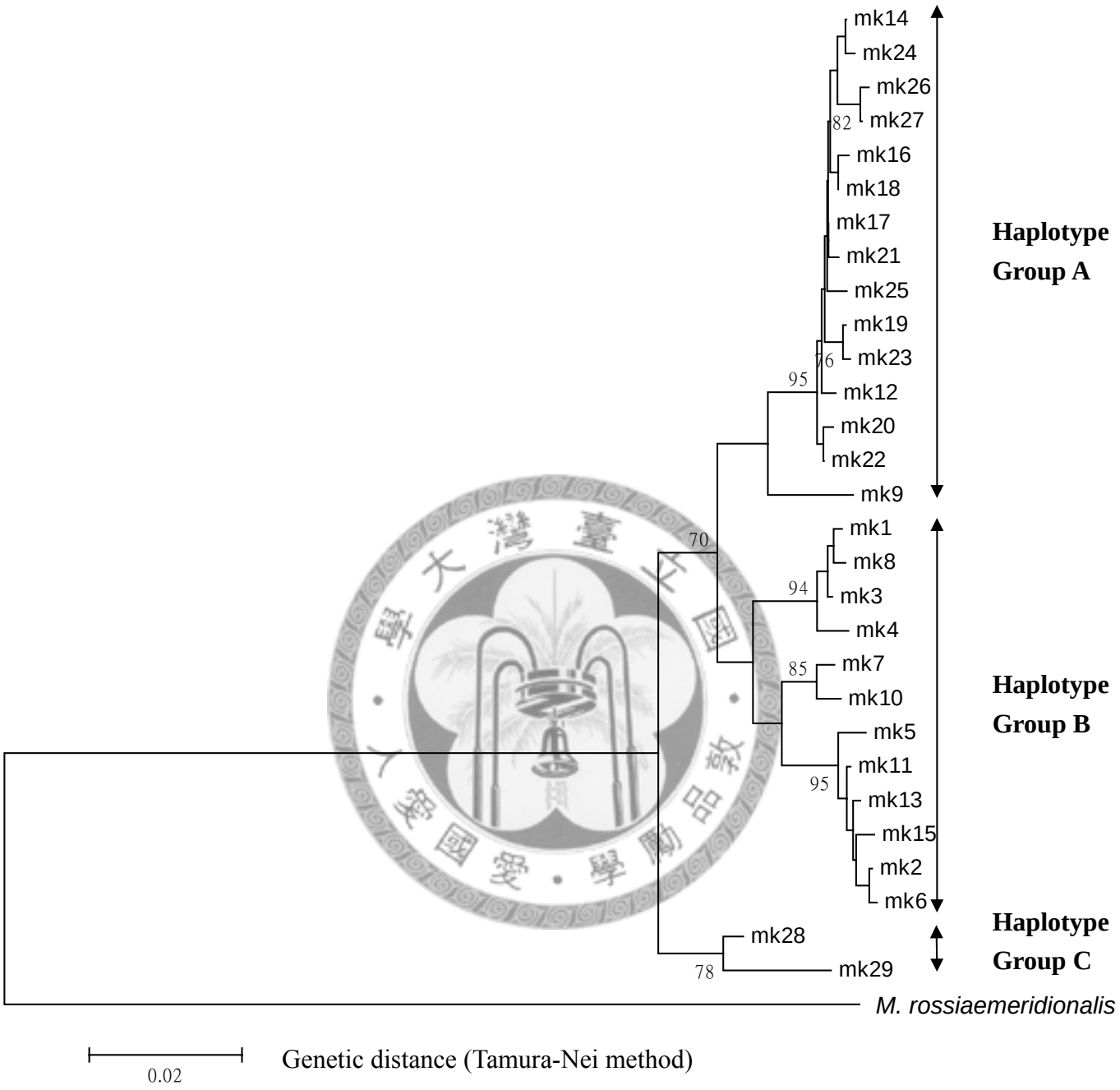
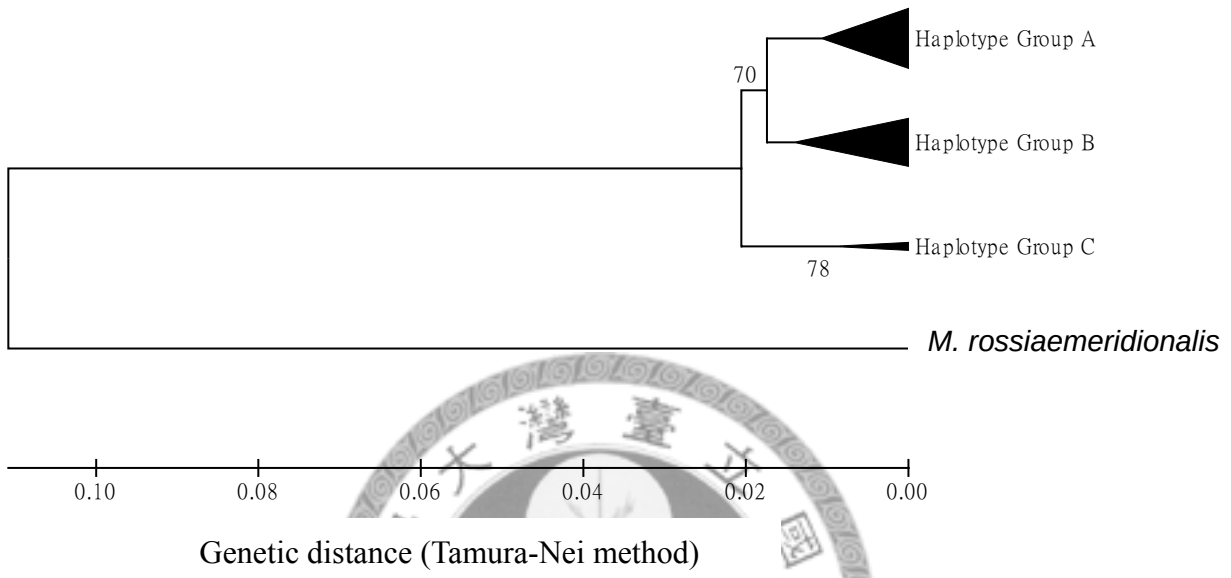


