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（英文）

Landscape genetics of *Niviventer coninga* in a fragmented forest landscape of low elevation in central west Taiwan

經本委員會審定通過，特此證明。

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東海大學生命科學系博士論文

台灣中西部低地破碎森林地景中刺鼠之地景遺傳學研究

Landscape genetics of *Niviventer coninga* in a fragmented low elevation forest landscape in west-central Taiwan

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Dedications

To the land, sky, and wildlife, especially, *Niviventer coninga*, of Sanyi, for nurturing this research

To my dear wife, our parents, our two children, and my sisters and brothers, for supporting me with endless love and encouragement

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摘　　要

土地利用變遷的空間格局與過程會改變地景的連結度(landscape connectivity)，因而影響了野生動、植物族群的散佈與基因交流。為評估土地利用變遷對野生動物族群遺傳結構和基因交流的影響，我選擇在苗栗三義地區破碎的森林地景中，從 2002 至 2007 年，進行刺鼠(*Niviventer coninga*)的地景遺傳學研究。刺鼠為台灣特有種，且為棲息於低海拔森林中唯一的鼠科物種；因其棲地限於森林環境，而適於作為探討人為開發對野生動物影響的研究對象。三義地區的地景變遷(1904–2001)顯示此地區百年來承受森林砍伐、農墾與造林等干擾，研究區域於 1904 年森林覆蓋約為 30.8%，1977 年約為 55.7%，2001 年約為 58.6%。然此歸因為相思樹(*Acacia confusa*)和油桐(*Aleurites montana*)單一林相的造林，雖增加了森林的覆蓋面積，卻大幅減少了森林的多樣性和森林野生動物適於生存的棲地。1950 年以降，省道台 13 線的路面拓寬與柏油化，高速公路於 1979 年通車，造就了與日俱增的車速和車流量，及沿路日漸發展的住宅、街區，因此，影響了三義地區森林地景的連結度。棲地適宜性模式(habitat suitability modelling)顯示，目前三義地區的人為開發限縮適合刺鼠生存的棲地空間。本研究顯示棲地適宜性模式結合最小成本路徑分析(least-cost path analysis)可以較客觀的評估研究區域的地景連結度。刺鼠個體間的遺傳距離(pairwise genetic distance)與個體間的最小成本距離(pairwise least-cost path distance)呈現較高的相關，顯示地景結構影響刺鼠的基因交流。人為開發降低了地景連結度，降低刺鼠的基因交流，而導致被高速公路和省道所分隔的東、西區域間的刺鼠次

族群有顯著的遺傳分化($\Phi_{RT} = 0.021$, $P = 0.0001$)；且於各區域內，被縣道所分隔的刺鼠次族群間，也有顯著的遺傳分化($\Phi_{PR} = 0.036$, $P = 0.0001$)。刺鼠雌雄間顯著的空間遺傳結構(spatial genetic structure)差異反映出刺鼠的散佈偏向為雄性(male-biased dispersal)；雌性間在短距離範圍內的相鄰個體具有較高的遺傳相關，顯示雌性的基因交流可能較容易受地景連結度的影響。土地利用和適宜棲地的空間分佈可以說明遺傳邊界(genetic boundaries)的形成與近期的人為開發有關。本研究說明近期的人為開發對刺鼠的族群遺傳結構和基因交流造成顯著的影響，其他棲息於人為開發壓力大的低海拔環境、族群量小且散佈能力差的野生動物，可能面臨比刺鼠更為嚴重的狀況。因此，我建議以發展觀光為重的苗栗縣政府或其他地方政府，應該將地景規劃和生態廊道的設置納入區域發展計畫中，為野生動物族群的續存，恢復並維護地景連結度。本研究的架構可應用於其他物種及區域，相關研究的資料與結果將有助於地方政府致力於野生動物和地景的保育規劃和實踐。

Abstract

Spatial patterns and processes of land changes affect landscape connectivity, thus, affecting dispersal and gene flow of wildlife over time. To assess the effects of land changes on the population genetic structure and gene flow of wildlife, I studied the landscape genetics of an endemic forest rodent, the spiny rat (*Niviventer coninga*), in a fragmented forest landscape in Sanyi Township, southern Miaoli County, western Taiwan. Study of the landscape history of the past century (1904–2001) showed that a process of deforestation-reforestation increased forest cover, but lowered forest diversity, reducing quality of forest habitat for wildlife. Since 1950, modernization of roads and expansion of buildings began to affect landscape connectivity of Sanyi. Currently, habitats suitable for *N. coninga* are limited. Landscape connectivity as estimated by habitat suitability also affected *N. coninga* gene flow, resulting in significant genetic differentiation between regions east and west of primary roads ($\Phi_{RT} = 0.021$, $P = 0.0001$) and among sites within regions ($\Phi_{PR} = 0.036$, $P = 0.0001$). Significant differences in spatial genetic structure showed that *N. coninga* had male-biased dispersal. Gene flow of females was more susceptible to landscape connectivity. Land-use and distribution of suitable habitats accounted for the location of genetic discontinuities. Other forest animals in the study area, especially those with relatively smaller population sizes and limited dispersal ability, may show even greater

genetic differentiation. Therefore, I suggest the Miaoli County government conserve landscape connectivity for wildlife by including greenway design and corridor construction in its regional development goals. The framework of my dissertation is applicable to other species in other regions. These methods can provide data and results to help local governments facilitate wildlife and landscape conservation.

Chapter 1

Introduction

Economic development in the past century has greatly altered the landscape. Deforestation, cropland expansion, urbanization, road construction, and globalization cause habitat loss, erosion of human cultural and wildlife genetic diversities, and a rapid decline in biodiversity (Forman et al. 2003; Lambin et al. 2003; Antrop 2005; Lindenmayer and Fischer 2006). These conditions have worsened since the 1990s, especially in tropical regions (Lambin et al. 2003). Since monitoring of global environmental change and implementation of truly sustainable development practices are urgently needed, there is a need for development in land change science (Rindfuss et al.2004; Turner et al. 2007). Land change science is an important planning tool for the future because it explains the driving forces of landscape changes, helps identify the effects and consequences of landscape changes, and predicts future changes (Marcucci 2000; Bürgi et al. 2004; Rindfuss et al. 2004; Antrop 2005; Turner et al. 2007).

Landscapes are rapidly changing in the modern world. Disturbances from economic development affect the distribution and amount of habitat and fracture landscape connectivity necessary for wildlife dispersal and gene flow. Therefore, any conservation measures counteracting the effects of economic development on wildlife should incorporate information from land change science and landscape ecology (Lindenmayer et al 2008). Landscape change study can help identify disturbances driving ecological change over time (Olsen et al. 2007). Recent development of habitat suitability modelling (Guisan and

Zimmermann 2000; Guisan and Thuiller 2005) can predict the amount and distribution of suitable habitat that affect the size and fate of a metapopulation. Conservation of landscape connectivity is also important (Crooks and Sanjayan 2006). Landscape connectivity broken by economic development can have severe consequences for wildlife populations. For example, limited dispersal among subpopulations can break up metapopulation dynamics, eventually resulting in local extinction of a species (Hanski and Gilpin 1997; Moilanen and Hanski 2006). Blocked gene flow among isolated small populations can cause inbreeding and genetic differentiation (Hartl and Clark 1997; Halliburton 2004). Recent developments of landscape genetics incorporate population genetics with spatial analyses to reveal the spatial genetic structure of wildlife in fragmented landscapes (Manel et al. 2003; Storfer et al. 2007). Interpreting the spatial structure of wildlife with environmental or landscape data also facilitates identifying landscape features or barriers producing that structure (Manel et al. 2003; Storfer et al. 2007). Therefore, a trans-disciplinary study that incorporates landscape change, habitat suitability modelling, and landscape genetics can provide comprehensive information important for regional conservation planning.

Taiwan has been developed for > 350 years. The original landscape of Taiwan's low elevations was forests dominated by Fagaceae, Lauraceae, and Moraceae trees (Chen 1960; Liu 1972). In the past three centuries, exploitation of these forests and colonization by the Han people completely cleared the original low elevation forests of western Taiwan. In the twentieth century, monocultures of economically valuable non-native trees and secondary succession have reforested parts of the landscape, but the ecological function and quality of these forests are completely different from the fragments of original forest remaining at the beginning of twentieth century. Economic development over the twentieth century largely decreased forest habitats of low elevation wildlife and compelled low elevation mammals to move to higher elevations (Lin and Lin 1983). Pei and Chen (2006) found that the low elevations of southern Miaoli County, western Taiwan, remain important for wildlife because five rare mammal species continue to inhabit this fragmented forest landscape. Recent economic development, however, has encroached on and degraded these fragmented wildlife habitats. The local government of Miaoli County has plans to build information technology (IT) industrial districts, construct new roads, broaden current roads, and promote tourism (Miaoli County Government 2008). This is part of a single-minded pursuit of economic growth without considering the facts of ecological sustainability. In the long run, these developments will reduce viability of wildlife and render null the goals of tourism development.

To assess the effects of recent economic development on wildlife and to provide information of current wildlife habitat distribution and population genetic structure, I studied a native forest rodent, the spiny rat (*Niviventer coninga*), in the fragmented forest landscape of Sanyi Township, Southern Miaoli County. This rat is distributed in forests below 2000 m and is common at 1300 m in elevation (Yu 1994; Wu and Yu 2000; Wilson and Reeder 2005). It has a low population density, high winter mortality (Chang-Chien 1989; Chang 1991; Wu and Yu 2000) and slow juvenile growth (Yu 1983; Yu and Lin 1999). Among the murids of Taiwan's low elevations, *N. coninga* is the only forest species (Wu and Yu 2000). Development of residential areas, farmlands, and transportation infrastructure potentially affect dispersal of *N. coninga*. This is because all records of *N. coninga* are from forests (Yu 1983; Chang-Chien 1989; Chang 1991; Tsai 1997; Wang et al. 2008). During the course of my graduate studies, I never trapped any *N. coninga* in urban, grassland, and farmland habitats (unpublished data).

In Chapter 2, I describe the land change history of Sanyi from 1904–2001, revealing the driving forces of landscape change and highlighting factors potentially affecting viability of wildlife. In Chapter 3, I evaluate the current habitat distribution of *N coninga* by habitat suitability modelling. I also developed a method to assess landscape connectivity for gene flow by incorporating habitat suitability modelling with population genetics. In Chapter 4, I examine the landscape genetics of *N. coninga* to reveal current spatial genetic structure and to map discontinuities in this structure. I identified landscape features affecting genetic structure and causing discontinuities by interpreting genetic data in light of data from landscape change, current land use, and habitat distribution.

Although the methods and results presented in this dissertation focus on *N. coninga* in the Sanyi Township, the framework should be applicable to species in other regions. The spatial pattern of *N. coninga* genetic structure and the causes of this structure can indicate challenges faced by rare animals in the fragmented landscapes of Taiwan.

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Chapter 2

Landscape changes from 1904–2001 in Sanyi Township, Miaoli County, Taiwan

This chapter has been submitted with the same title to the journal *Landscape and Urban Planning* by Yu-Huang Wang, Liang-Kong Lin, Kuoh-Cheng Yang, and Hsiung-Ming Liao

My use of "we" refers to my co-authors and me.

Abstract

Landscape history provides basic knowledge for landscape planning and wildlife conservation. Although the low elevations of Taiwan have been developed for over 350 years, modernization in the past century has completely changed low elevation ecosystems. To provide background information for regional conservation planning of the low elevations in western Taiwan, we analyzed land-cover maps digitized from historical maps and ortho-images for studing the patterns and processes of landscape changes from 1904–2001 in Sanyi Township, Miaoli County. Harvest of camphor trees (*Cinnamomum camphora*) and associated agro-forestry activities before 1940 almost eliminated original forests. Reforestation since 1960 greatly increased the structural connectivity of forests since 1904, with forests covering about 60% of the landscape in 2001. These forests, however, largely resulted from reforestation with monocultures of economically valuable trees (*Acacia confusa* and *Aleurites montana*). Modernization of the road system and residential development along roads since 1950 may have affected the landscape connectivity by blocking wildlife dispersal and gene flow.

Introduction

Studies of landscape changes are becoming more and more important for the changing world (Rindfuss et al. 2004; Bürgi et al. 2007; Turner et al. 2007). Landscape change history can provide essential knowledge for future landscape conservation and planning (Marcucci 2000; Antrop 2005; Lindenmayer et al. 2008). Many landscape change studies explain the driving forces and consequences of these changes (Coppedge et al. 2001; Fukamachi et al. 2001; Turner et al. 2003; Jomaa et al. 2008; Serra et al. 2008; Zomeni et al. 2008). Some studies use landscape indicators to evaluate ecosystem quality (Lathrop et al. 2007) or to monitor ecological changes in landscapes over time (Olsen et al. 2007). Because current landscape results from past land-use policies and land-cover conditions, landscape changes also affect current distributions of flora and fauna (Farina 2006). To understand processes shaping current landscapes and to plan for the future, we need to study landscape changes of the past through multiple data sources, e.g., oral history, historical maps, pictures, records, and aerial or satellite images (Petit and Lambin 2002; Bürgi et al. 2007).

The low elevations of western Taiwan have been developed over 300 years. Forest exploitation by the Han people during the Ching Dynasty (1684–1895) and during the Japanese colonial government (1895–1945) depleted natural forests at the low elevations of western Taiwan (Chen 1960). Agro-forestry activities and modernization over the past century greatly changed the original landscape. These events were documented in historical records (Editorial Board of Miaoli County History 1953, 1956; Chen 1959, 1960; Hong 1983). The locations and spatial extents of these events, however, were not completely or continuously mapped. Understanding the spatially explicit dynamic processes of land changes in this area is important for assessing economic development effects on landscape functions and for regional development and wildlife conservation decision-making.

Wildlife research and conservation in Taiwan has focused on the Central Mountain Range, where forests are more natural and continuous, resulting in neglect of the low elevations of western Taiwan (Pei and Chen 2006). Taiwan has eleven carnivore species, including two likely extinct species and four middle or high elevation species. The other five species are in low elevations (Chiang 2007). Pei and Chen (2006) used auto-cameras to survey the distribution of wild mammals in the low elevations of southern Miaoli County in west-central Taiwan. They found that carnivores in this area include all five low elevation species, the first four of which are rare: Formosan gem-faced civet (*Paguma larvata taivana*), small Chinese civet (*Viverricula indica pallida*), crab-eating mongoose (*Herpestes urva*), leopard cat (*Felis bengalensis chinensis*), and the Formosan ferret badger (*Melogale moschata subaurantiaca*). This area also contains the rare pangolin (*Manis pentadactyla*) and the first four low elevation carnivores (Pei and Chen 2006). Therefore, the low elevation forests in western Taiwan are important for wildlife. This area, however, suffers some human-wildlife conflicts. Wild animals are trapped or killed by poison in attempts to reduce damage to crops or poultry (Pei and Chen 2006). Around 2000, the tung-oil tree (*Aleurites montana*) blossom festival promotes tourism in Miaoli County (Council for Hakka Affairs 2007; Miaoli County Government 2008). Pressures from tourism and economic development are also causing habitat loss, increasing isolation among habitats, and probably suppressing survival of wildlife. These pressures include construction of new roads, widening of roads, increasing traffic speeds, and construction of tourism facilities, inns, and industrial districts (Miaoli County Government 2008).

To ameliorate human-wildlife conflicts and pressures from economic development in the low elevations of western Taiwan, it is important to know the distribution of forest habitat, landscape change history, and spatial locations of land changes over time. This information can be incorporated into planning for future regional development in sustainable way. Therefore, we studied the dynamics of landscape change in the Sanyi Township (including parts of adjacent townships) of southern Miaoli County to explain the effects of landscape history on landscape structure and function. We analyzed changes in landscape structure from 1904–2001 and identified processes shaping the landscape at each year, especially focusing on changes in spatial structure and connectivity of forest cover and locations of deforestation and reforestation. This information may be important for explaining current distribution and isolation of wildlife populations. Thus, it can help with conservation management and planning in this area.

Methods

Study area

The study area included most of Sanyi Township and parts of townships adjacent to Sanyi in southern Miaoli County, including Dahu, Tongluo, Tongsiao, and Yuanli (Fig. 2.1a). Colonization of Sanyi and Tongluo by the Han people dates back to the 1740s in the Ching Dynasty (Hong 1983). Early immigrants logged forests for fuel wood, extracted camphor from camphor trees (Lauraceae: *Cinnamomum camphora*), and cleared forests for fields of rice and tea (Hong 1983). Thus, camphor, tea, and rice were the main agro-forestry products of Sanyi during the Ching Dynasty (Hong 1983). During Japanese rule (1895–1945), logging of camphor trees continued, but the Taiwan Governor-General Office encouraged reforesting with camphor trees and acacia (*Acacia confusa*) and released lands for mass production of tea. Tea and charcoal were important products this time (Editorial Board of Miaoli County History 1953). With Kuomintang rule beginning in 1945, production of camphor ceased and tea plantations decreased. Lemon grass (*Cymbopogon* *citratus*) became an important cash crop, peaked, and dropped during the ten years from 1950–1960 (Chen 1959; Chen et al. 2001). Then there was a peak and decline in plantations of acacia and tung-oil trees (*Aleurites montana*), followed by land abandonment (Chen et al. 2001).

Beginning in the late Ching Dynasty and continuing through Japanese rule, the present Provincial Road No. 13 (then unpaved and 3 m wide) and railway were the main north-south traffic routes through Sanyi. Provincial Road No. 13 was blacktopped and broadened to 6 m in 1951 (Editorial Board of Miaoli County History 2003). In 2006, Provincial Road No. 13 was 4 lanes (14–20 m wide) with a speed limit of 60 km/hr and traffic of 21,416 cars/day (http://www.thb.gov.tw/download/Volume95.rar). Highway No.1 was opened in 1979, promoting industrial development in Sanyi Township (Editorial Board of Miaoli County History 2003). In 2006, Highway No.1 was 6 lanes (30–40 m wide) with a speed limit of 100 km/hr and traffic of 67,594 cars/day (http://www.freeway.gov.tw/Publish.aspx?cnid=187). The population of Sanyi was 5,861 in 1915, 9,629 in 1946, 16,951 in 1980, and 18,020 in 2000 (Editorial Board of Miaoli County History 1953, 2003).

Data sources

Due to poor definition of land-cover boundaries of maps from the 1920s–1930s and the fact that historical aerial images of 1940–1950 remain unavailable to the public, we only used base maps from 1904 and 1977 and digital ortho-images from 2001 to study the landscape change of Sanyi Township. The base maps (1:20000) of 1904, published by the Provisional Land Survey Bureau, Taiwan Governor-General Office during Japanese rule are the earliest available maps recording detailed land cover of Taiwan. The aerial photographic maps (1:5000) of 1977 were published by the Aerial Survey Office, Taiwan Forestry Bureau, Taipei. The Geographic Information System Team of the Computing Center, Academia Sinica, Taipei, Taiwan provided the scans of the 1904 and 1977 base maps and coordinate transformation software. Digital ortho-images (pixel size = 0.5 m) of 2001 were also published by the Aerial Survey Office. To map landscape changes, we transformed the coordinate system of each map onto the 1997 Taiwan land base (TWD97; http://www.moidlassc.gov.tw/satellite/english/Satellite/ESatellite_08.htm).

Digitizing land cover map

We classified land cover into sixteen types: forest, bamboo plantation, shrub land, cemetery, farmland (rice paddy and dry farmland), tea plantation, orchard, building, industrial district, railway, primary road (highway and provincial roads), secondary road (county roads), tertiary road (township roads and other paved small roads), unpaved road, water (river, pond, and reservoir), and bare land (exposed land and river bed). Because traffic and car speeds were lower in 1977 than 2001, we classified the 1977 provincial road as a secondary road. We digitized the land cover of 1904, 1977, and 2001 in ArcMap (ESRI, Redland, California) at fixed zoom scales: 1:5,000 for the 1904 maps and 1:2,500 for the 1977 and 2001maps. Considering the scale (1:20,000) of the 1904 historical map, we assumed a minimum mapping unit of 20 × 20 m^2 on the ground to match 1 × 1 mm^2 resolution on the map. To analyze landscape change, we converted the vector format of land cover maps to raster (Fig. 2.1b–d) with cell size of 20 m. Cell value was assigned to the dominant land cover type.

Changes of landscape structure and dynamics of land covers

We used FRAGSTATS 3.3 (McGarigal et al. 2003) to quantify changes in landscape structure from 1904 to 1977 to 2001 based on proportion (%), cover class area (ha), patch number (N), patch density (N/100 ha), and edge density (total perimeter/total area) of each land-cover type (McGarigal and Marks 1995; Leitão et al. 2006). We used three categories of landscape metrics to quantify configuration and connectivity of forest cover and effects of forest edge, respectively. Metrics of forest configuration are mean patch size (ha), median patch size (ha), mean gyration radius (m), clumpiness, and area-weighted mean shape (McGarigal and Marks 1995; Leitão et al. 2006). Metrics of forest connectivity are area-weighted mean

Euclidean nearest neighbor distance, and area-weighted mean proximity (within 100 m, 200 m, 500 m, and 1,000–2,000 m threshold distances; McGarigal and Marks 1995; Leitão et al. 2006). Metrics of forest edge effect are total core areas (after removing edges of 40 m, 80 m , and 120 m widths) and core area as percent of landscape (after removing edges of 40 m, 80 m, and 120 m widths; McGarigal and Marks 1995; Leitão et al. 2006).

To reveal the processes of landscape change, we calculated transition matrices among land-cover types for 1904–1977 and 1977–2001 with the cross-tabulation analysis of IDRISI ANDES (Eastman 2006). To show the spatial distribution of historical changes in forest cover, we used the Land Change Modeler of IDRISI ANDES (Eastman 2006) to map changes (loss, forested, and gain) of forest cover from 1904–1977 and 1977–2001 (Fig. 2.2a–b). These two change maps were used to generate a map of forest stages from 1904–1977–2001 (Fig. 2.2c) with the image calculator of IDRISI ANDES (Eastman 2006).

Results

Landscape structure and changes: 1904, 1977, and 2001

From 1904–1977 and 1977–2001, the main process of land change was reforestation as forest replaced shrub (Table 2.1; Fig. 2.1b–d; Fig. 2.3a–b, d–e). Patch number, patch density, and edge density of both cover types increased from 1904–1977, but decreased from 1977–2001 (Table 2.1). All metrics of farmland increased from 1904–1977, but decreased from 1977–2001 (Table 2.1). Increased agro-forestry activities (bamboo plantation, orchard, farmland, and shrub land) resulted in loss of 1904 forest (Figs. 2.2a and 2.3a–c). Increased agricultural activities also resulted in partial loss of 1904 shrub land (Fig. 2.3c). Since 1904, coverage, patch number, and edge density of bamboo plantation and orchard increased, but tea plantation decreased (Table 2.1). Some forest of 1977 was transformed by 2001 into shrub land, bamboo plantation, orchard, farmland, building, and industrial district (Figs. 2.2b and 2.3e). Partial forest of 2001 was transformed from shrub land, bamboo plantation, orchard, farmland, and tea plantation in 1977 (Figs. 2.2b and 2.3e). Coverage and edge density of buildings increased since 1904. Patch number and density of buildings increased from 1904–1977, but remained unchanged from 1977–2001 (Table 2.1). Industrial district appeared during 1977–2001. Unpaved roads disappeared after 1904 (Table 2.1). Secondary roads appeared by 1977 and primary and tertiary roads appeared by 2001 and (Table 2.1).

Changes in forest configuration and connectivity

Mean and median patch size, mean gyration radius, and clumpiness of forest cover were highest in 1904 and lowest in 1977 (Table 2.2). Area-weighted mean shape was highest in 1977 and lowest in 1904 (Table 2.2). Compared to 1977 and 2001, these four indices indicated that forest patches in 1904 were largest and most contiguous. Deforestation and reforestation from 1904–1977 created many small forest patches in 1977 (Fig. 2.2). Although deforestation and reforestation continued from 1977–2001, some reforestation connected forest fragments increasing patch size and compacting forest patches (Fig. 2.2). Compared to 1904 and 2001, core forest areas and percent of landscape in 1977 decreased drastically as edge width increased (Table 2.2), indicating that forest cover in 1904 and 2001 had larger, intact patches and was more resistant to edge effects. Area-weighted Euclidean nearest-neighbor distance was highest and proximities within 100–2000 m threshold distances were lowest in 1904 (Table 2.2), showing that forest patches were more isolated in 1904. With reforestation, distances between forest patches decreased as forest patches became more connected, resulting a high level of structural connectivity of forest cover in 2001 (Fig. 2.1).

Discussion

Human activities changed landscape structure and characteristics from 1904–1977–2001

Our analysis only includes maps from 1904, 1977, and 2001 and cannot reflect the momentous and profound changes that occurred between these dates. Forest cover in 1977 (Fig. 2.1c and 2.2b) was the result of many complex land conversions, mainly extensive reforestation with pure stands of acacia and tung-oil trees after 1950 (Fig. 2.4). Forest cover in 1977 was comprised of many small fragments (Tables 2.1 and 2.2; Figs. 2.1c and 2.2b). Although forest covered 58.6% of the landscape in 2001, the function of this landscape changed greatly compared to 1904 and 1977. This was because of the greatly increased human population (about three times that of 1915 and almost two times that of 1946; Editorial Board of Miaoli County History 1953, 2003) and the development of industry and modern road systems.

Original vegetation in the low elevations of western Taiwan was forest (Hong 1983; Keliher 2004) with trees in the Lauraceae, Moracea, and Fagaceae families (Chen 1960; Liu 1972; Su 1984). Of these trees, the native camphor tree was very economically important in early Taiwan. It is the source of distilled camphor oil and crystals. Forest exploitation in Taiwan by Han people at the end of the Ming Dynasty (1663–1683) targeted the camphor tree (Chen 1960). Large-scaled and haphazard logging of camphor trees and subsequent colonization of Taiwan by Han people during the Ching Dynasty (1684–1895) meant the original forests of central-west Taiwan's low elevations were largely destroyed by the time Japanese began governance of Taiwan (1895–1945), as evidenced by the 1904 base maps.

Thus, dominance of shrub land in 1904 (Table 2.1; Fig. 2.1b) resulted from logging for camphor trees and fuel wood and from clearing for farmland by Han people during the Ching Dynasty. Forests were limited to a few large interspersed patches (Table 2.1; Fig. 2.1b). Forest patches in 1904 were probably remnants of original forest as there was no management or reforestation policy until Japanese rule when the Taiwan Governor-General Office controlled harvest of camphor trees and began reforestation in 1900 (Fig. 2.4; Chen 1960). Farmland and tea plantations were the main land-cover types, representing the agricultural activities during the late Ching Dynasty (Table 2.1; Fig. 2.1b).

During Japanese rule, the Taiwan Governor-General Office encouraged governmental and private reforestation in 1900 and 1904 for sustainable exploitation of camphor trees and for other tree species, e.g., acacia (Fig. 2.4; Editorial Board of Miaoli County History 1953; Chen 1960). By 1941, the accumulated reforestation area of camphor trees in Taiwan was 47,000 ha (Chen 1960). From 1918–1924, the Taiwan Governor-General Office estimated that the stock of camphor trees in Taiwan could support an annual camphor production of 3,600 tons from 1924 to 1952 (Chen 1960). From 1939–1940, the Taiwan Governor-General Office surveyed each camphor trees in Taiwan, reporting that the stock volume was 2,690,000 m^3 and covered 694,250 ha (Chen 1960). By 1960, when Taiwan was governed by the Kuomintang party (1945–2000), camphor trees covered only 19,160 ha and all trees were < 60 years old (Chen 1960), indicating that original camphor trees were completely gone and that all remaining trees were from reforestation. In Sanyi Township, however, original camphor trees were almost exhausted by 1920 (Fig. 2.4; Editorial Board of Miaoli County History 1953) because of the continued exploitation of remnant forests after 1904. Production of camphor drastically decreased after 1945 and almost ceased by 1960 (Fig. 2.4; Chen 1960).

By 1941, the reforested area in Taiwan was 307,000 ha, largely of pure stands of acacia (Chen 1960). Acacia was an important tree for making charcoal and is easy to grow (Chen 1960). Charcoal production was important to Sanyi's economy under the mid–late Japanese rule (Figure 4; Editorial Board of Miaoli County History 1953). In Sanyi Township after 1920, intensive and extensive reforestation with acacia and plantings of tung-oil tree (*Aleurites montana*) replaced the shrub land resulting from camphor exploitation (Fig. 2.4; Editorial Board of Miaoli County History 1953).

As human population increased, coverage of farmland, orchards, and buildings also increased (Table 2.1; Fig. 2.3a). Other than farming, tea plantations and production of charcoal were the main agro-forestry activities causing deforestation during Japanese rule (Fig. 2.4). Large-scale plantations of tea in Sanyi began in 1915 (Fig. 2.4; Editorial Board of Miaoli County History 1953). Production of tea decreased by 1940 (Fig. 2.4) because of reduced exports during and after World War II and increased plantings of lemon grass (Chen 1960). Reduction in tea plantations (Fig. 2.4) and increase in orchards (Fig. 2.3a) showed how agricultural activity changed from 1904–1977.

After 1945, the Kuomintang government effected a change in land use. Mass plantings of lemon grass (Fig. 2.4) greatly reduced the forested landscape before World War II (Chen 1959, 1960). Lemon grass, the raw material for distilling citronella oil, was one of Taiwan's important cash crops in the 1950s (Chen 1959, 1960). In 1956, export of citronella oil at Keelung Harbor in northern Taiwan was 2,168 tons and worth 132.1 million New Taiwan Dollars (Chen 1959). Production of lemon grass centered on the Dahu, Jhuolan, Sanyi, and Tongsiao Townships of southern Miaoli County in western Taiwan. In 1952, the area under cultivation for lemon grass in Miaoli County was 19,950 ha, accounting for 77% of the total lemon grass cultivation in Taiwan (Editorial Board of Miaoli County History 1956; Chen 1959). In the Sanyi Township, the center of lemon grass production, plantations occupied 66% (ca. 2,600 ha) of arable land in the 1950s (Editorial Board of Miaoli County History 1956; Chen 1960). A flood on 7 August 1959 heavily damaged this area (Chen et al. 2001). Interviews with the elders of Sanyi Township (Chen et al. 2001) indicated that lemon grass plantations drastically declined after the 1959 flood, with farmers reforesting the hillsides with acacia and tung-oil trees (Fig. 2.4). By 1969, competition from lemon grass plantations in South-East Asia and the rise of the petrochemical industry quickly dropped the market price of lemon grass. Production in Taiwan waned.

After 1959, extensive reforestation of acacia supplemented with interspersed tung-oil tree plantations seemed to restore Sanyi's landscape to conditions of mid–late Japanese rule. Around 1975, however, the Kuomintang government encouraged mass planting of tung-oil trees (Fig. 2.4) for industrial use (Chen et al. 2001). Extracted tung-oil was used to paint wood and tung wood was used to make TV's cases and furniture (Chen et al. 2001). With the rise of the petroleum industry in 1970–80, acacia and tung-oil tree lost their economic value as charcoal was replaced by natural gas and tung-oil was replaced by petroleum byproducts (Chen et al. 2001). When Highway No. 1 opened in 1979, improved accessibility resulted in industrial development in Sanyi Township and adjacent areas (Editorial Board of Miaoli County History 2003). After the 1980s, decline in agro-forestry activities and associated land abandonment left acacia and tung-oil trees to grow unmanaged, dominating today's forested landscape (Fig. 2.4; Chen et al. 2001). Around 2000, the blossoming tung-oil trees during April and May attract large crowds of tourists to Miaoli County, resulting in development that promotes tourism (Council for Hakka Affairs 2007; Miaoli County Government 2008).

Forest habitat quality in 1904, 1977, and 2001

There were large continuous patches of original forest in 1904 (Table 2.2), making these forests the most suitable for forest interior species (Fig. 2.1b). In 1977, even though the amount of forest was greater, the forest was not suitable for forest interior species because it was broken into many small, isolated patches. After the 1980s, abandoned monocultures of acacia and tung-oil trees aggregated forest patches. Forest change maps (Fig. 2.2c) and landscape history (Fig. 2.4) show that monocultures of economically valuable trees occupied most of the forest landscape in 2001 through the complex deforestation and reforestation processes. In interviews, local eiders pointed out that Formosan wild boar (*Sus scrofa taivanus*) and Formosan Reeve's muntjacs (*Muntiacus reevesi micrurus*) lived in this area during Japanese rule, but are locally extinct for decades (Chen et al. 2001; Pei and Chen 2006). Monocultures of economically valuable trees replacing original vegetation and other disturbances related to landscape changes (Fig. 2.4) probably account for the local extinction of these two species. They generally have large home ranges and require large forest habitats (Pei and Chen 2006).

Landscape connectivity and development of road networks

Roads are notorious for their adverse effects on ecological processes (reviewed in Forman and Alexander 1998; Forman et al. 2003). In 1904, roads were unpaved and < 3 m wide. Road coverage in 1904 was probably over-estimated due to problems of map scale and mapping accuracy. Therefore, roads probably negligibly affected landscape connectivity in

1904 because of the relatively narrow road widths, low levels of traffic, and the slow speeds of animal-powered vehicles. Modernization of Provincial Road No. 13 began in the 1950s (Fig. 2.4). Increased north-south traffic, increased vehicle speed, and broadened road surfaces began to break east-west landscape connectivity. Animals may not succeed in crossing modern roads. High traffic and high vehicle speeds kill animals attempting to cross or generate noise that discourage crossing (Brody and Pelton 1989; Rolley and Lehman 1992; Forman and Alexander 1998; Inbar and Mayer 1999; Forman et al. 2003). Highway No. 1 opened in 1979 (Fig. 2.4) and parallels Provincial Road No. 13 (Fig. 2.1a). This greatly increased the effect of both road barriers. Buildings also developed along the roads (Fig. 2.1c). These, too, reinforced the barrier effects on east-west animal dispersal because of the accumulated risks of crossing parallel barriers (Forman et al., 2003) as well as areas of high human activity (Bashore et al. 1985). Modernization of county and township roads, beginning in the 1980s, further broke landscape connectivity (Figs. 2.1a and 2.4). Although, linear infrastructures such as roads occupy a very small proportion of the landscape (Table 2.1), their barrier effects can be severe (Forman and Alexander, 1998; Forman et al. 2003).

Area-weighted mean Euclidean nearest-neighbor distances and proximities to forest patches within 100–2,000 m threshold distances (Table 2.2) showed that forest connectivity was highest in 2001. These metrics are probably misleading because they only consider straight-line distances between forest patches and neglect effects of inter-patch environments on animals. Thus, they cannot reflect the barrier effects of modern linear infrastructure. Recent studies combining population genetics and landscape ecology show that transport infrastructures are barriers to animal dispersal and gene flow, resulting in loss of genetic diversity and population differentiation (Gerlach and Musolf 2000; Epps et al. 2005; Riley et al. 2006; Kuehn et al. 2007; Wang et al. 2008). Landscape genetics (Manel et al. 2003; Storfer et al. 2007) can help assess effects of modern linear infrastructures on landscape connectivity.

Conclusions

Tourism is the focus of Miaoli County's economic development policy (Miaoli County Government 2008). Our analysis provides basic information on land use, land changes, and their potential effects on wildlife. This information can be useful for tourism development in Sanyi Township. That recent economic development, especially increased road density and traffic volume, severely affects wildlife viability and landscape integrity is indicated by local extinction of two of Taiwan's common ungulate species within the past 60 years. Continuation of current trends will decrease the intrinsic value of local tourism. Incorporation of landscape change studies into other disciplines will generate useful information for local governments. Combining landscape history with landscape genetics can help identify the effects of recent development on wildlife populations. Landscape change study needs access to historical maps, aerial images, and information on landownership and regional planning. In Taiwan, however, these data are not usually opened to public, inhibiting studies of landscape change.