Fig. 3 Neighbor-joining (NJ) tree of the haplotypes of *M. kikuchii* based on partial sequence of mtDNA control region. The sequence of *M. rossiaemeridionalis* was used as an outgroup. Bootstrap probabilities larger than 70% are shown next to the branches basd on 1000 replications.



Region		WS				SY	ES			Total
Population		W1	W2	W3	W4	M2	E1	E2	E3	
Haplotype group	A	1	0	8	0	6	12	17	7	51
	B	12	9	4	7	0	0	0	0	32
	C	0	0	0	0	0	0	0	4	4

Fig. 4 The condensed neighbor-joining (NJ) tree of the haplotypes of *M. kikuchii* and outgroup *M. rossiaemeridionalis* based on partial sequence of mtDNA control region. Frequencies of individuals in each haplotype group were shown in the table below.

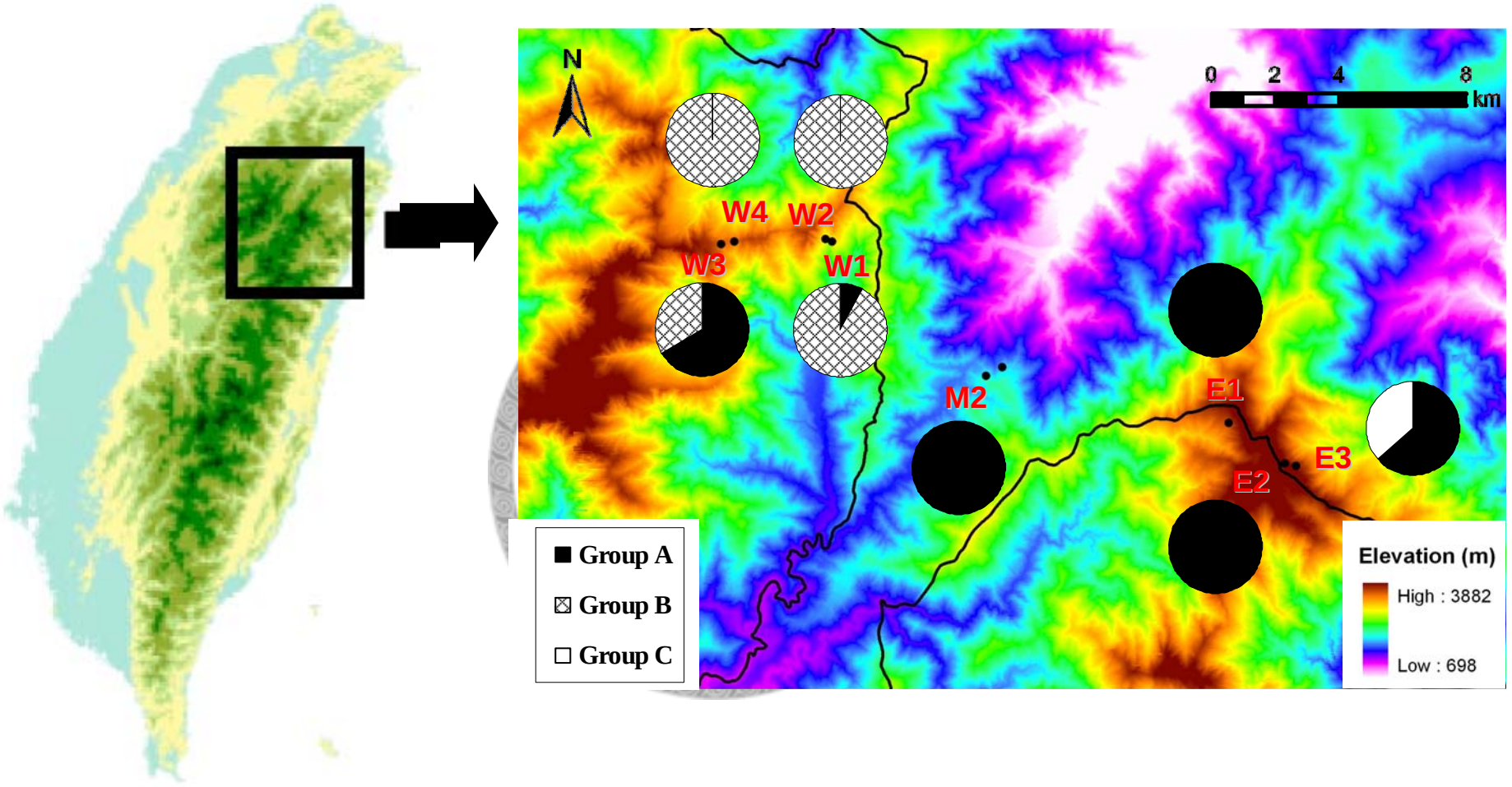


Fig. 5 The distribution of three haplotype groups in eight populations of *M. kikuchii*. The frequency of each haplotype group is given in the pie diagrams.