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Table 2.1 Selected landscape metrics for land-cover types in 1904, 1977, and 2001 in Sanyi Township and adjacent areas, Miaoli County, Taiwan

	Proportion (%)			Area (ha)			Patch number (N)			Patch density (N/100 ha)			Edge density (m/ha)		
Land-cover type	1904	1977	2001	1904	1977	2001	1904	1977	2001	1904	1977	2001	1904	1977	2001
Forest	30.8	55.7	58.6	3417.1	6182.3	6509.4	39	385	171	0.4	3.5	1.5	22.1	115.8	81.4
Shrub land	49.4	11.0	6.0	5490.0	1217.1	669.9	30	990	488	0.3	8.9	4.4	38.8	58.3	30.4
Cemetery	0.0	0.1	0.1	0.0	15.4	14.8	0	11	10	0.0	0.1	0.1	0.0	0.7	0.5
Bamboo plantation	0.2	2.0	6.2	21.0	225.9	690.5	12	138	329	0.1	1.2	3.0	0.8	9.1	26.6
Orchard	0.0	8.0	9.2	0.0	889.8	1020.1	0	549	429	0.0	4.9	3.9	0.0	38.3	40.0
Tea plantation	4.7	3.1	0.6	524.0	348.0	69.4	123	86	28	1.1	0.8	0.3	13.4	10.3	1.9
Farmland	5.1	10.1	5.2	562.8	1127.0	574.6	243	529	301	2.2	4.8	2.7	19.6	46.5	22.9
Building	0.6	1.4	3.3	71.5	154.4	364.2	319	617	617	2.9	5.6	5.6	5.3	11.4	18.9
Industrial district	0.0	0.0	0.9	0.0	0.0	103.5	0	0	5	0.0	0.0	0.0	0.0	0.0	1.5
Railroad	0.2	0.1	0.1	16.7	12.8	13.1	46	84	84	0.4	0.8	0.8	2.1	1.6	1.6
Unpaved road	0.9	0.0	0.0	101.2	1.5	0.1	432	24	2	3.9	0.2	0.0	12.4	0.2	0.0
Primary road	0.0	0.0	0.6	0.0	0.0	72.1	0	0	60	0.0	0.0	0.5	0.0	0.0	5.5
Secondary road	0.0	0.1	0.2	0.0	9.2	17.3	0	127	209	0.0	1.1	1.9	0.0	1.4	2.6
Tertiary road	0.0	0.0	0.5	0.0	0.6	53.0	0	13	601	0.0	0.1	5.4	0.0	0.1	7.7
Waters	0.1	1.4	1.3	12.9	155.2	139.8	26	112	169	0.2	1.0	1.5	1.3	6.3	7.4
Bare land	1.7	0.6	0.9	190.5	68.6	95.9	67	223	307	0.6	2.0	2.8	10.5	5.1	7.5

Table 2.2 Selected landscape metrics for 1904, 1977, and 2001 forest cover in Sanyi Township and adjacent areas, Miaoli County, Taiwan

Landscape metrics (unit)	1904	1977	2001
Mean patch size (± SD[1]; ha)	88 ± 223	16 ± 230	38 ± 308
Median patch size (range; ha)	8 (1156)	0.2 (4298)	0.5 (3425)
Mean gyration radius (± SD[1]; m)	271 ± 360	51 ± 214	86 ± 301
Clumpiness	0.95	0.77	0.83
AM[2] shape	3.5	25.6	16.0
AM[2] Euclidean nearest-neighbor distance (m)	106.8	40.7	40.4
AM[2] proximity within 100 m	5	3681	8435
AM[2] proximity within 200 m	27	3695	8463
AM[2] proximity within 500 m	42	3703	8542
AM[2] proximity within 1000–2000 m	61	3705	8543
TCA[3] – 40 m edge (ha)	2697	3194	4243
TCA[3] – 80 m edge (ha)	2049	1525	2705
TCA[3] – 120 m edge (ha)	1517	702	1744
TCA[3] – 40 m edge (% of landscape)	24	29	38
TCA[3] – 80 m edge (% of landscape)	18	14	24
TCA[3] – 120 m edge (% of landscape)	14	6	16

[1] Standard deviation

[2] Area-weighted mean

[3] Total core area after removing edge

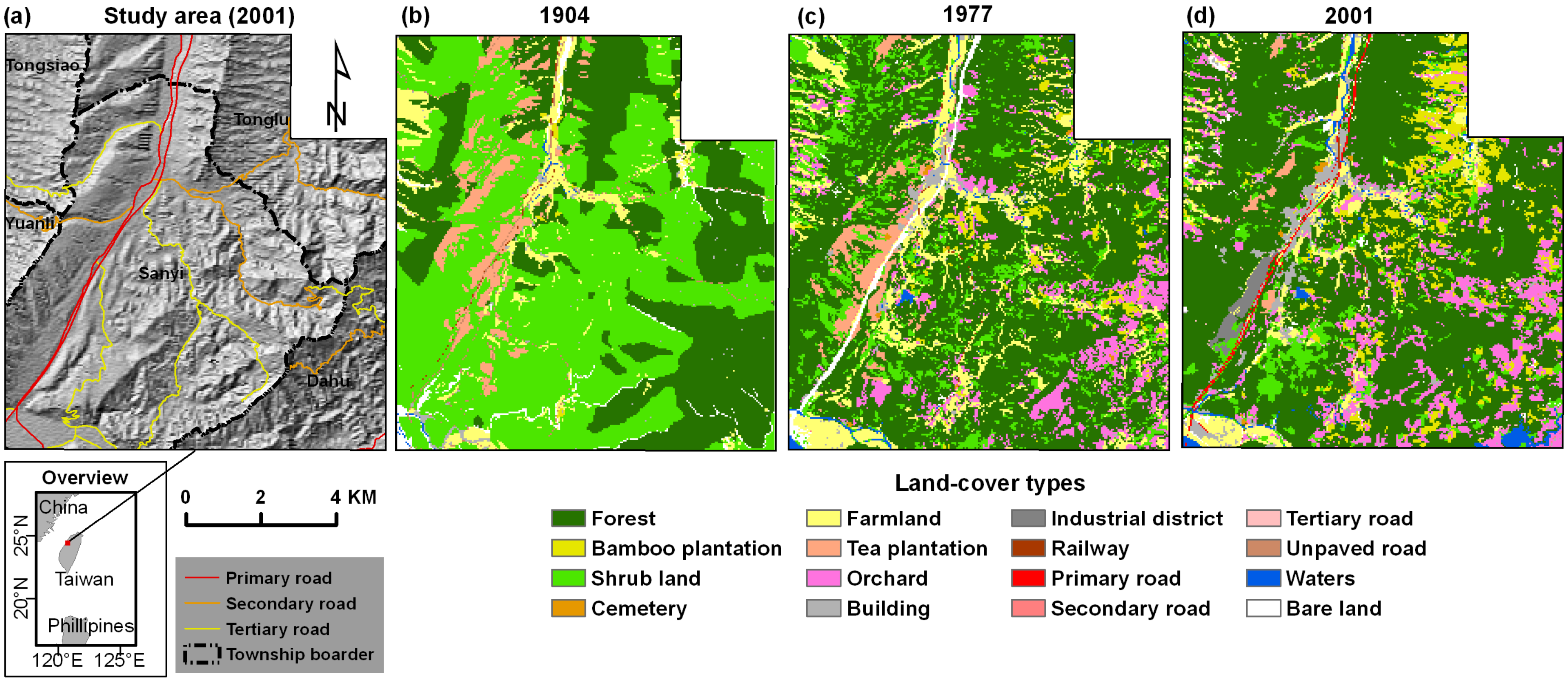


Fig. 2.1 Location and extent of study area (a) and land-cover maps of Sanyi Township and adjacent areas, Miaoli County, Taiwan, in 1904 (b), 1977 (c), and 2001 (d).

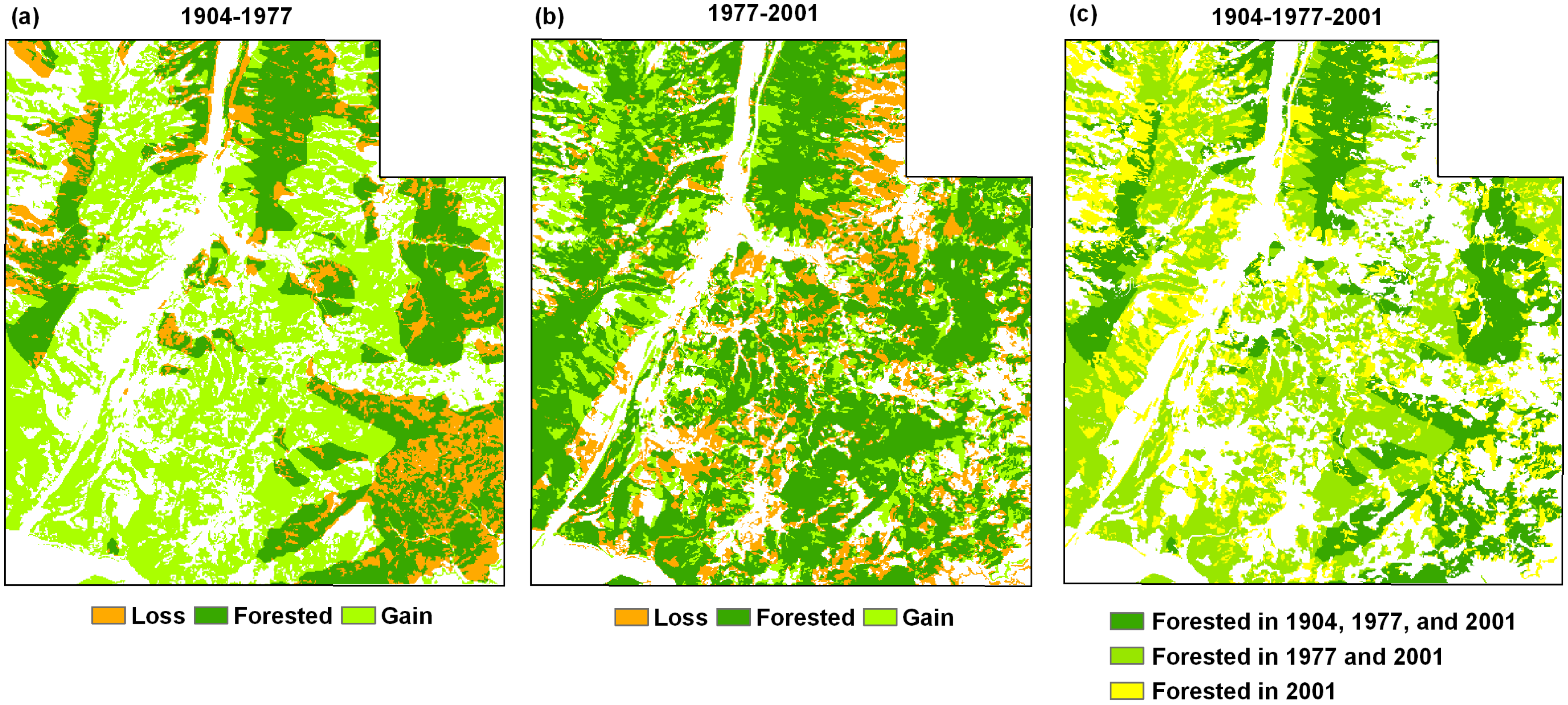


Fig. 2.2 Spatial distribution of forest-cover changes in Sanyi Township and adjacent areas, Miaoli County, Taiwan, from 1904–1977 (a) and 1977–2001 (b) and forest patches sustained over time from 1904–1977–2001 (c). White indicates non-forested areas.

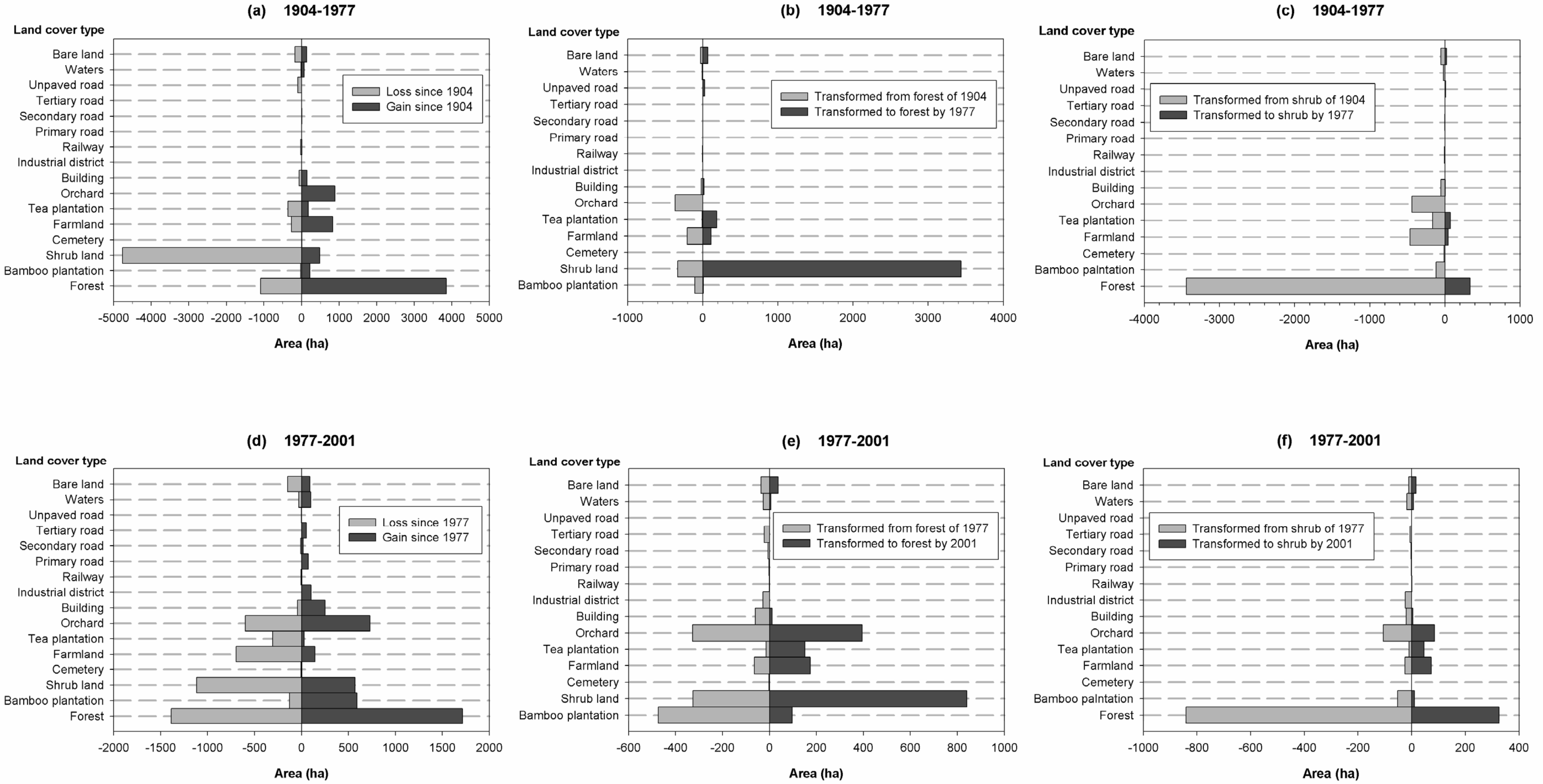
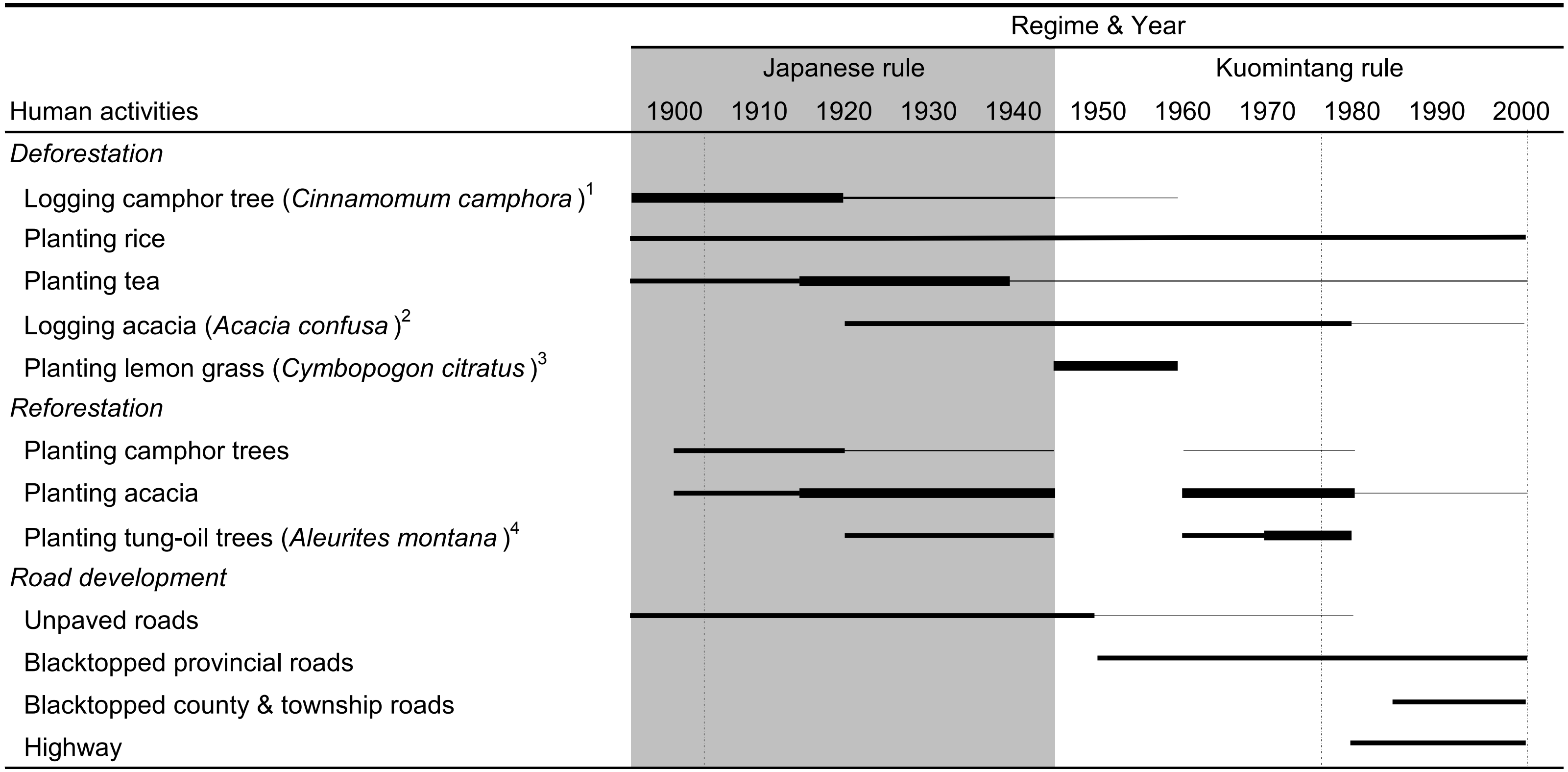


Fig. 2.3 Dynamics of land-cover changes (a, d), area of land-cover types transforming into and from forest cover (b, e) and shrub land (c, f) from 1904–1977 and 1977–2001 in Sanyi Township and adjacent areas, Miaoli County, Taiwan.



[1] Source of camphor

[2] For making charcoal

[3] Source of citronella oil

[4] For extracting tung oil

Fig. 2.4 Relative intensities of human activities driving landscape changes in Sanyi Township and adjacent areas, Miaoli County, Taiwan, from 1895–2000. Thickness of solid lines indicates intensity of each activity. Thin dashed lines indicate years of snapshots of landscapes analyzed in this study: 1904, 1977, and 2001. Data sources for this figure are Editorial Board of Miaoli County History 1953, 1956, and 2003; Chen 1959, 1960; and Chen et al. 2001.

Chapter 3

Habitat suitability modelling to correlate gene flow with landscape connectivity

This chapter is a lightly revised version of the paper which has been published
with the same title in *Landscape Ecology (2008) 23:989–1000*
by Yu-Huang Wang, Kuoh-Cheng Yang, Cara Lin Bridgman, and Liang-Kong Lin,
and can be accessed from *http://dx.doi.org/10.1007/s10980-008-9262-3*

My use of "we" refers to my co-authors and me.

Abstract

Landscape connectivity is important in designing corridor and reserve networks. Combining genetic distances among individuals with least-cost path (LCP) modelling helps to correlate indirect measures of gene flow with landscape connectivity. Applicability of LCP modelling, however, is reduced if knowledge on dispersal pathways or routes is lacking. Therefore, we integrated habitat suitability modelling into LCP analysis to avoid the subjectivity common in LCP analyses lacking knowledge on dispersal pathways or routes. We used presence-only data and ecological niche factor analysis to model habitat suitability for the spiny rat, *Niviventer coninga*, in a fragmented landscape of western Taiwan. We adapted the resultant habitat suitability map for incorporation into LCP analyses. Slightly increased Mantel correlations indicated that a class-weighted suitability map better explained genetic distances among individuals than did geographical distances. The integration of habitat suitability modelling into LCP analysis can thus generate information on distribution of suitable habitats, on potential routes of dispersal, for placement of corridors, and evaluate landscape connectivity.

Introduction

Landscape connectivity is important for animal dispersal and gene flow in fragmented landscapes (Verbeylen et al. 2003; Coulon et al. 2004; Spear et al. 2005; Vignieri 2005; Broquet et al. 2006; Stevens et al. 2006). In a continuous landscape, animal dispersal can involve back-and-forth routine movements to exploit different resources (Van Dyck and Baguette 2005; Baguette and Van Dyck 2007). Gene flow is usually restricted by distance, with individuals being genetically more related at shorter than at longer geographical distances (Wright 1943). In a fragmented landscape, animal dispersal shifts to faster, directed searches for suitable habitat or resource patches (Van Dyck and Baguette 2005; Baguette and Van Dyck 2007). Dispersal routes through the matrix are likely to include environments perceived by an animal as hospitable since dispersal risks or costs are increased in hostile environments (Baguette and Van Dyck 2007). Dispersal distance increases greatly when the dispersal route meanders through a fragmented landscape. Therefore, land use and habitat fragmentation affect landscape connectivity and potentially reduce gene flow (Berry et al. 2005). To provide information for designing reserve networks and planning dispersal corridors, landscape connectivity must be assessed.

Least-cost path (LCP) analysis is widely used to estimate landscape connectivity (Adriaensen et al. 2003; Verbeylen et al. 2003). Gene flow estimated through population genetics using highly polymorphic genetic markers, e.g., microsatellites or amplified fragment length polymorphisms (AFLPs; Lowe et al. 2004) helps to validate landscape connectivity modelled by LCP (European roe deer, *Carpreolus carpreolus*, Coulon et al. 2004; Pacific jumping mouse, *Zapus trinotatus*, Vignieri 2005; American marten, *Martes americana*, Broquet et al. 2006; desert bighorn sheep, *Ovis canadensis*, Epps et al. 2007). In these cases, the shortest path with the lowest accumulated cost of dispersing through different components in a landscape is traced between locations of two individuals or populations and the length of that path is calculated (Adriaensen et al. 2003; Verbeylen et al. 2003). Correlating effective landscape distances calculated from LCP analysis or straight-line geographic distances with genetic distances among individual tests whether genetic distance is better explained by LCP distance than by straight-line distance.

Before using LCP analysis, the cost or resistance of each landscape component must be weighted to generate a cost layer (Adriaensen et al. 2003). Assigning this cost, however, relies on expertise (Coulon et al. 2004; Vignieri 2005; Broquet et al. 2006; Epps et al. 2007) or experiments designed to estimate resistance values for each landscape component (Stevens et al. 2006). In studies with insufficient knowledge of dispersal or lacking experimental estimates of resistance, a solution is to repeat the LCP analysis with different combinations of cost values (Chardon et al. 2003; Verbeylen et al. 2003; Schweiger et al. 2004). Another way of weighting the cost of each landscape component might be achieved by using habitat suitability models or resource selection functions (Hirzel et al. 2006).

Habitat suitability is defined as the probability that a species uses a particular habitat. Models of habitat suitability or species distribution (Guisan and Zimmerman 2000; Guisan and Thuiller 2005; Pearce and Boyce 2006) can help select reserve networks (Tole 2006; Zielinski et al. 2006) and evaluate connectivity (Binzenhöfer et al. 2005), because these models predict the distribution of suitable habitats or resource patches in a landscape. The resultant suitability map gives the probability for each patch to be used by a given species (Boyce et al. 2002). The information in a suitability map is the distribution and configuration of resource availability for a given species in a given landscape. The cost for an animal to disperse through a landscape matrix partly includes mortality from predation and energy exhaustion (Baguette and Van Dyck 2007). The relative dispersal cost of a location can be reflected by resource availability, including cover for hiding from predators and food for energy replenishment during dispersal. Therefore, a habitat suitability map provides a basis for objectively generating cost layers for LCP analysis.

Habitat suitability models are based on the environmental characteristics of locations used or not used by the species in question: i.e. presence-absence or presence-only data. Models using presence-absence data are more accurate than models using presence-only data (Brotons et al. 2004). Presence-only data, however, are far easier to get from existing biodiversity databases, mainly because of the scarcity of reliable absence data (Boyce et al. 2002; Zaniewsky et al. 2002; Graham et al. 2004). Field studies using radio or global positioning system (GPS) telemetry and non-systematic surveys collect presence-only data precluding habitat suitability models requiring presence-absence data. To translate a habitat suitability map into a cost layer for LCP analysis, we must first evaluate the reliability of the habitat suitability model before assigning cost weights to suitability values based on objective criteria. Hirzel et al. (2006) proposed the k-fold cross validation procedure and used the continuous Boyce index to help evaluate habitat suitability models using presence-only data. This procedure produces a curve of the ratio of predicted/expected frequency of evaluation data (P/E) versus the mean suitability value of a suitability class. The P/E curve can serve as a more objective means for reclassifying habitat suitability maps (Hirzel et al. 2006) to estimate dispersal costs.

Because the applicability of evaluating landscape connectivity by incorporating a habitat suitability model into LCP analysis has not yet been assessed (Guisan and Thuiller 2005; Broquet et al. 2006), we used ecological niche factor analysis (ENFA, Hirzel et al. 2002) on the presence-only data of an endemic forest rodent, the spiny rat (*Niviventer coninga*), to model the distribution of habitat suitable for the rat in a fragmented forest landscape in western Taiwan. We evaluated this model by k-fold cross validation and the P/E curve (Hirzel et al. 2006). We then used the P/E curve to incorporate the habitat suitability model into LCP analysis. Finally, we correlated the LCP distances with the genetic distances among individuals. The latter were considered as indirect estimates of gene flow. Our objective was to test the usefulness of a habitat suitability model for assessing landscape connectivity.

Methods

Study area

The study area was ca. 10 × 10 km^2 and included most of the Sanyi Township in Miaoli County, western Taiwan (Fig. 3.1), at an elevation of 200–980 m above sea level. Mean January temperature was 15˚C, mean July temperature 28˚C, mean annual temperature 22˚C and annual precipitation 1,500–2,000 mm (http://www.sanyi.gov.tw/b/b01_3.htm). In 2004, residents in Sanyi Township numbered 17,990 (http://www.sanyi.gov.tw/b/b07_1.htm) and economic activities included agriculture, tourism, and industry. Heavy traffic on Highway No. 1 (6 lanes, traffic flow = 67594 cars/day, speed limit = 100 km/hr; http://www.freeway.gov.tw/02_01_06.asp) and Provincial Road No. 13 (4 lanes, traffic flow = 21,416 cars/day, speed limit = 60 km/hr; http://www2.thb.gov.tw/download/Volume95.rar) as well as adjacent residential areas separated the area's forests into a western and eastern part (Fig. 3.1). Forest cover of the entire landscape was about 60% in 2001 (Fig. 3.1). Other types of land cover (e.g., bamboo plantations, agricultural land, additional smaller roads and buildings) further fragmented the forest cover. Because the spatial pattern of forest cover was different in the western and the eastern part, effects of landscape connectivity on gene flow may be different within each part and/or the entire landscape.

Therefore, we assessed landscape connectivity by correlating genetic distances with LCP distances among individuals for each part separately as well as for the entire landscape.

Study species

The spiny rat, *Niviventer coninga*, is endemic to Taiwan. It is distributed in forests below 2000 m and common at 1,300 m above sea level (Yu 1994; Wu and Yu 2000; Wilson and Reeder 2005). It has a low population density, high winter mortality (Chang-Chien 1989; Chang 1991; Wu and Yu 2000) and slow juvenile growth (Yu 1983; Yu and Lin 1999). Among the murids of Taiwan's low elevations, *N. coninga* is the only forest species (Wu and Yu 2000). All records of *N. coninga* are from forests (Yu 1983; Chang-Chien 1989; Chang 1991; Tsai 1997; Y-H Wang, unpublished data). A pilot study (2002-2004, Y.-H. Wang, unpublished data) in west-central Taiwan did not trap *N. coninga* in urban, grassland, and farmland habitats. Thus, human land uses such as residential areas, farmlands and transportation infrastructure were expected to affect the dispersal of *N. coninga*.

Trapping

Since we had no prior information on the distribution of *N. coninga* in the study area, we placed trapping sites in accessible public and private land. From March 2005 to March 2006, we chose eight sites covered by forest and shrub in which we placed 758 mesh-wired live traps (Fig. 3.1 and Appendix 1). Neighbouring traps were 15–30 m apart. We recorded the geographical coordinate of each trap location using a global positioning system receiver (3–5 m accuracy without differential correction). We anaesthetised trapped *N. coninga* with isoflurane, recorded sex and body weight and individually marked each rat by toe clipping. Because amplified fragment length polymorphisms (AFLPs) DNA analysis demands large quantities of high quality DNA (Lowe et al. 2004), we augmented tissue from toes with snips of ear tissue or tail tissue if a rat had severely torn ears from fighting. Tissues were preserved in 99.9% alcohol.

Genetic analysis

We extracted genomic DNA using the MasterPure Complete DNA Purification Kit (Epicentre Biotechnologies), checked DNA quality and its concentration by a UV/Vis Spectrophotometer SSP-3000 (Infinigen Biotechnology). Extracted DNA was diluted to 50 ng/μL.

We followed the AFLP protocol of Vos et al. (1995) with some modifications. Combinations of EcoRI/MseI and EcoRI/Taq$^{\alpha}$I restriction enzymes digested genomic DNA. Two fluorophores, 6-FAM or NED, labelled the 5’ ends of the selective EcoRI primers. Conditions of pre-selective polymerase chain reactions (PCR) and selective PCR are described in the Appendix 2. We used twelve selective primer combinations (Appendix 3).

We analysed fragment lengths on a 3100 Genetic Analyzer with GS500 ROX (Applied Biosystems) as internal standard and GENESCAN (Applied Biosystems). GENEMAPPER 4.0 (Applied Biosystems) was used to identify and score peaks as present or absent within a fragment-length range of 75–500 bp. The peak-height threshold was set to 50 relative fluorescent units to exclude false peaks due to background noise. To reduce genotyping error, we randomly chose fourteen individuals and independently repeated the AFLP analysis from DNA extraction to peak scoring twice. Fragments with a reproducibility ≥ 86% (12/14) were retained. We identified a total of 172 polymorphic AFLP loci (Appendix 3) after dismissing loci with fragment frequencies higher than 95% or lower than 5%. GENALEX

(Peakall and Smouse 2006) was used to calculate pair-wise genetic distances between individuals: $D = N(1 - 2N_{xy} / 2N)$, where $2N_{xy}$ is the number of shared loci of two individuals and N is the total number of polymorphic loci (Huff et al. 1993).

Niviventer coninga presence data

We used a geographic information system to create a 20-m buffer around each point where a *N. coninga* was trapped. The buffered area (1,257 m^2) approximated Chang's (1991) report of *N. coninga* core home range (1,550 m^2). We transformed this buffer polygon layer into a raster of 10-m grid cells to match the resolution of SPOT5 satellite images and coded the cell value as '1' for cells within buffer polygons and as '0' for cells outside buffer polygons.

Environmental variables for modelling habitat suitability

We used twelve variables in the habitat suitability model, including vegetation growth, topography, and distance to various types of human land uses (Table 3.1). We used two scenes of cloud-free and geo-referenced SPOT5 satellite images of the study area (recorded 16 October 2005 and 30 March 2006, processed by the Center for Space and Remote Sensing Research, Central University, Chungli, Taiwan) to calculate a mean normalized difference vegetation index (NDVI; Bawa et al. 2002), which averaged the seasonal variation of NDVI in the study area to indicate vegetation growth of each location. We derived aspect and slope layers from a digital elevation model (DEM) created by the Aerial Survey Office, Forestry Bureau, Taipei, Taiwan. The DEM was resampled to 10 m with nearest neighbour without interpolation. Additionally, we used a land cover map (Y.-H. Wang, unpublished data) digitised from ortho-rectified images photographed in 2001 by the Aerial Survey Office, Forestry Bureau, Taipei, Taiwan, to calculate nine distance layers (Table 3.1). To match the resolution of SPOT5 satellite images, we transformed the polygon layer of the land cover map of the study area into a raster with 10-m cell size by the rule of maximum combined area of a cover type in one cell. All distance layers were calculated from this raster layer of the land cover map. Methods used to normalise the variables for factor analysis are listed in Table 3.1. Box-Cox transformation was a robust way to normalise most of these variables.

Habitat suitability model

We used ENFA (Hirzel et al. 2002) as implemented in BIOMAPPER (http://www2.unil.ch/biomapper/) to develop and validate a habitat suitability model for *N. coninga*. Based on the ecological niche concept, ENFA extracts factors of marginality and specialization to quantify the environmental variation of locations with the species compared to the environmental variation of the entire study area (Hirzel et al. 2002). Global marginality measures how the average environmental conditions of locations occupied by a species differ from the average of the entire study area (Hirzel et al. 2002). Tolerance, i.e., reciprocal of global specialization, measures the range of a species' distribution along environmental gradients (Hirzel et al. 2002).

We used an algorithm of geometric mean distance (Hirzel and Arlettaz 2003) to produce the habitat suitability map. To validate this model, we divided the presence data into 10 partitions with zero randomness for k-fold cross validation (Hirzel et al. 2006). We used the continuous Boyce index and a curve of the P/E ratio versus habitat suitability values (Hirzel et al. 2006) to evaluate model quality. The continuous Boyce index is the Spearman rank correlation of the P/E ratio with the habitat suitability value (Hirzel et al. 2006). If the P/E ratio increases with increasing habitat suitability (Fig. 3.2), the model has a good predictive ability (Hirzel et al. 2006).

Dispersal distances among individuals in a fragmented landscape

We used LCP modelling in ARCVIEW 3.2 (Environmental System Research Institute) and PATHMATRIX script (Ray 2005) to calculate the pair-wise LCP distances among individuals. Six cost layers (Cost 1–Cost 6) were used to model the least-cost dispersal paths among individuals. In the Cost 1 layer, each cell had the same cost value of one (Fig. 3.3a), assumed that landscape had no effect on the dispersal of *N. coninga*. This resulted in straight-line geographical paths among individuals. In the Cost 2 layer, the cost value of each cell was weighted between 1 and 101 by subtracting the habitat suitability value from 101 (Fig. 3.2; Fig. 3.3b). Since Hirzel et al. (2006) pointed out that uncertainty in a model may make continuous habitat suitability values too accurate to reflect actual habitat conditions, we produced Cost 3–Cost 5 layers by reclassifying the habitat suitability into 2–4 classes. For the Cost 3 layer, we reclassified habitat suitability (HS) into four equal interval classes (class 1: $0 \leq HS < 25$; class 2: $25 \leq HS < 50$; class 3: $50 \leq HS < 75$; class 4: $75 \leq HS \leq 100$) and gave each class a respective cost weight of 1,000, 100, 10, and 1 (Fig. 3.2; Fig. 3.3c). Hirzel et al. (2006) also suggested using the line P/E = 1 and steps in the P/E curve to define the class boundaries (Fig. 3.2). Hence, we produced Cost 4 by reclassifying habitat suitability into four classes according to the defined boundaries (class 1: $0 \leq HS < 16$; class 2: $16 \leq HS < 38$; class 3: $38 \leq HS < 60$; class 4: $60 \leq HS \leq 100$) and weighted cost of each class as 1,000, 100, 10, and 1, respectively (Fig. 3.2; Fig. 3.3d). We overlaid the land-cover map onto the habitat suitability map and found that most land-cover types had low mean suitability values (Appendix 4). Because our habitat suitability model used presence-only data recorded within or close to forest cover, it mainly reflected suitability of forest habitat. Therefore, we developed the Cost 5 and Cost 6 layers to examine landscape connectivity if habitat suitability above a threshold (Cost 5 layer: cost = 1 if HS $\geq$ 16 or cost = 1,000 if HS < 16; Fig. 3.3e) or if all forested patches (Cost 6 layer: cost value of forest = 1; cost value of all other land cover types = 1,000; Fig. 3.3f) were suitable for *N. coninga* dispersal. We modelled the among-individual LCP within each part and within the entire landscape to assess the landscape connectivity at two spatial scales.

Correlating indirect estimate of gene flow with landscape connectivity

Gene flow of *N. coninga* would be restricted by distance if dispersal is spatially homogeneous (Rousset 2004). To test whether indirect estimates of gene flow of *N. coninga* were correlated with landscape connectivity in western and eastern parts and the entire landscape, we used Mantel tests (9,999 permutations) in GENALEX (Peakall and Smouse 2006) to correlate pair-wise genetic distance matrices with logarithmic landscape distance matrices calculated from the six LCP models (Cost 1–Cost 6).

Results

A total of 134 *N. coninga* (49 females and 85 males) were trapped during 6,098 trap-nights, including four individuals trapped from pilot study in 2003 (Appendix 5). Because the range of body weight of adult *N. coninga* is 55–228 g (Yu and Lin 1999), we classified seven individuals with bodyweight $\leq$ 55 g as sub-adult (Appendix 5). Global marginality and tolerance for *N. coninga* in the study area were 0.745 and 0.279, respectively. The four marginality and specialization factors retained to calculate the habitat suitability map explained 87% of the variation in twelve environmental variables describing *N. coninga* distribution (Table 3.1). The marginality factor explained 60% of the variation (Table 3.1). Distance to forest, bamboo plantation, bare land, buildings and orchards contributed most to the marginality factor (factor scores ≤ -0.3 or ≥ 0.3; Table 3.1). Specialization factors 1–3 explained another 27% of the variation (Table 3.1). Distance to forest, bare land, buildings, bamboo plantations and water contributed most to specialization factors 1 and 2 (Table 3.1). Distance to farmland and orchards and the mean NDVI contributed most to specialization factor 3 (Table 3.1). The continuous Boyce index (window size = 20) was 0.888 ± 0.168. The P/E ratio increased with habitat suitability in an exponential way (Fig. 3.2).

Figure 3.3 illustrates the least cost paths traced from six LCP models (Cost 1–Cost 6) that one *N. coninga* could use to disperse to other locations with *N. coninga*. Mantel correlation in the entire landscape as well as within each of the two parts separately slightly increased. The LCP distances of Cost 4 exhibited the strongest correlation with genetic distances in entire landscape and in both parts (Table 3.2).

Discussion

HS model and characteristics of *N. coninga* habitat

Niviventer coninga had specific habitat requirements: forests and areas distant from human activities (factor scores ≤ -0.3 or ≥ 0.3 in Table 3.1). As shown by high global marginality (0.745) and low tolerance (0.279), suitable habitat was not common in the study area. Although *N. coninga* inhabits low elevation forests, its presence within these forests is hard to predict (Wu and Yu 2000; Wang, unpublished data). Dense shrub cover is an important habitat variable determining the species' habitat use and spatial distribution (Chang-Chien

1989; Chang 1991; Tsai 1997). For *N. coninga* nesting on ground or in trees, shrub cover may provide shelter and access to tree canopy (Chang 1991; Tsai 1997). According to our habitat suitability map, some habitats of low suitability were in the interior of large forest patches (Fig. 3.3b), even though *N. coninga* is considered a forest species (Wu and Yu 2000; Wilson and Reeder 2005). Dense shrub cover is unevenly distributed in canopy gaps within forests. This heterogeneous distribution may result in low capture rates at locations away from canopy gaps. The continuous Boyce index and P/E curve showed that our model reflected the general distribution of suitable habitats and indicated its high predictive ability.

However, to refine the model's predictive ability for forest interiors, we would need to incorporate micro-environmental variables characterizing complex three-dimensional forest structures at a landscape scale. Point surveys measuring height and coverage of shrubs under canopy cannot be extrapolated to the entire area without the help of remote sensing. Recent advances in remote sensing technology using light detection and ranging (LiDAR) promise to map forest structure accurately at the landscape scale (Zimble et al. 2003; Hyde et al. 2005; Nelson et al. 2005).