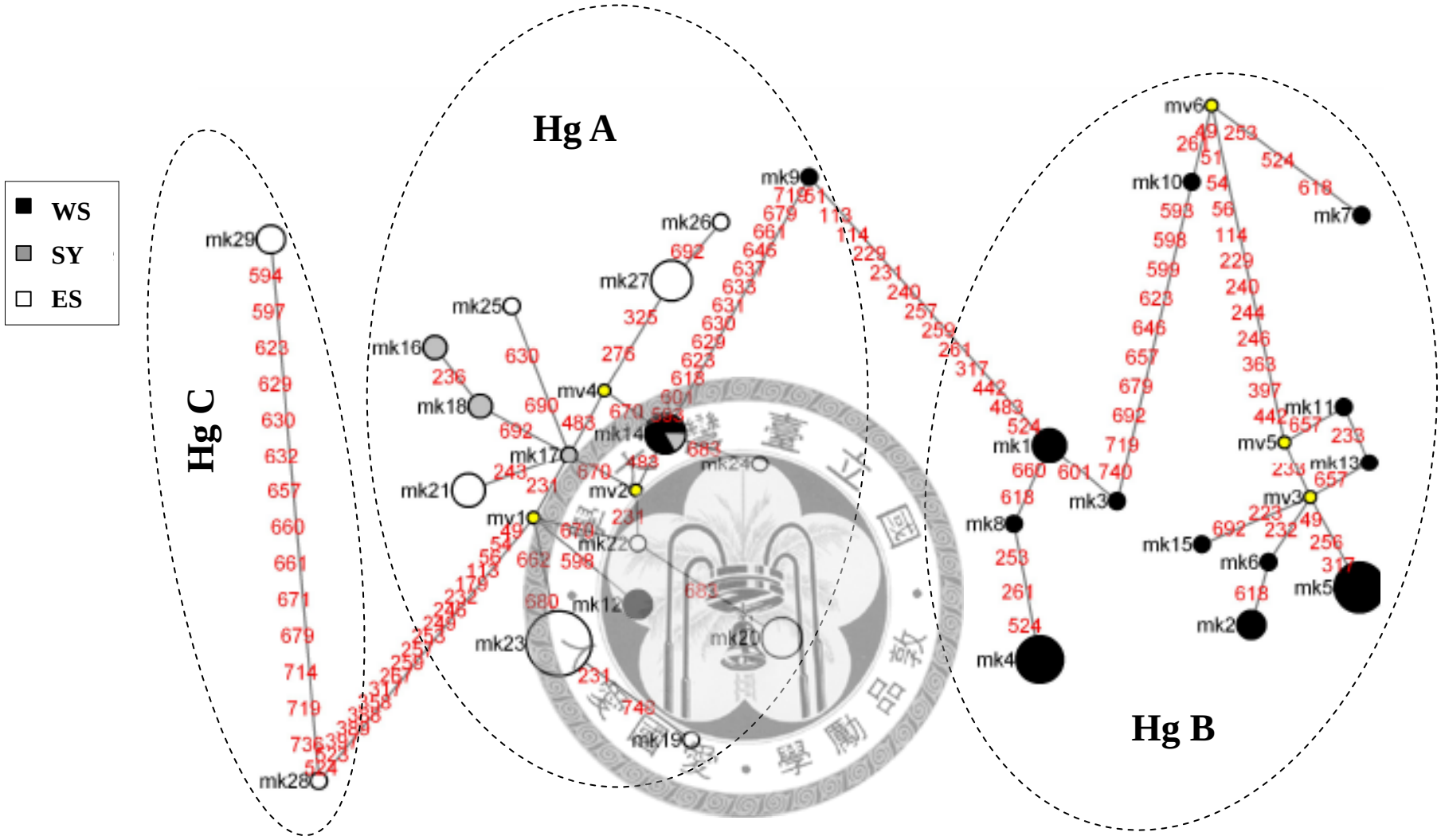


Fig. 6 Median Joining Network of 29 haplotypes of *M. kikuchii*. Circles represent haplotypes, with sizes proportional to frequencies. The pie diagrams inside each circle indicate the relative frequency in each region (black: WS; grey: SY; white: ES). Values on each connecting line are polymorphic sites between haplotypes. Nodes that named as "mv#" are median vectors. Dotted areas indicate the haplotype groups identified in NJ tree, and "haplotype group" is abbreviated as "Hg" here.