Landscape connectivity and *Niviventer coninga* gene flow

Niviventer coninga gene flow may be partially affected by fragmentation in Sanyi's forests. This is because the dispersal distance estimated from Cost 1 LCP model (Fig. 3.3a), which gave the same cost weight to all land cover types, had the lowest correlation with genetic distance (Table 3.2). Path distances of other LCP models (Fig. 3.3c-f; Table 3.2), giving different cost weights to each location according to habitat suitability, improved the correlations with genetic distance. The Cost 2 model (Fig. 3.3b) showed almost the same correlation as Cost 1 (Table 3.2), indicating that weighting dispersal cost for each location according to habitat suitability produced a contrast too weak to reflect the potential dispersal routes and did not have a better predictive ability than straight lines. Reclassifying habitat suitability into few classes (Fig. 3.2; Fig. 3.3) produced a better contrast of relative dispersal cost of each location, as indicated by the correlations of models Cost 3 to Cost 5 (Table 3.2; Fig. 3.3c-e).

When reclassifying a habitat suitability map it is important to determine thresholds of class boundaries based on the quality of a habitat suitability model as shown in Figure 3.2: an ideal model should have a linearly increasing trend and small uncertainty (low variance of P/E ratio) in the P/E curve (Hirzel et al. 2006). If a model has large uncertainty, it is impractical to reclassify habitat suitability into a large number of classes, because adjacent classes would have a similar P/E ratio. If a model's predictive ability does not linearly increase with habitat suitability, adjacent classes also have similar P/E ratios: these situations would erroneously weight different dispersal costs of adjacent classes with similar P/E ratios. Therefore, we determined class boundaries based on the line P/E = 1 and steps in the P/E curve (Hirzel et al. 2006) to weight classes in the Cost 4 model (Fig. 3.2). The correlation of genetic distance with the Cost 4 LCP distance was the highest of all models for both spatial scales, the entire landscape and its western and eastern parts (Table 3.2).

Because we used presence-only data sampled from forests or locations close to forests to build a habitat suitability model for *N. coninga*, our model mainly contrasted the habitat suitability with respect to forest cover (Fig. 3.3b). Although the study area was about 60% forest cover, the model showed that many small forest patches were unsuitable for *N. coninga* (small fragments in Fig. 3.3a are shown as unsuitable in Fig. 3.3b). When correlated with genetic distance, the landscape connectivity estimated by Cost 5 better explained estimated gene flow than the Cost 6, which assumed all forest patches were suitable. Among the six LCP models, the LCP distances in Cost 4 best explained estimated gene flow. This showed that reclassifying a habitat suitability map based on trends in the P/E curve helped to objectively weight the cost of dispersing through each location with given habitat quality. Although the area of a specific land cover type is not necessarily equal to the area of suitable habitat for a species (Lindenmayer et al. 2008), the distribution and amount of suitable habitat can be mapped through habitat suitability modelling to reclassify the suitability map.

Least-cost path analyses of previous studies had two main problems when assigning dispersal costs to land cover types. First, they did not consider patch size and shape. Expert opinions (Spear et al. 2005; Vignieri 2005; Broquet et al. 2006; Compton et al. 2007) or experiment-based resistance values (Stevens et al. 2006) give the same cost to a particular land cover type regardless of patch size, shape, and edge effects. Since small, complexly shaped patches may have low suitability due to edge effects, the relative cost of dispersing through these patches should be higher (e.g., small fragments of forest in Fig. 3.3a are shown as unsuitable in Fig. 3.3b-e). By including distance or proximity variables, habitat suitability modelling takes edge effects into account. A reclassified suitability map may hence more objectively reflect dispersal costs. The second problem with assigning dispersal costs is incorporating knowledge from other studies on the species' dispersal behaviour. This information may not be transferable from one area to another because of variation in environment, differences in landscape history, and local adaptation of animal behaviour. Because habitat suitability models are based on information of local environmental conditions and species presence (and absence) in the landscape of the study area, translating habitat suitability into dispersal cost can sidestep problems of patch size, patch shape, and transferring dispersal knowledge from other areas.

Table 3.3 correlated pair-wise genetic distances with landscape connectivity, even though they varied with respect to the mobility of the species studied and size of study area. The correlation coefficient of the genetic distance and Cost 4 LCP distance of *N. coninga*

(0.161; Table 3.3) was almost five times larger than that for the European roe deer (Coulon et al. 2004) and five times smaller than that for the natterjack toad (Stevens et al. 2006). Studies showing low correlation with landscape connectivity may result from the assumptions that genetic structure reflects gene flow without considering differential dispersal by sex, age, and social status (Broquet et al. 2006). It is also possible that landscape structure is not correlated with gene flow because of landscape history.

Advantages and disadvantages of ENFA in LCP modelling

We incorporated ENFA into LCP analysis to assess landscape connectivity for *N. coninga*. Factor scores from ENFA quantified the contribution of environmental variables to the habitat suitability model (Table 3.1). Understanding the importance of each environmental variable is necessary for habitat management, but such quantification is not available from other models using presence-only data (Sérgio et al. 2007). Furthermore, the P/E curve from k-fold cross validation (Hirzel et al. 2006) not only helped with describing the predictive ability of our model based on presence-only data, but it also provided an objective basis for reclassifying the habitat suitability map.

Using presence-only data, ENFA can overestimate suitable habitat (Hirzel et al. 2001; Pearce and Boyce 2006) and may be less accurate than models including absence or pseudo-absence data, e.g., generalized linear models (GLM; Hirzel et al. 2001; Brotons et al. 2004; Engler et al. 2004) and generalized additive models (GAM; Zaniewsky et al. 2002). In cases where reliable absence data are not available, pseudo-absence data may be acceptable, provided they are adequately generated. Researchers can more objectively select pseudo-absence data points from locations predicted as unsuitable by ENFA and then use these data for GLM (Engler et al. 2004; Chefaoui and Lobo 2007, 2008) or GAM. By including ENFA-generated pseudo-absence data and modelling approaches, GLM or GAM may also help to contrast habitat suitability and dispersal costs in all land cover types. We did not adopt this approach because validation of GLM and GAM using the method of area under curve (AUC) does not provide convenient and objective basis for reclassifying habitat suitability as compared with k-fold cross validation and P/E curve (Hirzel et al. 2006). Although the k-fold cross validation and P/E curve are applicable to validate GLM and GAM (Hirzel et al. 2006), the complexity of these techniques may prevent general users of geographic information systems from performing this validation procedure. Hence, a program imbedded in geographic information system software that automatically cross validates GLM or GAM and generates a P/E curve would greatly facilitate incorporation of habitat suitability modelling using presence/pseudo-absence data into LCP analysis.

Integrating habitat suitability model, LCP, and genetic analysis to conserve biodiversity in fragmented landscapes

The LCP analysis in a geographic information system is routinely used to assess landscape connectivity by modelling animal dispersal and corridor use. The effectiveness of these models can be validated by population- or individual-based genetic analyses (Coulon et al. 2004; Vignieri 2005; Broquet et al. 2006; Epps et al. 2007). If knowledge of a species' habitat preference and dispersal is lacking, LCP analysis loses effectiveness. This is because the costs of dispersing through a landscape matrix must be weighted subjectively. When collecting genetic samples, the collection locations can be mapped. If the result of random sampling, these presence data are useful for habitat suitability modelling, especially in areas lacking information of a species' distribution. Through habitat suitability modelling, it is possible to identify potentially suitable habitats not yet sampled within the study area.

This technique also allows quantification of a study species' ecological niche and habitat requirements (Guisan and Thuiller 2005). Here, we showed how habitat suitability modelling can be adapted for LCP analysis to assess landscape connectivity and validated the usefulness of LCP models by using landscape genetics.

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Table 3.1 Environmental variables for ecological niche factor analysis (ENFA) for *Niviventer coninga* in Sanyi Township, Miaoli County, Taiwan, including factor scores of marginality and specialization

				Specialization		
Environmental variable	Transformation	Value range	Marginality factor (60%)	Factor 1 (11%)	Factor 2 (11%)	Factor 3 (5%)
Mean NDVI[a]	None	-0.321–0.476	0.226	0.116	-0.161	-0.728[b]
Aspect	Square root	0.017–18.974	-0.038	0.039	-0.019	-0.054
Slope	Box-Cox	-1.789–16.721	-0.191	0.086	0.149	0.032
Distance to forest	Plus 0.5	0.5–475.9	-0.315[b]	-0.494[b]	-0.628[b]	-0.226
Distance to bamboo plantation	Box-Cox	0–30.641	0.414[b]	0.217	-0.389[b]	0.244
Distance to bare land	Box-Cox	0–52.756	0.301[b]	0.514[b]	-0.465[b]	-0.042
Distance to building	Box-Cox	0–44.568	0.442[b]	-0.477[b]	0.093	0.134
Distance to shrub	Box-Cox	0–34.395	0.103	-0.29	-0.04	-0.093
Distance to farmland	Box-Cox	0–30.412	0.276	-0.182	0.168	0.37[b]
Distance to orchard	Box-Cox	0–24.981	0.472[b]	-0.257	0.017	-0.315[b]
Distance to road	Box-Cox	0–31.564	0.123	0.096	0.076	0.26
Distance to water	Box-Cox	0–57.532	0.174	-0.043	0.38[b]	-0.153

[a] Mean normalized difference vegetation index (indicates vegetation growth)

[b] Indicates variables with factor scores ≤ -0.3 or ≥ 0.3

Table 3.2 Mantel tests of a pair-wise genetic distance matrix against six least-cost path (LCP) distance matrices (Cost 1 - Cost 6) among individuals of *Niviventer coninga* within the western and eastern parts and the entire landscape covering the Sanyi Township, Miaoli County, Taiwan.

	Mantel r (P-value)[b]		
LCP model[a]	Entire landscape	Western part	Eastern part
Cost 1	0.123 ($P < 0.0001$)	0.115 ($P = 0.0012$)	0.151 ($P < 0.0001$)
Cost 2	0.127 ($P < 0.0001$)	0.116 ($P = 0.0016$)	0.160 ($P < 0.0001$)
Cost 3	0.147 ($P < 0.0001$)	0.138 ($P < 0.0001$)	0.162 ($P < 0.0001$)
Cost 4	0.161 ($P < 0.0001$)	0.142 ($P < 0.0001$)	0.169 ($P < 0.0001$)
Cost 5	0.158 ($P < 0.0001$)	0.139 ($P < 0.0001$)	0.159 ($P < 0.0001$)
Cost 6	0.154 ($P < 0.0001$)	0.120 ($P = 0.0008$)	0.149 ($P < 0.0001$)

[a] Models defined in text and Figs. 3.2 and 3.3
[b] Mantel r is the Pearson correlation coefficient

Table 3.3 Mantel tests of genetic distances against least-cost path distances for selected species in fragmented landscapes

Species	*R*	*P*	Level of analysis	Study area (km^2)	Reference
European roe deer (*Carpreolus carpreolus*)	0.031	0.005	individual	2200	Coulon et al. 2004
American marten (*Martes americana*)	0.043	0.026	individual	800	Broquet et al. 2006
Spiny rat (*Niviventer coninga*)	0.161	< 0.0001	individual	100	This study
Land snail (*Cepaea nemoralis*)	0.374	0.007	population	16	Schweiger et al. 2004
Mountain vizcacha (*Lagidium viscasia*)	0.40	0.008	population	12000	Walker et al. 2007
Pacific jumping mouse (*Zapus trinotatus*)	0.420	0.029	individual	945	Vignieri 2005
Natterjack toad (*Bufo calamita*)	0.75[a]	0.036	population	100	Stevens et al. 2006

[a] Partial Mantel test

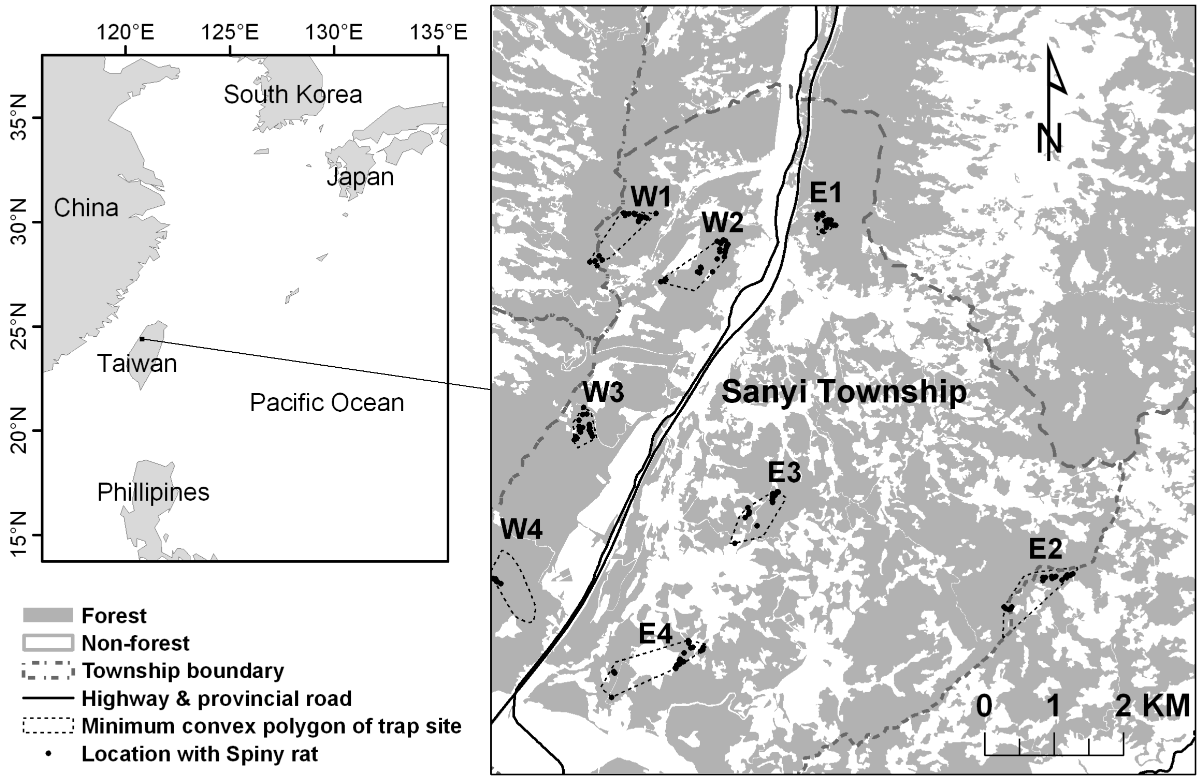

Fig. 3.1 Study area and trap sites of the spiny rat (*Niviventer coninga*) in the Sanyi Township, Miaoli County, Taiwan. Trap sites are numbered and labelled W or E depending on whether they were west or east of the highway and the provincial road

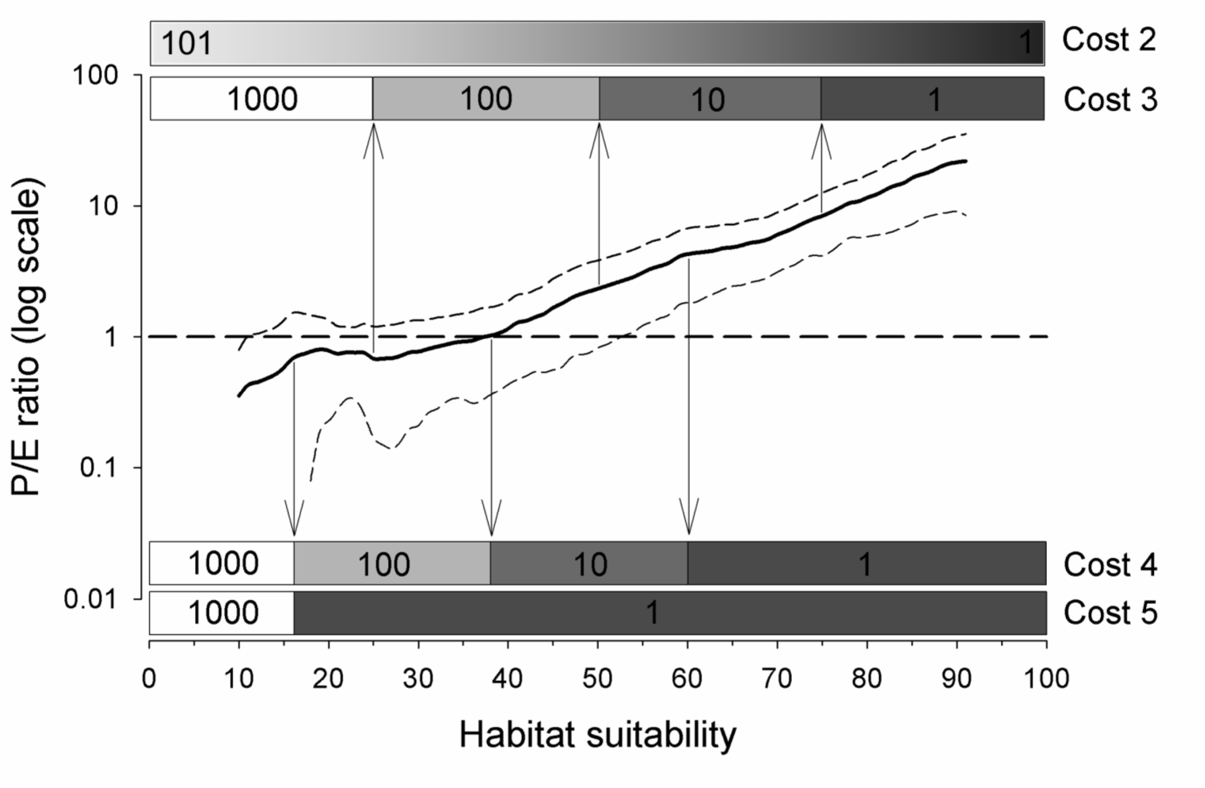

Fig. 3.2 The ratio of predicted/expected (P/E) frequency of evaluation data versus values of mean suitability. Trends in the P/E curve help with reclassifying habitat suitability and weighting costs. Solid line indicates mean P/E ratio. Short dashed lines are standard deviations of P/E. Long dashed line (P/E = 1) indicates a random model. Grey scale bars indicate four cost weighting methods (Cost 2–5) for each suitability value or class. Numbers in the bars indicate relative dispersal cost for each suitability value or class