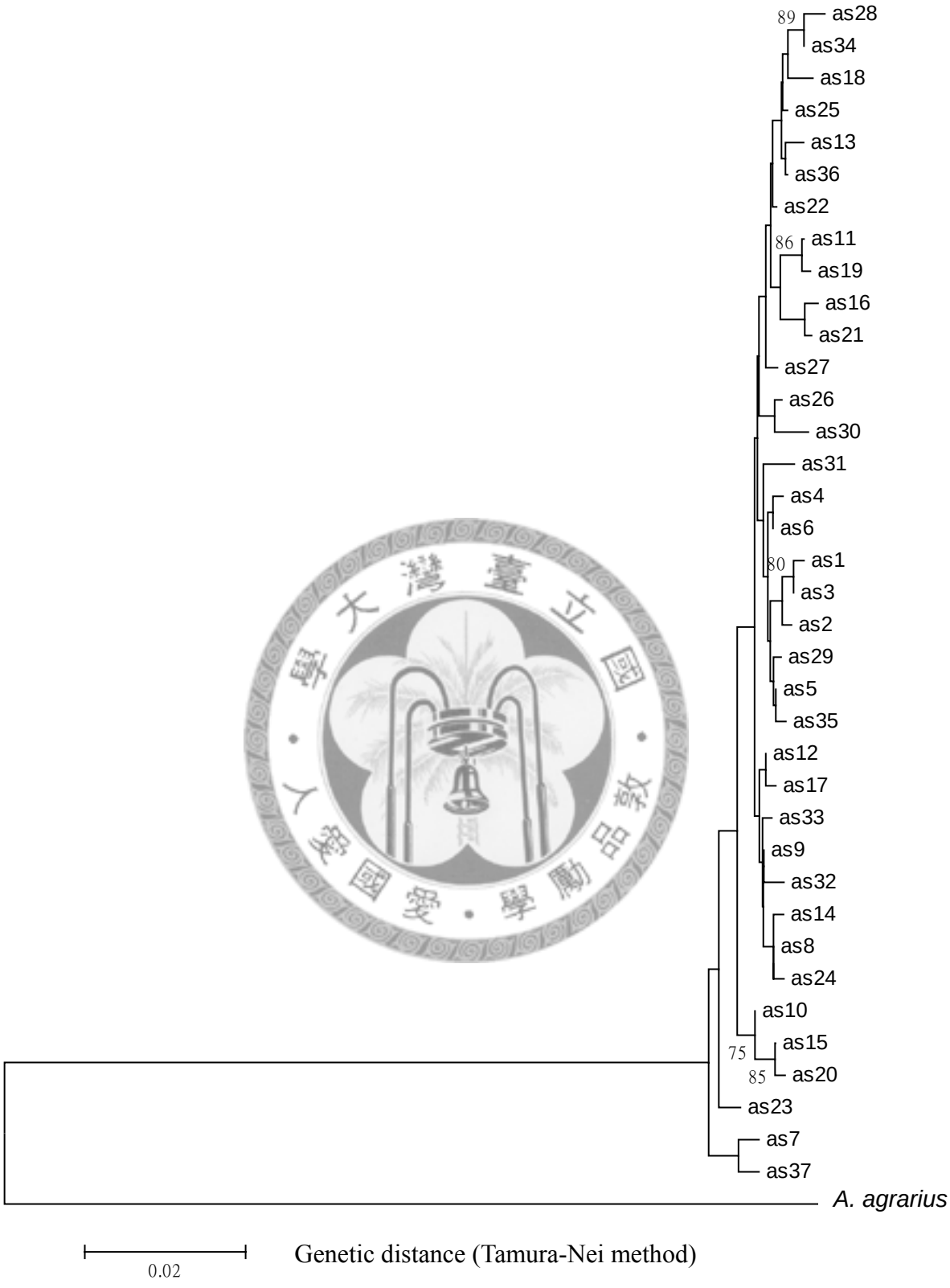


Fig. 7 Neighbor-joining (NJ) tree of the haplotypes of *A. semotus* based on partial sequence of mtDNA control region. The sequence of *A. agrarius* was used as an outgroup. Bootstrap probabilities larger than 70% are shown next to the branches with a star sign based on 1000 replications.

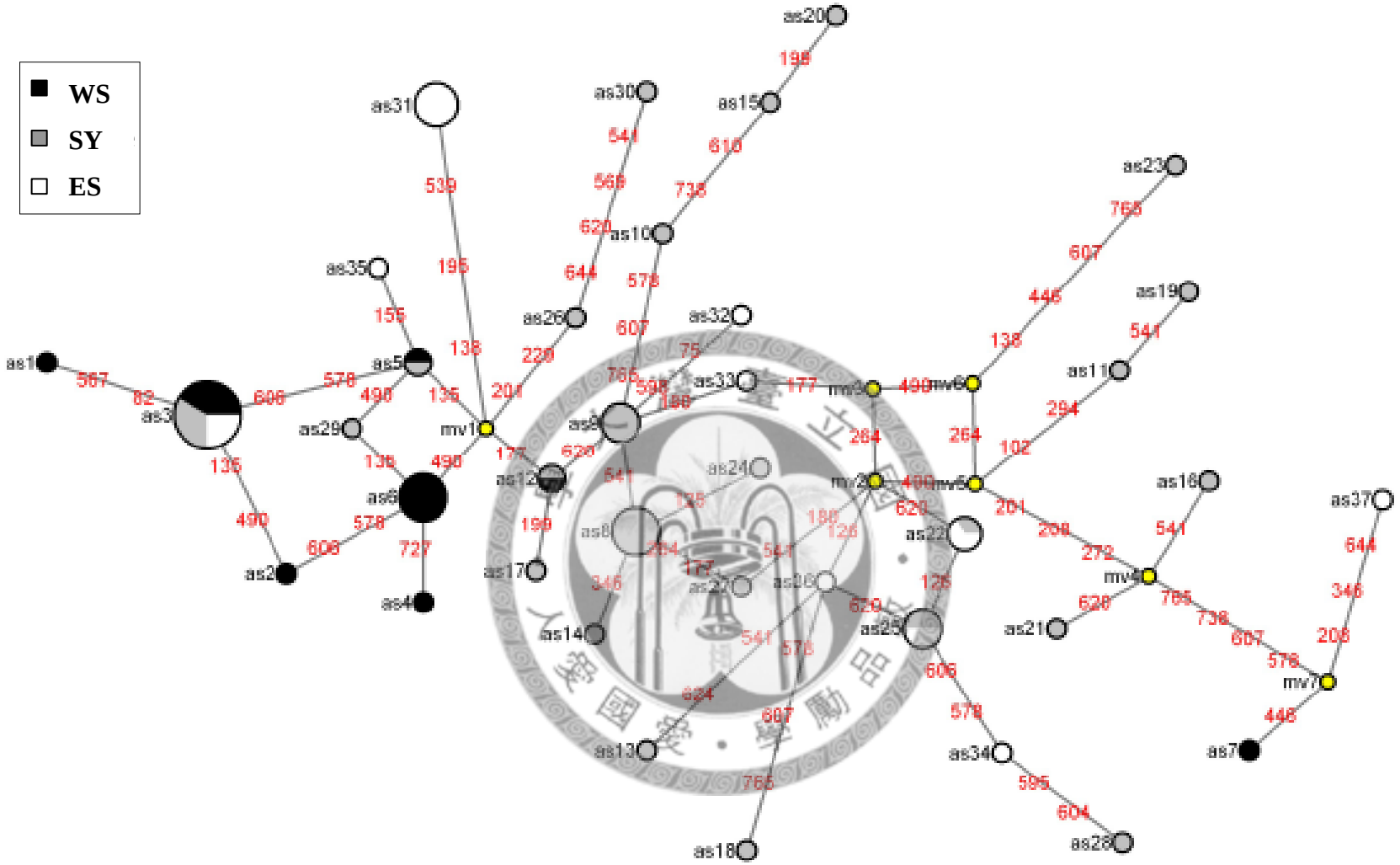


Fig. 8 Median Joining Network of 37 haplotypes of *A. semotus*. Circles represent haplotypes, with sizes proportional to frequencies. The pie diagrams inside each circle indicate the relative frequency in each region (black: WS; grey: SY; white: ES). Values on each connecting line are polymorphic sites between haplotypes. Nodes that named as "mv#" are median vectors.

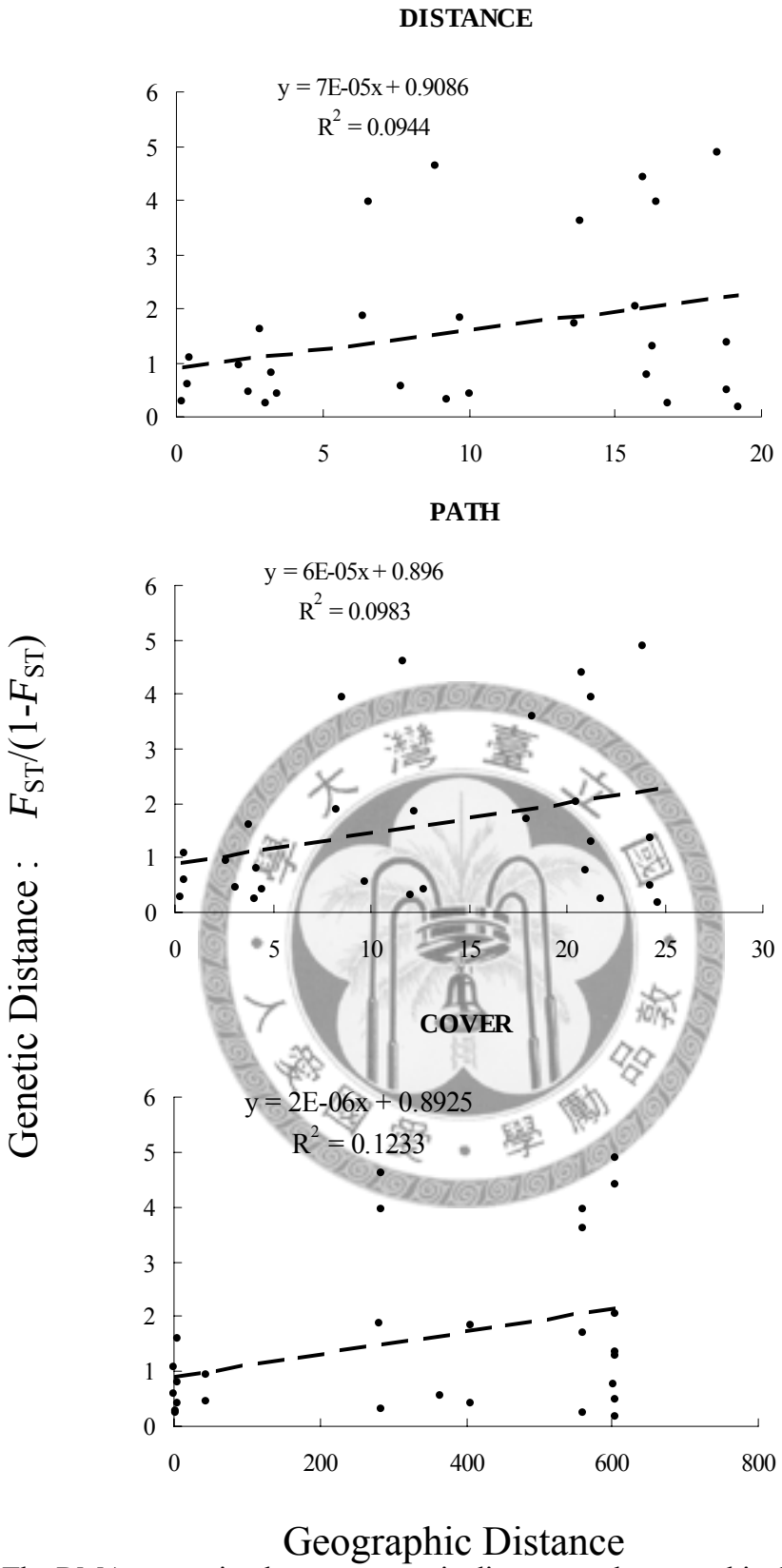


Fig. 9 The RMA regression between genetic distance and geographic distance (measured by three models, respectively) among populations of *M. kikuchii*. In model DISTANCE and PATH, the geographic distances were represented by physical distances measured in kilometer, while in model COVER, the geographic distance was represented by accumulated cost (unit-free) along the least-cost path among populations.