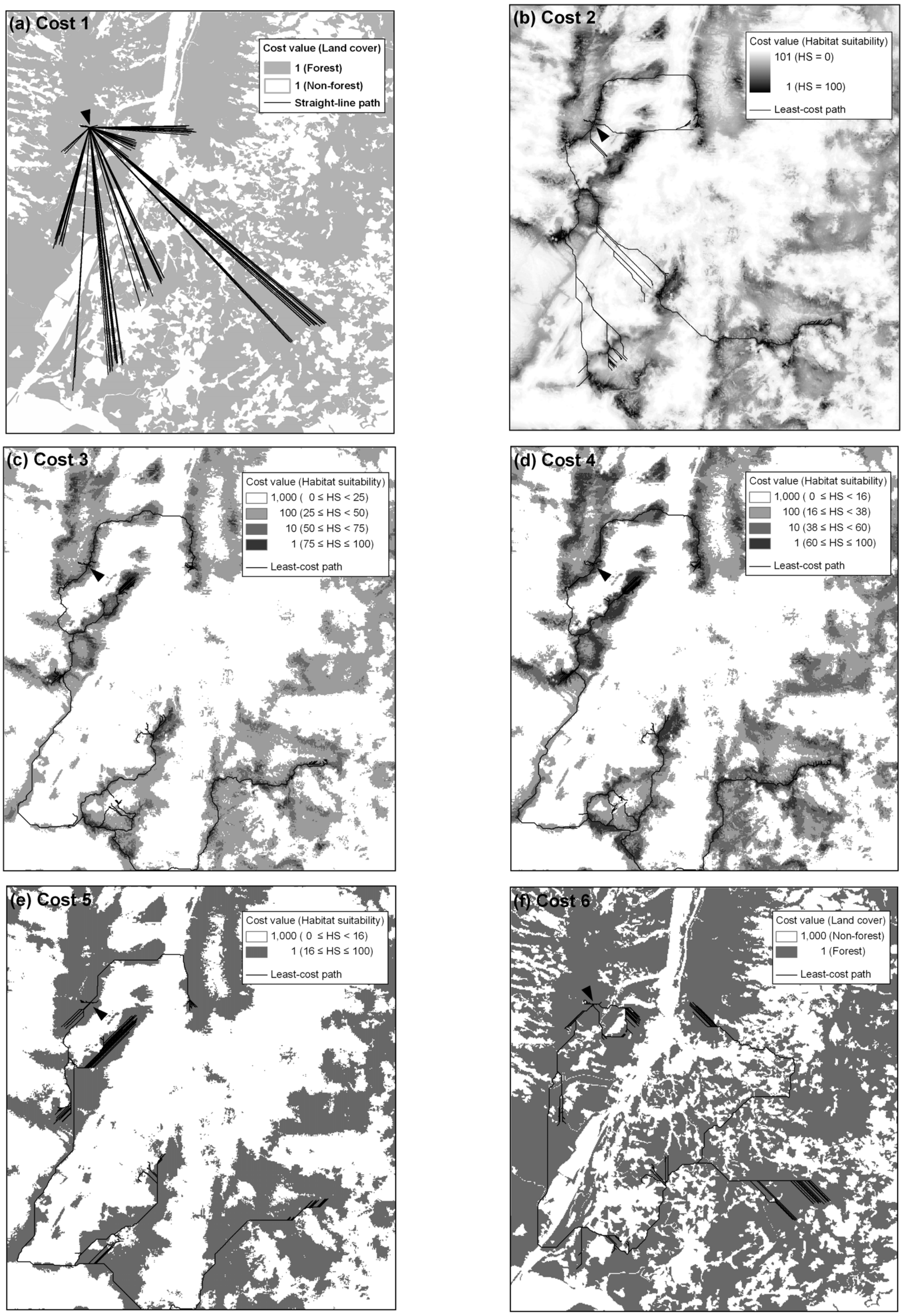

Fig. 3.3 Dispersal paths of a *Niviventer coninga* (arrow) to all others trapped in the Sanyi Township, Miaoli County, Taiwan, as delineated by least-cost path (LCP) models using six different cost layers: (**a**) Cost 1, forest and non-forest received same cost of one, resulting straight-line dispersal paths; (**b**) Cost 2, each cell received a cost between 1 and 101 that was opposite its habitat suitability (HS); (**c–d**) Cost 3 and Cost 4, cost value of 1, 10, 100, and 1,000 assigned to each of four HS classes; (**e**) Cost 5, cost value was one if HS $\geq$ 16 or 1000 if HS < 16; and **(f)** Cost 6, forest received a cost value of one and non-forest was 1000

Chapter 4

Landscape genetics of *Niviventer coninga* in Sanyi Township, Miaoli County, Taiwan

Abstract

Recent economic development, including development of road systems, breaks landscape connectivity and blocks gene flow, causing genetic differentiation among isolated populations. To mitigate or remedy barrier effects of economic development, the extent of genetic differentiation and landscape features causing that differentiation must be identified. To show how recent developed techniques in population genetics and spatial analyses can help resolve the problems, I studied the landscape genetics of the spiny rat, *Niviventer coninga*, as a model. Analysis of molecular variance ($\Phi_{RT} = 0.021$, $\Phi_{PR} = 0.036$, $P = 0.0001$) and pairwise Φ_{PT} (0.022–0.091, $P < 0.015$) indicated that primary and secondary roads and buildings along roads blocked *N. coninga* gene flow, resulting in genetic differentiation between regions and among sites within regions. Individual-based spatial autocorrelation analysis revealed significant spatial genetic structure (both sexes: r = 0.08–0.02, $P = 0.0001$) and male-biased dispersal (male: r = 0.058–0.029, $P \leq 0.001$; female: r = 0.112–0.062, $P = 0.0001$) in *N. coninga*. Land use and distribution of habitats within the landscape accounted for genetic boundaries detected by the Wombling method. Rare animal species in the study area may suffer severe genetic differentiation because of their relatively small population sizes. Methods and results of this study can help regional planning include measures to restore landscape connectivity for wildlife.

Introduction

Economic development fractures landscape connectivity, impeding dispersal or gene flow of local faunal and floral populations. Loss of habitat and landscape connectivity can cause different levels of genetic erosion, isolation, and extinction depending on life history traits and dispersal abilities (Vucetich & Waite 1998; With & King 1999). Modern road systems with wide road surfaces, high vehicle speeds, and large traffic volumes are notorious for their ill effects on wildlife and ecological processes, e.g., road kill, habitat loss, barrier effects, and reduced dispersal or gene flow (reviewed in Forman and Alexander 1998; Forman et al. 2003). Several studies of wildlife show that modern road systems reduce genetic diversity or block gene flow (Gerlach and Musolf 2000; Epps et al. 2005; Coulon et al. 2006; Riley et al. 2006; Kuehn et al. 2007).

Because of the barrier effects of roads, genetic drift of isolated populations and blocked gene flow among populations may create sharp genetic discontinuities among populations, i.e., genetic boundaries (Manel et al. 2003). Understanding spatial genetic structure of wildlife populations and mapping genetic boundaries are important for mitigating and remedying effects of human disturbances on wildlife. Landscape genetics helps resolve this problem because it uses highly polymorphic DNA markers to discern individual genetic variation and applies spatial analyses to reveal spatial genetic structure and map genetic boundaries (Manel et al. 2003; Storfer et al. 2007). Interpreting spatial genetic structures and genetic boundaries in light of data of land use and habitat distribution helps identify landscape features or barriers creating observed spatial structure and genetic boundaries (Coulon et al. 2006; Epps et al. 2005; Kuehn et al. 2007; Wang et al. 2008).

The low elevations in west-central Taiwan have been developed for > 350 years. The original ecosystem was of forest abundant with Fagaceae, Lauraceae, and Moraceae trees.

This was almost completely cut by 1945. Sanyi Township, Miaoli County is an example of original broadleaf forests replaced by forests of economically valuable but non-native trees. Subsequent land abandonment following reforestation increased forest coverage and connectivity (see Chapter 2). Development of modern road systems since 1950 and increased buildings along these roads are recent forces (see Chapter 2) that may create barriers and break landscape connectivity. To clarify effects of recent economic development on landscape connectivity and forest wildlife in Sanyi Township, I studied spatial genetic structure and identified genetic boundaries of an endemic forest rodent, the spiny rat (*Niviventer coninga*). I overlaid genetic boundaries of *N. coninga* on maps of forest change and habitat suitability to interpret effects of economic development on its population genetic structure and to identify landscape features potentially causing the genetic boundaries. This information can be useful for land use and landscape management policies for conserving wildlife in the low elevations of western Taiwan.

Methods

Study area, study species, and trapping

The study area (ca. 10 × 10 km^2) included most of the Sanyi Township and parts of adjacent townships in southern Miaoli County, western Taiwan. Forest covered about 60% of study area in 2001 (see center map in Appendix 1). Primary roads (highway and provincial) and adjacent buildings separate the study area into western and eastern regions (see center map in Appendix 1). Secondary and tertiary roads further subdivided each region. Wang et al. (2008) provide detailed information of study area.

The spiny rat (*Niviventer coninga*) is distributed in forests below 2000 m in elevation

(Yu 1994; Wu and Yu 2000; Wilson and Reeder 2005). This rat is the only murid species inhabiting the low-elevation forests of Taiwan (Wu and Yu 2000; Wang et al. 2008). Development of residential areas, farmlands, and transportation infrastructure potentially affect dispersal of *N. coninga*. This is because all records of *N. coninga* are from forests (Yu 1983; Chang-Chien 1989; Chang 1991; Tsai 1997; Wang et al. 2008). No *N. coninga* were trapped in urban, grassland, and farmland habitats (Y.-H. Wang, unpublished data).

I placed 758 mesh-wired live traps in eight sites, four sites in each western and eastern region (Appendix 1) and used a global positioning system receiver to record the geographical coordinates of each trap location. I recorded sex and body weight of trapped animals and marked each individual by toe clipping. Tissue for genetic analysis was preserved in 99.9% alcohol. Wang et al. (2008) describe the details of animal trapping and handling.

DNA extraction and genotyping

I extracted the genomic DNA of *N. coninga* with a DNA purification kit and checked DNA quality and concentration by a UV/VIS spectrophotometer (Wang et al. 2008). I genotyped 134 *N. coninga* by 172 amplified fragment length polymorphism (AFLP) DNA loci from twelve EcoRI/MseI and EcoRI/Taq$^{\alpha}$I primer combinations (Wang et al. 2008; Appendix 3). I checked genotype quality by independently running AFLP analysis two more times for 14 randomly chosen individuals (Wang et al. 2008).

Genetic analysis

Due to the small sample size (3 individuals) from site W4, I omitted these samples from subsequent genetic analyses. Total sample size for analysis was 131. To assess the effects of roads on population genetic structure of *N. coninga*, I used a hierarchical analysis of molecular variance (AMOVA in GenAlEx 6; Peakall et al. 1995) to estimate the total genetic variation partitioned into east-west regions, among sites within regions, and within sites. Three statistics, Φ_{RT}, Φ_{PR}, and Φ_{PT} (Peakall et al. 1995), respectively indicated levels of genetic differentiation between regions, among sites within regions, and within sites. I calculated with GenAlEx 6 (Peakall and Smouse 2006) pair-wise Φ_{PT} between any two sites to compare levels of inter-site genetic differentiation. Significance of population genetic structure was determined from 9,999 permutations. To show effects of north-south primary roads on east-west gene flow of *N. coninga*, I plotted pair-wise Φ_{PT} against inter-site distances (Fig. 4.1) and tested for significance of difference in pair-wise Φ_{PT} within- and between-region by exact Wilcoxon rank sum test in the exactRankTests package for R (Hothorn and Hornik 2006).

To reveal the spatial pattern of genetic structure of *N. coninga*, I analyzed spatial autocorrelations (Smouse and Peakall 1999) for each interval on Fig. 4.2 from 0–8000 m among individuals across the entire study area for each sex and both sexes (Fig. 4.2a–c). To evaluate difference in spatial autocorrelation between sexes, I did a heterogeneity test of the autocorrelation (Smouse et al. 2008) between sexes. GenAlEx 6 (Peakall & Smouse 2006) was used for spatial autocorrelation analysis and the heterogeneity test of the autocorrelation between sexes. Significance of spatial autocorrelation coefficient was determined from 9,999 permutations.

I used WOMBSOFT (Crida and Manel 2007) to identify genetic boundaries by computing systemic function grids (i.e., gradient surface of allele frequency) with the Wombling method (Cercueil et al. 2007; Crida and Manel 2007) and to test significance of genetic boundaries with the binomial test (Crida and Manel 2007). In computing systemic function, genetical bandwidth (h) must be set first. Genetical bandwidth (h) is a scalar determining the decay of distance weighting in estimating the allele frequency of each location (Cercueil et al. 2007). Determining optimal h is difficult because too small or too large a value will lead to over- or under-estimated local structures, respectively (Cercueil et al. 2007). Half distance range of spatial autocorrelation ≥ 0 may help to determine h because this range reflects the potential scale of spatial genetic structure. Therefore, I set h to approximate the half distance range of spatial autocorrelation ≥ 0 (600 m based on Fig. 4.2a). In detecting boundaries, I set percentile (p_b) = 0.3 for systemic function of grids as candidate boundary elements. I set *P*-value = 0.05 for significance level of candidate boundaries (Crida and Manel 2007).

Landscape data

Current genetic structure may result from past landscape change or current land use. To identify potential causes of the spatial genetic structure of *N. coninga*, I overlaid genetic boundaries on current road system layers, a forest change map (Fig. 2.2c in Chapter 2) and a habitat suitability map to produce Fig. 4.3. Wang et al. (2008) generated the habitat suitability map by ecological niche factor analysis (ENFA; Hirzel et al 2002) using presence data of *N. coninga* and 12 environmental variables of the study area.

Results

Analysis of molecular variance indicated slight genetic differentiation between eastern and western regions and among sites within regions (Table 4.1). Partial genetic variation existed between east-west regions and among sites within regions. Most genetic variation was within sites (Table 4.1). All inter-site genetic differentiations were significant (pairwise Φ_{PT}

= 0.022–0.091, $P < 0.015$). Mean $\pm$ standard deviation of between- and within-region pairwise Φ_{PT} were 0.054 $\pm$ 0.017 and 0.036 $\pm$ 0.011, respectively. Exact Wilcoxon rank sum test showed that between-region pairwise Φ_{PT} was greater than within-region ($W = 88$, $P = 0.015$), but inter-site genetic differentiation did not show a pattern of isolation by distance (Fig. 4.1).

Spatial autocorrelation analysis indicated significant spatial genetic structure for all *N. coninga* with positive correlations in the first four distance classes ($r = 0.08$–0.02, $P = 0.0001$; Fig. 4.2a). This positive spatial structure extended to 1,464 m ($r = 0$; Fig. 4.2a). There was no significant difference in overall autocorrelations between sexes ($\Omega = 25.823$, $P = 0.173$). Within 250–500 m distances, however, there was significant difference in the autocorrelation between sexes ($t^2 = 3.785$, $P = 0.051$, for 0–100 m distances; $t^2 = 0.957$, $P = 0.326$, for 100–250 m distances; $t^2 = 4.96$, $P = 0.027$, for 250–500 m distances; Fig. 4.2b–c). For females, positive autocorrelation sharply decreased by 488 m. Females only showed positive correlations at 0–100 m and 100–250 m ($r = 0.112$–0.062, $P = 0.0001$; Fig. 4.2b). Males showed significant positive spatial autocorrelations at 0–100 m, 100–250m, 250–500 m, and 500–1000 m ($r = 0.058$–0.029, $P \leq 0.001$). Positive spatial autocorrelation for males, however, extended to 2,449 m (Fig. 4.2c). This showed that the females had more limited ranges of positive autocorrelations than males.

I set a 600-m bandwidth (h) to compute systemic function to detect boundaries. Significant genetic boundaries are identified in Fig. 4.3. These boundaries did not conform to the spatial pattern of forested areas in 1904, 1977, and 2001. They seemed to conform to current road systems (Fig. 4.3a), especially primary and secondary roads. Primary roads outlined boundaries between east and west regions. Secondary roads corresponded with boundaries within each region (Fig. 4.3a). These boundaries also conformed to the habitat suitability map (Fig. 4.3b). In the west region, site W3 was separated from W1 and W2 by a secondary road. In the east region, sites E1 and E2 were separated from each other and sites E3 and E4 by secondary roads and the spatial distribution of suitable habitat patches (Fig. 4.3b).

Discussion

Roads and adjacent buildings as barriers to *Niviventer coninga* gene flow

Most genetic variation of *N. coninga* existed within sites but significant genetic differentiation between east and west regions and among sites within regions (Table 4.1) was caused by the current landscape structure. Greater inter-site genetic differentiation between regions than within regions (Fig. 4.1) supported primary roads and buildings along these roads blocking or reducing east-west dispersal and gene flow. This result is comparable to previous studies which show that road systems caused genetic differentiation in bank vole *Clethrionomys glareolus* (Gerlach and Musolf 2000) and roe deer *Carpreolus carpreolus* (Coulon et al. 2006; Kuehn et al. 2007) populations. For *N. coninga*, secondary roads also produced significant inter-site genetic differentiation within each region, even though their effect was less than that of primary roads (Fig. 4.1).

Inter-site genetic differentiation did not show the spatial pattern of isolation by distance. This also supported the conclusion that road systems blocked or reduced between- and within-region gene flow. Without gene flow, the driving forces of genetic differentiation among populations would be mutation, genetic drift, or bottleneck (Hartl and Clark 1997; Halliburton 2004). Since the modernization of roads in Sanyi Township began in 1950, separation among sites between east-west regions was not long enough (< 60 years) to accumulate mutations resulting in significant differentiation among sites. Genetic drift can cause significant differentiation within few generations if the effective sizes of isolated populations are small (Hartl and Clark 1997; Halliburton 2004). *Niviventer coninga* generally has low population density and high winter mortality (Chang-Chien 1989; Chang 1991; Wu and Yu 2000). Suitable habitat in the study area was limited (Fig. 4.3b). These factors may reduce the population size in each isolated habitat patch, initiating genetic drift to cause significant differentiation among sites instead of showing an isolation-by-distance spatial pattern. The landscape change of Sanyi Township is highly dynamic, resulting from the complex deforestation and reforestation processes of the past 100 years (see Chapter 2). Human activities accompanying this land change may include calamities for *N. coninga* populations, especially the spreading of rat poisons in fields and orchards to control plague and crop damage. These calamities may create bottlenecks in *N. coninga* that enhance any genetic differentiation among isolated sites.

Spatial genetic structure of *Niviventer coninga* as revealed by individual-based genetic analysis

Population-based genetic analyses, e.g., AMOVA and pairwise F_{st} (comparable to Φ_{PT}) depend on the subjective delineation of a population unit and may reflect historical instead of current gene flow (Manel et al. 2003; Neville et al. 2006). Individual-based methods do not need to define a population priori and can reveal current gene flow at fine spatial scales (Manel et al. 2003; Neville et al. 2006). Individual-based spatial autocorrelation analysis revealed significant spatial genetic structure not identified by population-based analysis (Fig. 4.2). That spatial autocorrelation declined as distance increased (Fig. 4.2) suggests a patchy distribution of genetically similar clusters (Diniz-Filho and Telles 2002) of *N. coninga* across the study area. The extent of positive spatial autocorrelation (1,464 m for *N. coninga*) can be used to define population or conservation units (Diniz-Filho and Telles 2002).

Sex-biased dispersal can be detected by spatial autocorrelation analysis. The expected philopatric sex should show higher positive spatial autocorrelation within shorter distance classes (Peakall et al. 2003; Hazlitt et al. 2004; Piggott et al. 2006). Dispersal in *N. coninga* is male-biased because females show higher positive correlation within shorter distances (Fig. 4.2b–c). The range of female genetic clusters is 488 m (Fig. 4.2b). The tendency to form genetic clusters was less strong in males, but the range extended to 2,449 m. These results were comparable to the fact that female *N. coninga* have smaller home range sizes than males (1,745 m^2 for female and 5,200 m^2 for male, Chang-Chien 1989; 3,911 m^2 for female and 4,471 m^2 for male, Chang 1991; 1,440 m^2 for female and 2,928 m^2 for male, Wu and Yu 2004). The short extent of positive spatial autocorrelation correlates with low dispersal ability (Bowman et al. 2000; Peakall et al. 2003). Therefore, female dispersal and gene flow may be more susceptible to habitat fragmentation.

Causes of *Niviventer coninga* genetic boundaries

Analysis of molecular variance and spatial autocorrelation analysis in this study revealed the intensity and spatial extent of significant genetic structure among sites and individuals. Genetic boundaries define the location and extent of each genetic cluster. By overlaying the genetic boundaries onto landscape data, it is easier to identify landscape features causing these boundaries. The spatial pattern of forest changes in Sanyi Township did not conform to the locations of these boundaries. These boundaries better conformed with the spatial pattern of current primary and secondary roads, supplying more evidence of road as barriers to gene flow (Fig. 4.3a). Road development improves accessibility for human, but it is also accompanied by additional disturbances, e.g., buildings and farms. Therefore, ascribing the cause of genetic boundaries to road systems seems an oversimplification. The habitat suitability map for *N. coninga* reflects the effects of human activities on the location of habitat patches (Fig. 4.3b; Wang et al. 2008). Overlaying these boundaries with road systems and habitat suitability map (Fig. 4.3b) identified major roads and adjacent structures (farms and buildings) as barriers blocking the gene flow and causing these boundaries.

Conclusions

I used *Niviventer coninga* as a model animal and combined genetic and landscape data to show that modern road systems and economic development along roads blocked gene flow of *N. coninga* and caused genetic discontinuities among sites. If the rare animals inhabiting the forests of Sanyi Township (Pei and Chen 2006) are unable to cross primary road systems and adjacent areas of intense human activity, they may suffer even more severe genetic differentiation than *N. coninga* in the long run because of their relatively small population sizes. My results indicate that wildlife conservation measures should include corridors that restore landscape connectivity. This is because *N. coninga*, the only forest-dwelling murid, may be an important food resource for forest predators. Wang et al. (2008) and this study can help indicate locations that may be suitable for corridors of *N. coninga* by predicting the distribution of suitable habitats and least-cost paths (potential dispersal routes). Least-cost paths that follow suitable habitat and that link separated genetic boundaries may be the suitable locations for corridors. By facilitating *N. coninga* dispersal, these corridors may attract species with similar habitat preferences and the predators that feed on it.

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Table 4.1 Analysis of molecular variance for *Niviventer coninga* in Sanyi, Miaoli County, Taiwan. Φ statistics are indices of genetic differentiation

Source of variation	d.f.[a]	Total variation %	Φ statistics	*P*-value
Between regions	1	2.116	$\Phi_{RT} = 0.021$	0.0001
Among sites/regions	5	3.557	$\Phi_{PR} = 0.036$	0.0001
Within sites	124	94.326	$\Phi_{PT} = 0.057$	0.0001

[a] Degree of freedom

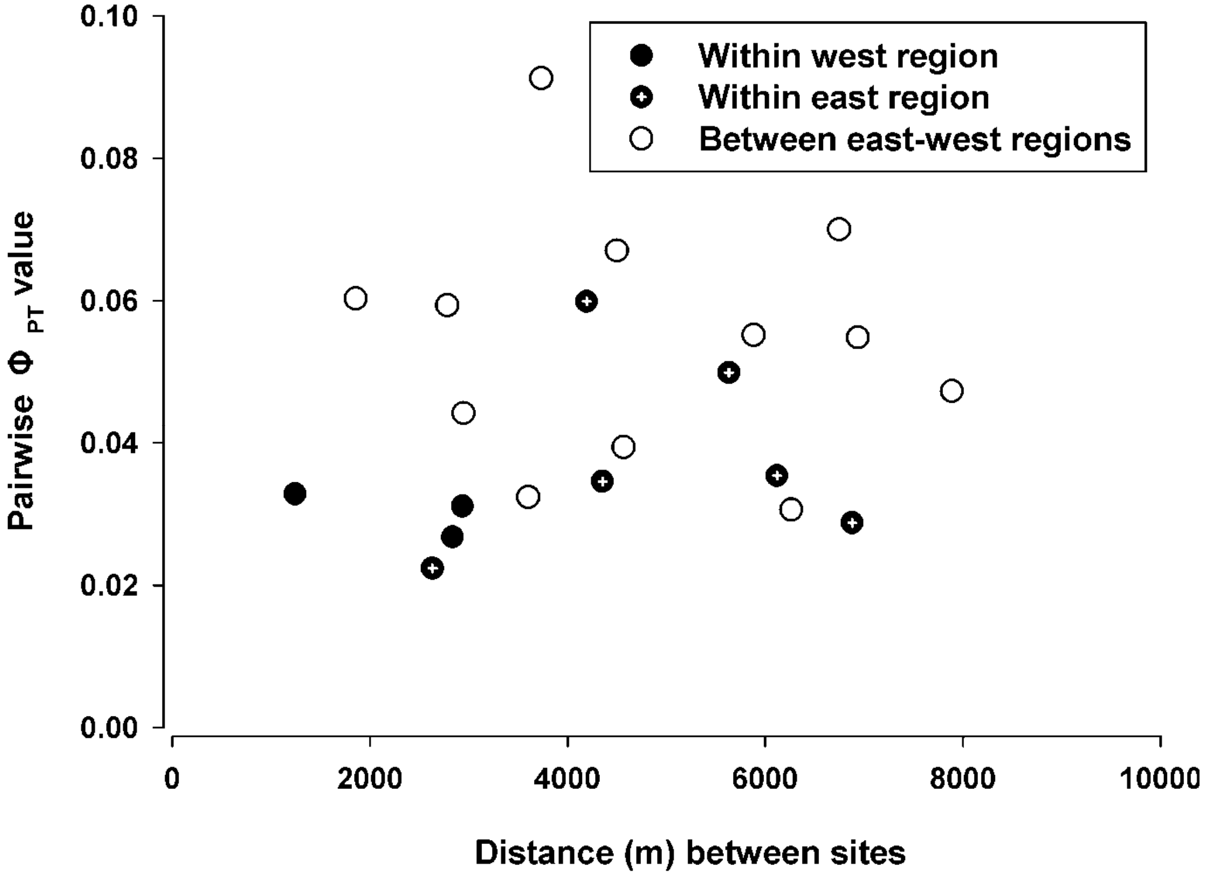
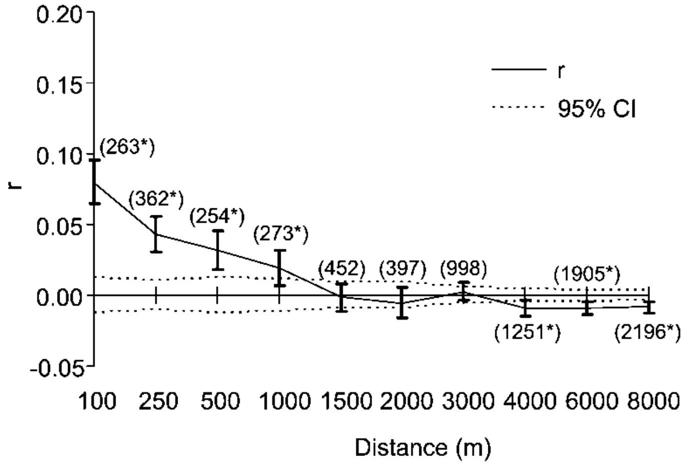
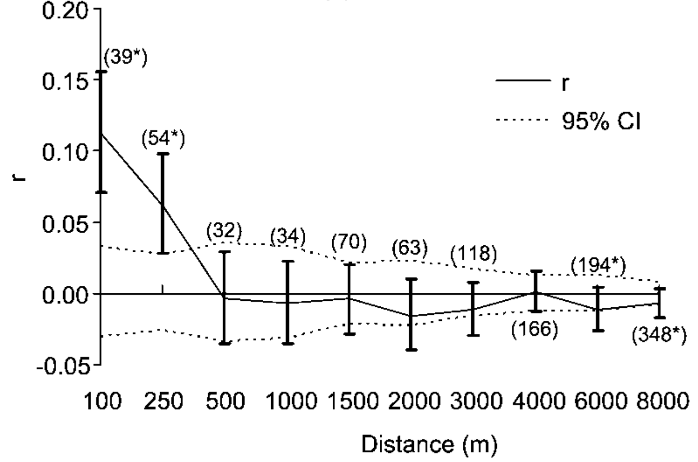


Fig. 4.1 Pairwise inter-site genetic differentiation (Φ_{PT}) in *Niviventer coninga* with respect to geographical distances between sites within east and west regions and between east-west regions in Sanyi Township, Miaoli County, Taiwan

(a) Both sexes



(b) Females



(c) Males

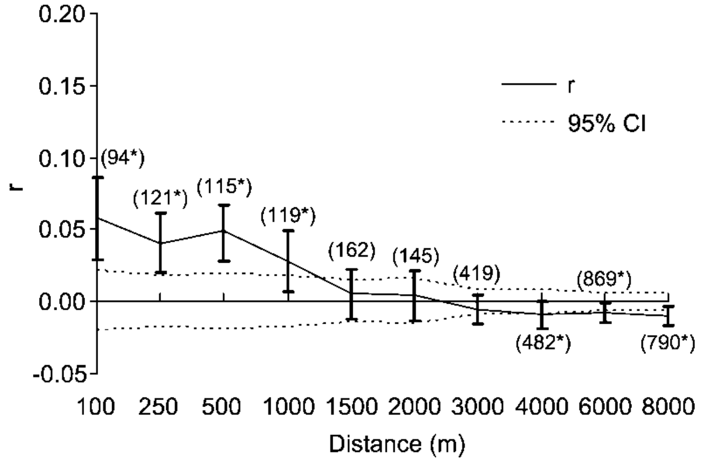


Fig. 4.2 Spatial autocorrelation in variable distance-class sizes for *Niviventer coninga* in Sanyi Township, Miaoli County, Taiwan: (**a**) both sexes, (**b**) females, and (**c**) males. Error bars are 95% confidence intervals (CI) of the correlation coefficient (r). Dotted lines show upper and lower limits of 95% CI of the null hypothesis for random distribution (r = 0). Number in parentheses is sample size of each distance class. Asterisks indicate significant (*P*-value <0.05) r value

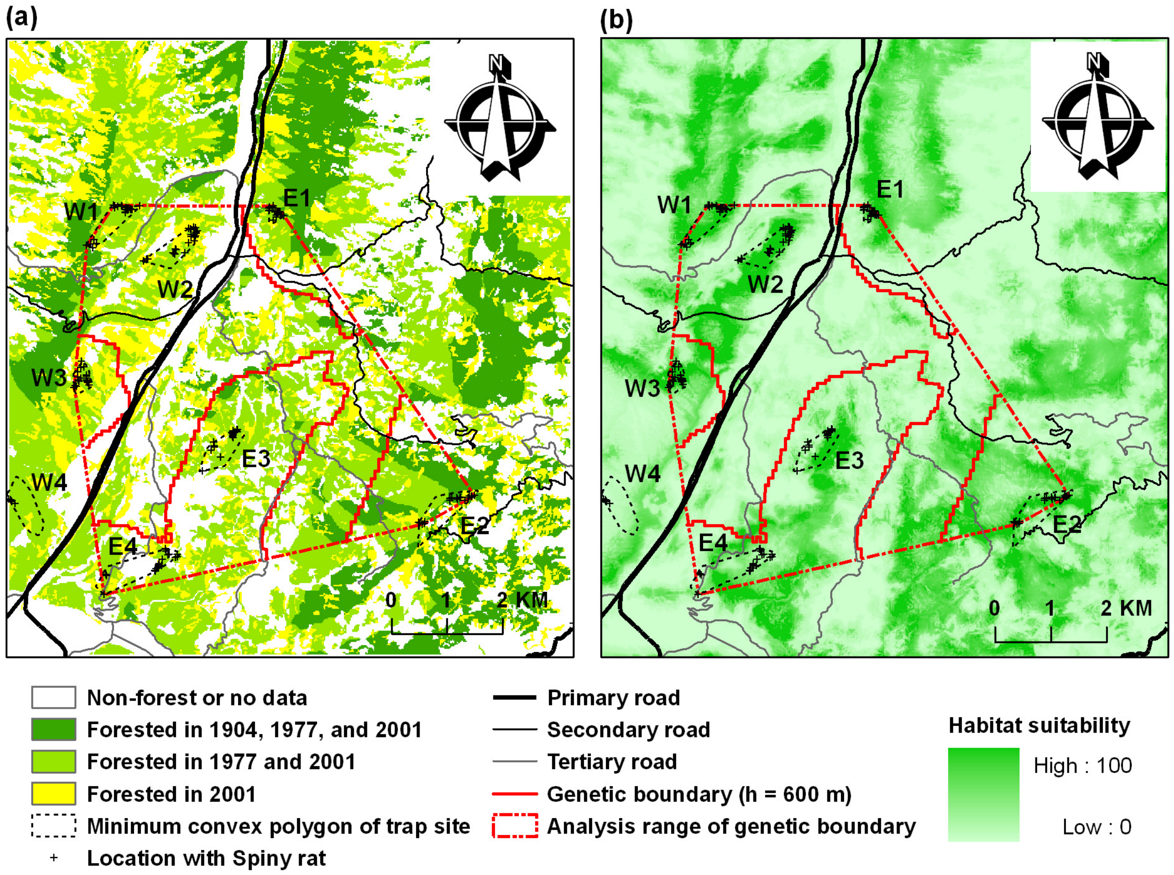


Fig. 4.3 Genetic boundaries of *Niviventer coninga* in Sanyi Township, Miaoli County, Taiwan, overlain on (**a**) forest change map of Chapter 2 and (**b**) habitat suitability map of Chapter 3

Chapter 5

Conclusions

Recent development in the disciplines of landscape change, habitat suitability modelling, spatial analysis, and population genetics (Manel et al. 2003; Rindfuss et al. 2004; Guisan and Thuiller 2005; Storfer et al. 2007; Turner et al. 2007; Jacquez et al. 2008) can help incorporate spatial and temporal dimensions important for understanding the effects of landscape change (Kienast et al. 2007). In this dissertation, I tried to integrate methods in these disciplines to study the effects of landscape change on the population genetics of wildlife in Taiwan's low elevations.

In Chapter 2, landscape history highlighted the potential effects of economic development on wildlife. Study of landscape change in Sanyi Township revealed that a complex deforestation-reforestation process associated with development of agriculture has changed the 1904 landscape of large interspersed remnant forest patches to the 2001 landscape dominated by monocultures of acacia and tung-oil trees. Although forest cover increased since 1904, the diversity of forest tree species has declined considerably. Since 1950, modernization of road systems and expansion of buildings along roads began to affect landscape connectivity.

In Chapter 3, incorporating habitat suitability modelling with least-cost path analysis avoided the subjectivity common in determining costs or frictions for animal dispersal in least-cost path modelling. Habitat suitability modelling revealed the effects of current land-use on the amount and distribution of suitable habitat for *Niviventer coninga*. Combining population genetics with habitat suitability modelling and least-cost path analysis helped evaluate how landscape connectivity affected *N. coninga* gene flow.

Study of population genetics in Chapter 4 revealed significant genetic differentiation among *N. coninga* populations. Integrating population genetics, spatial analyses (spatial autocorrelation and boundary detection), and landscape data (forest change, land use, and habitat suitability) evidenced that recent economic development affected population genetic structure and gene flow of *N. coninga*. Spatial autocorrelation analysis showed sexual dimorphism in *N. coninga* spatial genetic structure, suggesting that gene flow of females was more susceptible to breaks in landscape connectivity. I used the spatial extent of positive autocorrelations to help determine the bandwidth important to Wombling method of genetic boundary detection. Overlaying genetic boundaries onto maps of forest change, land use, and habitat suitability, confirmed that economic development over the past fifty years affected landscape connectivity and, thus, affected the spatial genetic structure of *N. coninga*.

This information indicates that other animals in Sanyi or in other regions of Taiwan already are confronting the same situation as *N. coninga*. This is probably worse for rare species unable to cross high traffic roads and areas of intense human activities. Recent development and the development scheduled in a white paper of the Miaoli County Government (2008) will worsen the situation. Regional development planning should find ways to mitigate or remedy the adverse effects of economic development on wildlife because priority local government is giving to tourism (Miaoli County Government 2008). Therefore, I recommend incorporation of greenway designs or corridors (Hellmund and Smith 2006; Hilty et al. 2006) into regional development projects. These activities will restore or improve landscape connectivity that not only conserves wildlife but also preserves the integrity of the landscape. Incorporation of wildlife and landscape conservation into regional development will eventually increase the intrinsic value of the area for tourism.

The framework of my research is applicable to other species and regions, but it costs much labor, time, and money. Trans-discipline cooperation will be the best use of research resources. For example, landscape genetic studies should cooperate with studies of landscape ecology and population genetics as suggested by Holderegger and Wangner (2006). To facilitate this type of research in Taiwan, increased access and availability of geographical information data is necessary. This includes historical maps, aerial photo images, landownership, digital elevation models, LiDAR data, and regional planning. Many of these data are important for landscape change studies and habitat modelling but are locked up in governmental database systems and unavailable to the public. This lack of access seriously restricts the potential of these studies and wastes precious research resources. On the other hand, any results of these integrated studies will be available to local governments, enriching governmental databases and supporting better planning and decision making.