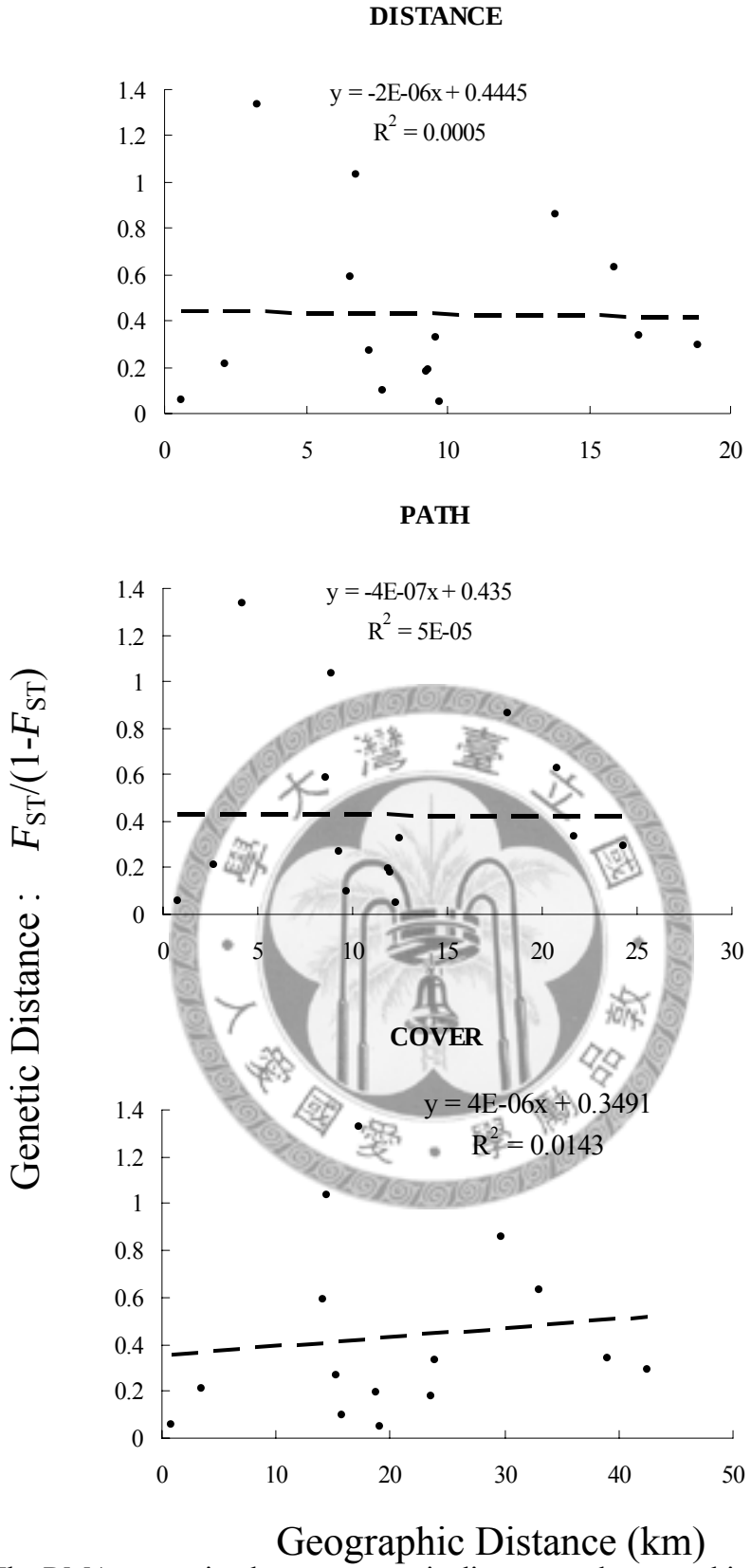


Fig. 10 The RMA regression between genetic distance and geographic distance (measured by three models, respectively) among populations of *A. semotus*. In model DISTANCE and PATH, the geographic distances were represented by physical distances measured in kilometer, while in model COVER, the geographic distance was represented by accumulated cost (unit-free) along the least-cost path among populations..

Appendix I Capture data of *M. kikuchii* and *A. semotus* at the study site during 2007~2008,

Species	ID	Population	Sex	Weight	Traping date
W	1110	M1	M	21.5	2007/1 /22
W	1111	M1	M	17.5	2007/1 /22
W	1102	M2	F	17.5	2007/1 /22
W	1104	M2	F	20.0	2007/1 /22
V	body	M2	M	35.0	2007/1 /22
W	1103	M2	M	20	2007/1 /22
V	1113	W1	M	36.0	2007/1 /25
V	1114	W2	F	35.6	2007/1 /25
W	1114	M1	M	?adult	2007/3 /27
W	2101	M1	F	?adult	2007/3 /27
W	2102	M1	F	?adult	2007/3 /27
W	2103	M1	M	?adult	2007/3 /27
W	2104	M1	F	?adult	2007/3 /27
W	1124	M2	F	?adult	2007/3 /27
W	3102	M2	M	?adult	2007/3 /27
W	1130	M1	F	?adult	2007/3 /28
W	1131	M1	M	?adult	2007/3 /28
W	1132	M1	F	?adult	2007/3 /28
W	2113	M2	M	?adult	2007/3 /28
W	2114	M2	F	?adult	2007/3 /28
W	3113	M1	F	?adult	2007/3 /29
W	3114	M1	M	?adult	2007/3 /29
W	3120	M1	M	?adult	2007/3 /29
W	3121	M1	M	?adult	2007/3 /29
W	3122	M1	F	?adult	2007/3 /29
W	2121	M2	M	?adult	2007/3 /29
W	2122	M2	F	?adult	2007/3 /29
W	2124	M2	F	?adult	2007/3 /29
W	2131	M2	M	?adult	2007/3 /29
W	3131	M1	F	24.5	2007/4 /29
W	4112	M2	M	25.5	2007/4 /29
W	4111	M1	M	29	2007/4 /29
V	1140	M2	M	42	2007/4 /30

Appendix I (continued)

Species	ID	Population	Sex	Weight	Traping date
W	4130	M1	M	32	2007/4 /30
W	4131	M1	F	21.5	2007/4 /30
W	4134	M2	M	28.2	2007/4 /30
V	1141	M2	F	46.5	2007/4 /30
V	0112	M2	F?	34	2007/5 /1
W	3141	M1	M	23.5	2007/5 /1
W	3144	M2	F	21.5	2007/5 /1
W	4143	M2	F	28.5	2007/5 /1
W	1021	E1	F	14	2007/7 /4
W	1022	E1	F	27	2007/7 /4
W	1014	E1	F	14.4	2007/7 /4
W	2012	E1	F	22.5	2007/7 /5
W	1023	E1	M	15.5	2007/7 /5
W	1024	E1	F	32.5	2007/7 /5
W	2013	E1	M	23	2007/7 /5
V	2021	E1	F	44.5	2007/7 /6
V	2022	E1	F	42	2007/7 /6
W	2023	E1	F	15.5	2007/7 /6
W	2014	E1	F	19.5	2007/7 /6
W	2024	E1	F	11	2007/7 /6
V	3011	W1	M	36	2007/7 /18
V	0212	W2	F	37.1	2007/7 /18
W	3013	W2	F	28	2007/7 /18
W	0211	W2	F	26.7	2007/7 /18
W	3012	W2	j	9.5	2007/7 /18
V	3023	W1	M?	38.5	2007/7 /19
V	0221	W1	M	32	2007/7 /19
V	3014	W2	F	31.5	2007/7 /19
V	3021	W2	F	38	2007/7 /19
V	0213	W2	F	45	2007/7 /19
V	0214	W2	M	34.8	2007/7 /19
W	3022	W2	F	19	2007/7 /19
V	0222	W2	M	39.2	2007/7 /20
V	0223	W2	F?	35	2007/7 /20

Appendix I (continued)

Species	ID	Population	Sex	Weight	Traping date
V	0231	W2	M	27.7	2007/7 /20
W	0224	W2	j	11	2007/7 /20
V	body	W1	?	?	2007/7 /20
V	0233	E2	F	41	2007/9 /5
V	0241	E2	F	35.5	2007/9 /5
V	0243	E2	M	33.5	2007/9 /5
V	0244	E2	F	40	2007/9 /5
W	0234	E2	F	18.5	2007/9 /5
W	0242	E2	F	21.5	2007/9 /5
W	0232	E2	F	27	2007/9 /5
W	1203	E1	F	26	2007/9 /6
W	1202	E2	M	28.5	2007/9 /6
V	1204	E1	M	32	2007/9 /6
V	1210	E1	M	27	2007/9 /6
W	1201	E2	F?	15.5	2007/9 /6
V	1213	W1	F	27	2007/9 /12
W	1211	W2	F	15	2007/9 /12
W	1212	W2	M	16.5	2007/9 /12
V	1220	W1	M	39	2007/9 /13
V	1223	W1	F	25	2007/9 /13
V	1214	W2	M	48	2007/9 /13
V	1222	W1	M	33.5	2007/9 /13
V	1224	W1	M	35	2007/9 /14
V	1231	W1	F	43	2007/9 /14
V	1232	W1	M	40	2007/9 /14
V	1233	W1	F	36	2007/9 /14
V	1234	W2	F	35	2007/9 /14
W	2212	M2	F	33.5	2008/6 /2
W	2214	M2	M	9.0	2008/6 /2
V	2213	M2	M	37.5	2008/6 /3
W	2221	M2	F	25.5	2008/6 /3
V	2222	M2	M	42.5	2008/6 /3
V	3213	W4	F	44	2008/7 /14
W	4011	W3	F	24	2008/7 /14

Appendix I (continued)

Species	ID	Population	Sex	Weight	Traping date
W	3212	W3	M?	20	2008/7 /14
V	3124	W3	M	39	2008/7 /14
W	3221	W3	M	12.5	2008/7 /15
W	(12)(12)xx	W3	?	?	2008/7 /15
W	xx(234)(23)	W3	?	?	2008/7 /15
V	4014	W3	F	47	2008/7 /15
V	4023	W3	F	35	2008/7 /15
V	4012	W3	M	34	2008/7 /15
V	4024	W4	M	37	2008/7 /15
V	4013	W3	M	35	2008/7 /15
V	4204	W3	F	54	2008/7 /16
V	4220	W3	F	40	2008/7 /16
V	4230	W4	F	26.5	2008/7 /16
V	0132	W4	F	31	2008/7 /16
V	1032	W4	F	39	2008/7 /16
V	2032	W4	F	36.5	2008/7 /16
W	4031	W3	F	22	2008/7 /16
V	4210	W3	F	29	2008/7 /16
W	3230	W3	M	12.0	2008/7 /16
V	0133	W3	F	38.5	2008/7 /16
W	2031	W3	F	28	2008/7 /16
V	4240	W3	F	40.5	2008/7 /17
V	3220	W3	M	41.5	2008/7 /17
V	4212	W3	M	36	2008/7 /17
V	4213	W4	F	51.5	2008/7 /17
V	4214	W4	M	42	2008/7 /17
V	4221	W4	M	46	2008/7 /17
W	3240	W3	M	25	2008/7 /17
V	4211	W3	M	35	2008/7 /17
V	0322	E1	M	36.5	2008/8 /29
V	0312	E2	F	37	2008/8 /29
V	0313	E2	M	29.5	2008/8 /29
V	0314	E2	F	47.0	2008/8 /29
V	0331	E2	F	28.5	2008/8 /29