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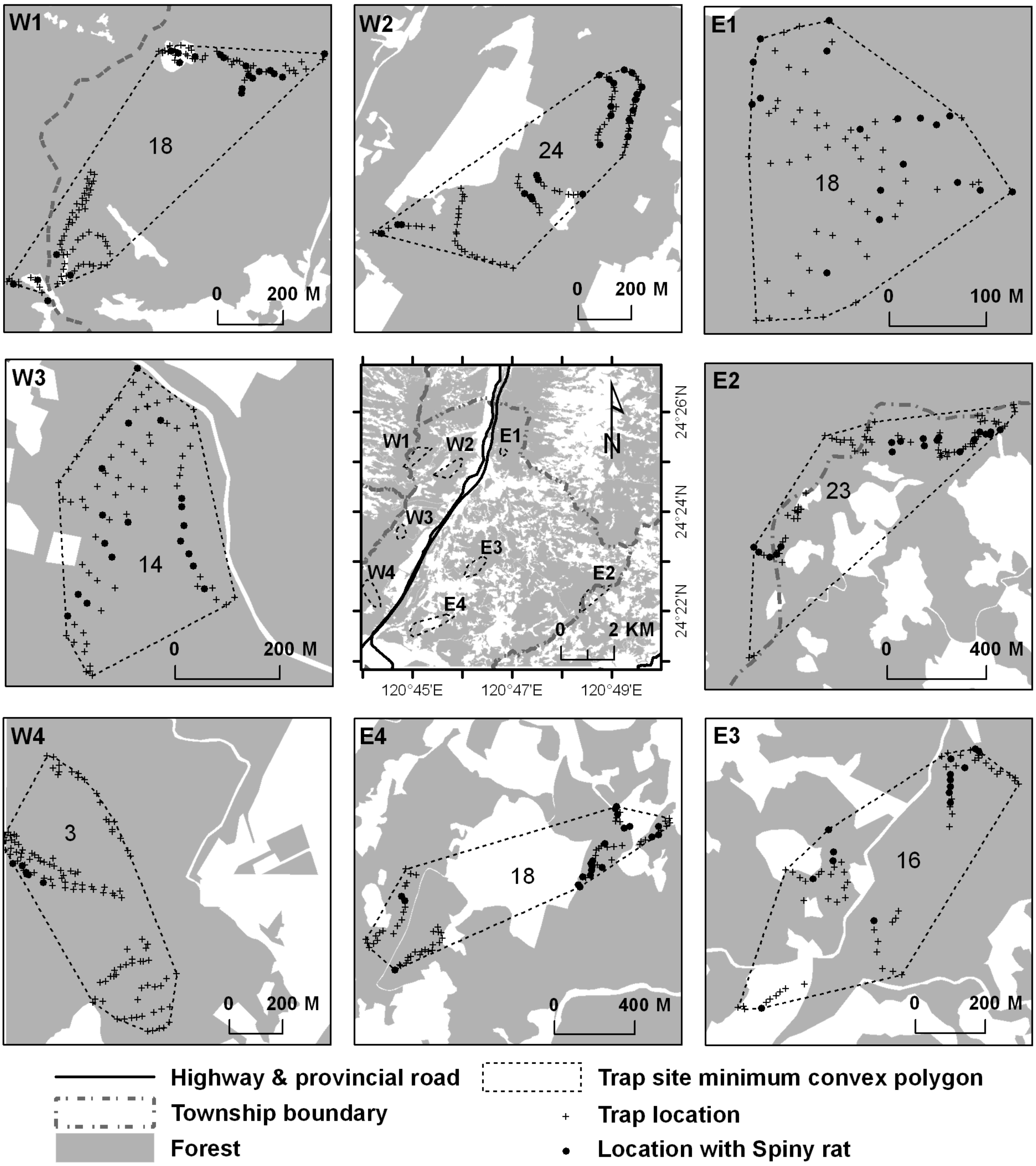
Appendices

Appendix 1 is the slightly revised version of Supplementary material S. 1 published in Landscape Ecology (2008) 23:989–1000



Appendix 1 Study area (center map) of *Niviventer coninga* in Sanyi Township, Miaoli County, Taiwan, and eight trap sites (surrounding maps) named W1–W4 and E1–E4). Number in minimum convex polygon indicates sample size of each trap site; white indicates non-forest

Appendix 2 is the slightly revised version of Supplementary material S. 2 published in Landscape Ecology (2008) 23:989–1000

Appendix 2 Protocol of AFLP analysis

We modified the AFLP protocol from http://ravel.zoology.wisc.edu/sgaap/AFLP_html/AFLP_protocol.htm (accessed 8 March 2005). We used EcoRI/MseI and EcoRI/Taq$^{\alpha}$I (New England Biolabs Inc.) restriction enzyme combinations to digest *N. coninga* genomic DNA. In the EcoRI/MseI restriction, 2 μL (100 ng) of DNA was digested with 5 units of each enzyme in 8 μL buffer containing 1X NEBuffer 2 (New England Biolabs Inc.) at 37°C for 3 hours followed by 65°C for 10 minutes. In EcoRI/Taq$^{\alpha}$I restriction, 2 μL (100 ng) of DNA was digested with 5 units of EcoRI enzyme in 3 μL buffer containing 1X NEBuffer 3 (New England Biolabs Inc.) at 37°C for 3 hours followed by 65°C for 10 minutes. This restriction mixture was further digested with 5 units of Taq$^{\alpha}$I enzyme in 5 μL buffer containing 1X NEBuffer 3 and 1X BSA (New England Biolabs Inc.) at 65°C for 3 hours followed by 80°C for 10 minutes. The restriction mixture from each restriction combination was ligated with corresponding adaptors at 16°C overnight in 10 μL ligation buffer solution containing 200 units of DNA ligase (New England Biolabs Inc.), 37.5 pmole of each corresponding adaptor, and 1X T4 DNA ligase reaction buffer. Ligation mixture was diluted to 100 μL with sterilized pure water for pre-selective PCR.

From the diluted ligation mixture, 5 μL was added into 20 μL of polymerase chain reaction (PCR) pre-mixed solution containing 0.5μL of deionized formamide, 1X PCR buffer (containing 2 mM $MgSO_4$), 0.5 μL of dNTP (10 mM each, New England Biolabs Inc.), 7.5 pmole of EcoRI primer, 7.5 pmole of MseI (or Taq$^{\alpha}$I) primer, and 1.25 units of Taq DNA polymerase (New England Biolabs Inc.). Pre-selective PCR used four primer-pair combinations (E-A/M-G, E-A/M-C, E-A/T-G, E-A/T-C). The PCR profile was initiated with 2 minutes of extension at 72°C followed by 20 cycles of 50 seconds at 94°C, 1 minute at

56°C, and 2 minutes at 72°C. Then, 5 µL of PCR product was diluted to 95 µL with sterilized pure water for selective PCR.

From the diluted pre-selective PCR product, 5 µL was added into 20 µL of selective PCR pre-mixed solution containing 0.5 µL of deionized formamide, 1X PCR buffer (containing 2 mM $MgSO_4$), 1 µL of dNTP, 5 pmole of fluorophore-labeled (6-FAM or NED, Applied Biosystems) selective EcoRI primer, 25 pmole of selective MseI (or Taq$^{\alpha}$I) primer, and 1.25 units of Taq DNA polymerase. All selective primers were purified by high performance liquid chromatography (HPLC). The PCR profile was initiated by 1 minute of denature at 94°C followed by 9 touchdown cycles of 50 seconds at 94°C, 1 minute at 65-57°C, and 2 minutes at 72°C, then 20 cycles of 50 seconds at 94°C, 1 minute at 56°C, and 2 minutes at 72°C. The final extension step, 10 minutes at 72°C, was to increase the specificity of amplified fragments.

Appendix 3 is the Supplementary material S. 3 published in Landscape Ecology (2008) 23:989–1000

Appendix 3 Polymorphic loci scored from twelve primer combinations in amplified fragment length polymorphisms (AFLP) analysis of 134 *Niviventer coninga* from Sanyi Township, Miaoli County, Taiwan

Primer combination	Fluorophore label	Polymorphic loci (N)
E-AAA/T-GAA	6-FAM	15
E-AAA/T-GAG	6-FAM	19
E-AAA/T-CAG	6-FAM	21
E-ACA/T-GAA	6-FAM	13
E-AAA/M-CCG	6-FAM	4
E-ACA/M-CCG	6-FAM	8
E-AGA/T-CAA	NED	21
E-AGA/T-CAG	NED	15
E-AAC/T-GAA	NED	20
E-AAC/T-GAG	NED	17
E-AAC/T-CAG	NED	12
E-AGA/M-CGA	NED	7
Total		172

Appendix 4 is the slightly revised version of Supplementary material S. 4 published in Landscape Ecology (2008) 23:989–1000

Appendix 4 *Niviventer coninga* habitat suitability value in each land-cover type of Sanyi Township, Miaoli County, Taiwan

		Habitat suitability value		
Land cover	Number of cells	Minimum	Maximum	Mean ± SD[a]
Forest	681592	0	100	23.7 ± 20.2
Shrub land	72259	0	98	13.2 ± 16.7
Unpaved road	32	3	26	11.5 ± 8.0
Lawn	3760	0	98	9.3 ± 13.2
Tea plantation	7026	0	45	7.3 ± 7.0
Small road	6146	0	80	5.2 ± 9.5
Township road	2412	0	84	5.1 ± 12.7
Orchard	115181	0	95	4.9 ± 8.8
County road	3006	0	77	4.4 ± 11.5
Bamboo	84534	0	96	3.8 ± 7.4
River	15420	0	49	2.3 ± 5.5
Pond	2168	0	35	2.2 ± 5.1
Buildings	48749	0	59	2.2 ± 5.4
Highway	7111	0	26	2.1 ± 2.6
Bare land	9838	0	28	2.0 ± 3.3
Cemetery	1489	0	8	1.7 ± 1.8
Dry farmland	38677	0	34	1.0 ± 2.4
Railway	1408	0	26	0.8 ± 1.9
Rice paddy	22610	0	26	0.7 ± 1.7

[a] Standard deviation

Appendix 5 Records of trapped *Niviventer coninga* from Sanyi Township, Miaoli County, Taiwan

Individual	Sex	Weight (g)	Age[1]	Site	X_97[2]	Y_97[3]	Trapping date
NC001	F	101	A	W2	226335	2701238	2005/3/5
NC002	F	57	A	W2	225909	2700856	2005/3/5
NC003	M	60	A	W2	225934	2700844	2005/3/5
NC004	F	141	A	W2	225444	2700740	2005/3/6
NC005	F	99	A	W2	226308	2701304	2005/3/7
NC006	M	169	A	W2	226321	2701205	2005/3/7
NC007	M	190	A	W2	226293	2701068	2005/3/11
NC008	F	96	A	W2	226298	2701130	2005/3/11
NC009	F	105	A	W2	226311	2701164	2005/3/11
NC010	M	152	A	W2	226308	2701304	2005/3/11
NC011	F	130	A	W2	226279	2701314	2005/3/11
NC012	F	111	A	W2	226188	2701038	2005/3/11
NC013	M	72	A	W2	226230	2701146	2005/3/11
NC014	M	173	A	W2	226186	2701297	2005/3/11
NC015	M	109	A	W2	226224	2701280	2005/3/11
NC016	M	64.5	A	W2	225451	2700740	2005/3/11
NC017	M	65.5	A	W2	225937	2700835	2005/3/11
NC018	M	52	SA	W2	225960	2700907	2005/3/11
NC019	F	87	A	W2	226124	2700853	2005/3/11
NC020	M	101	A	W2	225377	2700708	2005/3/12
NC021	F	119	A	W2	225952	2700923	2005/3/12
NC022	M	116	A	W2	226124	2700853	2005/3/12
NC023	M	124	A	W2	226238	2701267	2005/3/12
NC024	F	102	A	W2	226230	2701179	2005/3/12
NC025	F	75	A	E1	227803	2701599	2005/4/5
NC026	M	171.5	A	E1	227708	2701689	2005/4/6
NC027	F	66.5	A	E1	227803	2701599	2005/4/6
NC028	M	57	A	E1	227825	2701596	2005/4/6
NC029	M	121.5	A	E1	227633	2701616	2005/4/6
NC030	F	87	A	E1	227631	2701657	2005/4/7
NC031	F	72	A	E1	227780	2701599	2005/4/7
NC032	M	58	A	E1	227762	2701525	2005/4/7
NC033	M	120	A	E1	227898	2701524	2005/4/7
NC034	F	144	A	E1	227898	2701524	2005/4/8
NC035	M	101	A	E1	227638	2701681	2005/4/9
NC036	M	184	A	E1	227786	2701552	2005/4/9

[1] Adult (A): body weight > 55 g; sub-adult (SA): body weight ≤ 55 g

[2] X coordinate of the projected coordinate system of Taiwan, TWD97

[3] Y coordinate of the projected coordinate system of Taiwan, TWD97

Appendix 5 (continued)

Individual	Sex	Weight (g)	Age[1]	Site	X_97[2]	Y_97[3]	Trapping date
NC037	F	66.5	A	E1	227741	2701588	2005/4/10
NC038	M	69	A	E1	227865	2701525	2005/4/10
NC039	M	118	A	E1	227865	2701525	2005/4/10
NC040	F	74	A	E1	227841	2701533	2005/4/10
NC041	F	144	A	E1	227761	2701495	2005/4/10
NC042	M	176	A	E1	227707	2701440	2005/4/10
NC043	F	153	A	W3	224350	2698631	2005/4/29
NC044	M	162	A	W3	224150	2698455	2005/4/29
NC045	M	96	A	W3	224308	2698802	2005/4/30
NC046	M	113	A	W3	224247	2698796	2005/5/1
NC047	F	133	A	W3	224303	2698562	2005/5/2
NC048	M	158	A	W3	224196	2698710	2005/11/18
NC049	F	39	SA	W3	224209	2698554	2005/11/18
NC050	M	195	A	W3	224377	2698514	2005/11/18
NC051	M	94	A	W3	224222	2698614	2005/11/19
NC052	F	123	A	W3	224264	2698901	2005/11/20
NC053	F	71	A	W3	224348	2698645	2005/11/20
NC054	F	64.5	A	W3	224348	2698637	2005/11/20
NC055	F	72	A	W3	224370	2698524	2005/11/20
NC056	M	118	A	W3	224370	2698524	2005/11/21
NC057	F	73	A	W4	223021	2696380	2005/7/25
NC058	M	38.5	SA	W4	223000	2696401	2005/7/27
NC059	M	91	A	W4	222957	2696425	2005/7/27
NC060	M	89	A	E2	231015	2696426	2006/1/18
NC061	M	102	A	E2	231215	2696450	2006/1/19
NC062	M	131	A	E2	231011	2696455	2006/1/19
NC063	F	61	A	E2	231215	2696450	2006/1/20
NC064	M	84	A	E2	230435	2696017	2006/1/20
NC065	M	197	A	E2	231280	2696471	2006/1/21
NC066	F	46	SA	E2	230883	2696420	2006/1/21
NC067	M	61.5	A	E2	231321	2696492	2006/1/22
NC068	M	146	A	E2	231284	2696482	2006/1/22
NC069	F	108	A	E2	231065	2696448	2006/1/22
NC070	F	87.5	A	E2	231073	2696460	2006/1/22
NC071	F	114	A	E2	230919	2696445	2006/1/22
NC072	F	79	A	E2	230418	2695988	2006/1/22

[1] Adult (A): body weight > 55 g; sub-adult (SA): body weight ≤ 55 g

[2] X coordinate of the projected coordinate system of Taiwan, TWD97

[3] Y coordinate of the projected coordinate system of Taiwan, TWD97

Appendix 5 (continued)

Individual	Sex	Weight (g)	Age[1]	Site	X_97[2]	Y_97[3]	Trapping date
NC073	M	119	A	E2	230346	2695996	2006/1/22
NC074	F	120	A	E2	230326	2696016	2006/1/22
NC075	M	65	A	E2	231263	2696474	2006/1/23
NC076	M	114	A	E2	231238	2696481	2006/1/23
NC077	M	136	A	E2	231157	2696401	2006/1/23
NC078	F	118	A	E2	231073	2696460	2006/1/23
NC079	M	173	A	E2	230884	2696401	2006/1/23
NC080	M	144	A	E2	230390	2695976	2006/1/23
NC081	F	129	A	E2	230346	2695996	2006/1/23
NC082	M	73.5	A	E2	230326	2696016	2006/1/23
NC083	M	179	A	W1	225106	2701644	2006/3/7
NC084	F	101	A	W1	225068	2701654	2006/3/8
NC085	F	107	A	W1	225054	2701585	2006/3/9
NC086	M	137	A	W1	224498	2701070	2003/2/22
NC087	M	113	A	W1	224355	2700994	2003/2/25
NC088	M	155	A	W1	225053	2701578	2006/3/10
NC089	M	48	SA	W1	224430	2701008	2003/2/26
NC090	M	70	A	W1	224460	2700944	2003/2/26
NC091	F	160	A	W1	224993	2701690	2006/3/10
NC092	M	137	A	W1	225177	2701627	2006/3/11
NC093	F	100	A	W1	225145	2701643	2006/3/11
NC094	M	151	A	W1	225086	2701622	2006/3/11
NC095	M	88	A	W1	224859	2701699	2006/3/12
NC096	M	134	A	W1	225307	2701697	2006/3/13
NC097	M	148	A	W1	225153	2701636	2006/3/13
NC098	F	154	A	W1	224863	2701670	2006/3/13
NC099	M	174	A	W1	224910	2701690	2006/3/13
NC100	M	83	A	W1	224838	2701707	2006/3/13
NC101	M	184	A	E3	227016	2697621	2006/2/4
NC102	F	150	A	E3	227062	2697679	2006/2/4
NC103	M	46.5	SA	E3	226981	2697558	2006/2/4
NC104	M	195	A	E3	226984	2697584	2006/2/5
NC105	M	152	A	E3	226646	2697361	2006/2/5
NC106	M	109	A	E3	226589	2697308	2006/2/5
NC107	M	167	A	E3	226440	2696935	2006/2/5
NC108	M	200	A	E3	226986	2697654	2006/2/6

[1] Adult (A): body weight > 55 g; sub-adult (SA): body weight ≤ 55 g

[2] X coordinate of the projected coordinate system of Taiwan, TWD97

[3] Y coordinate of the projected coordinate system of Taiwan, TWD97

Appendix 5 (continued)

Individual	Sex	Weight (g)	Age[1]	Site	X_97[2]	Y_97[3]	Trapping date
NC109	M	147	A	E3	227061	2697680	2006/2/6
NC110	M	85	A	E3	226648	2697387	2006/2/7
NC111	M	118	A	E3	226765	2697187	2006/2/7
NC112	M	60	A	E3	227061	2697680	2006/2/8
NC113	F	118	A	E3	226648	2697387	2006/2/8
NC114	M	172	A	E3	226985	2697593	2006/2/9
NC115	F	149	A	E3	226985	2697528	2006/2/9
NC116	M	100	A	E3	226633	2697451	2006/2/10
NC117	M	104	A	E4	225702	2695229	2006/2/20
NC118	M	143	A	E4	225636	2695202	2006/2/20
NC119	M	66	A	E4	225961	2695385	2006/2/21
NC120	F	147	A	E4	225826	2695430	2006/2/22
NC121	M	142	A	E4	225654	2695263	2006/2/23
NC122	M	122	A	E4	225840	2695434	2006/2/23
NC123	F	83	A	E4	225771	2695530	2006/2/23
NC124	F	87	A	E4	225647	2695249	2006/2/24
NC125	F	52	SA	E4	224663	2694714	2006/2/24
NC126	M	158	A	E4	224706	2695071	2006/2/24
NC127	F	71.5	A	E4	225651	2695236	2006/2/25
NC128	M	199	A	E4	225586	2695134	2006/2/25
NC129	M	165	A	E4	225840	2695434	2006/2/25
NC130	M	77	A	E4	225812	2695427	2006/2/25
NC131	M	222	A	E4	225714	2695349	2006/2/26
NC132	M	179	A	E4	225984	2695434	2006/2/26
NC133	M	77.5	A	E4	225780	2695496	2006/2/26
NC134	M	181	A	E4	225812	2695427	2006/2/26

[1] Adult (A): body weight > 55 g; sub-adult (SA): body weight ≤ 55 g

[2] X coordinate of the projected coordinate system of Taiwan, TWD97

[3] Y coordinate of the projected coordinate system of Taiwan, TWD97

VITA

Yu-Huang Wang was born in Taoyuan, Taiwan, on June 30, 1971. He received a Bachelor of Science degree in 1993 and a Master of Science degree in 1996 from the Department of Environmental Science, Tunghai University, Taichung, Taiwan. He joined military service as a second lieutenant of the Land Survey Team from 1996–1998. From 1998–2000, as a research assistant of Institute of Zoology, Academia Sinica, Taipei, Taiwan, he was responsible for intertidal and estuarine benthic surveys in northern Taiwan and on the Kinmen and Matzu islands and for identifying polychaete and oligochaete specimens. From 2000–2001, as a research assistant of the Research Center of Taiwan's Ecology, Taichung, he assisted in forest surveys. He began his Ph.D. study in September 2001 and received Ph.D. degree in Science in January 2009 from the Department of Life Science, Tunghai University.