Appendix I (continued)

Species	ID	Population	Sex	Weight	Traping date
V	0324	E3	M	36.5	2008/8 /29
W	0311	E2	M	26	2008/8 /29
V	0321	E1	M	30.5	2008/8 /29
V	0323	E1	F	30.0	2008/8 /29
V	0332	E2	F	28.5	2008/8 /30
V	0333	E2	M	30	2008/8 /30
V	0334	E2	M	34.5	2008/8 /30
V	0341	E2	F	34.5	2008/8 /30
V	0342	E2	M	33	2008/8 /30
V	0343	E3	M	33	2008/8 /30
V	0344	E3	F	15	2008/8 /30
V	1313	E3	F	36.5	2008/8 /31
W	1302	E2	F	22	2008/8 /31
V	1303	E3	M?	22.5	2008/8 /31
V	1301	E2	F	20	2008/8 /31
V	1304	E1	M	31.5	2008/9 /1
V	2302	E2	F	29	2008/9 /1
V	2303	E2	M	28.5	2008/9 /1
V	2301	E2	F	32	2008/9 /1
V	1330	E3	F	39.5	2008/9 /1
V	1311	E1	M?	24.5	2008/9 /1
V	1312	E1	M	30.5	2008/9 /1
V	1314	E1	F?	24.5	2008/9 /1
V	1321	E1	M?	32.5	2008/9 /1
V	1310	E3	M?	20	2008/9 /1
V	1320	E3	M	30.5	2008/9 /1
V	2304	E3	F	38.5	2008/9 /2
V	2310	E3	F	27.5	2008/9 /2
V	2(34)12	E3	F	32	2008/9 /2

Appendix II The pairwise distance matrices between populations of *M. kikuchii*.

Population pair		Genetic distance	Geographic distance[1]		
		$F_{ST}/(1-F_{ST})$	DISTANCE	PATH	COVER
W1	W2	0.27	223.89	335.44	1416.32
W1	W3	0.41	3460.71	4488.26	3959.85
W1	W4	0.25	3040.00	4038.27	3667.32
W1	M2	1.87	6368.71	8291.28	282107.84
W1	E1	1.70	13596.77	17931.96	560166.31
W1	E2	2.03	15713.35	20491.20	603375.81
W1	E3	0.76	16065.38	20926.95	603189.56
W2	W3	0.80	3258.93	4214.71	4318.44
W2	W4	1.60	2836.59	3764.74	4025.92
W2	M2	3.95	6582.78	8567.87	282940.86
W2	E1	3.61	13820.63	18231.27	560999.44
W2	E2	4.41	15937.04	20790.51	604208.94
W2	E3	1.28	16289.12	21226.26	604022.69
W3	W4	1.09	425.00	451.00	327.44
W3	M2	0.30	9230.51	12025.47	283131.47
W3	E1	0.25	16781.62	21716.33	561196.84
W3	E2	0.48	18856.27	24269.64	604406.34
W3	E3	0.17	19214.37	24705.42	604220.09
W4	M2	4.62	8886.77	11609.52	282839.20
W4	E1	3.95	16408.43	21308.32	560903.44
W4	E2	4.88	18489.74	23867.59	604112.94
W4	E3	1.36	18847.15	24303.34	603926.69
M2	E1	0.57	7702.55	9701.33	364336.48
M2	E2	1.84	9698.62	12260.78	407535.98
M2	E3	0.43	10060.40	12696.55	407349.45
E1	E2	0.95	2152.35	2663.13	44853.29
E1	E3	0.45	2488.42	3089.84	44667.04
E2	E3	0.59	362.84	460.92	258.92

[1] In model DISTANCE and PATH, the geographic distances were represented by physical distances measured in kilometer, while in model COVER, the geographic distance was represented by accumulated cost (unit-free) along the least-cost path among populations.

Appendix III The pairwise distance matrices between populations of *A. semotus*.

Population pair		Genetic distance	Geographic distance[1]		
		$F_{ST}/(1-F_{ST})$	DISTANCE	PATH	COVER
W2	W3	1.33	3258.93	4214.71	17222.90
W2	M1	1.03	6809.94	8938.00	14528.93
W2	M2	0.59	6582.78	8567.87	14186.82
W2	E1	0.86	13820.63	18231.27	29667.33
W2	E2	0.63	15937.04	20790.51	33074.66
W3	M1	0.33	9571.84	12495.98	23897.01
W3	M2	0.18	9230.51	12025.47	23554.91
W3	E1	0.34	16781.62	21716.33	39035.28
W3	E2	0.29	18856.27	24269.64	42442.54
M1	M2	0.06	575.02	757.00	768.17
M1	E1	0.27	7268.80	9303.35	15325.28
M1	E2	0.19	9300.54	11862.79	18732.58
M2	E1	0.10	7702.55	9701.33	15716.19
M2	E2	0.05	9698.62	12260.78	19123.47
E1	E2	0.21	2152.35	2663.13	3515.17

[1] In model DISTANCE and PATH, the geographic distances were represented by physical distances measured in kilometer, while in model COVER, the geographic distance was represented by accumulated cost (unit-free) along the least-cost path among populations.