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巢鼠的族群生物學研究 (3/3)

Population biology of the Harvest Mouse, *Micromys minutus* (3/3)

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Abstract

The Guandu salt marsh in the Guandu Nature Park (Taipei, Taiwan) offered habitat patches of different qualities for the harvest mouse (*Micromys minutus*). The current research aimed to find out (1) if succession could influence the genetic structure of the harvest mouse and (2) how population dynamics affected the genetic structure of the harvest mouse. Study sites were categorized into dense, sparse, and mix patches. Dense patches were preferred by *Micromys minutus*. However, the overall area of dense patches decreased greatly during the succession occurred between 2000 and 2005. The decrease of dense patches not only reduced but also fragmented the habitats suitable for the harvest mouse. The harvest mouse population was fragmented along with dense patches, yet the population did not become structured genetically with significance. I detected bottleneck effect with M-ratio ($M < 0.68$) but not mode-shift and heterozygosity excess in both years, which indicated that bottleneck probably occurred in the past distant enough that the signature of bottleneck detectable by mode-shift and heterozygosity excess has been erased. Within each year, population size became very low in summer, and the genetic differentiation was significant between spring and autumn population in 2005. Overall, the results suggest that salt marsh succession can influence the genetic structure of the harvest mouse in Guandu salt marsh spatially and temporal in a short period of time.

Key words : **bottleneck, habitat heterogeneity, harvest mice, *Micromys minutus*, microsatellite, population dynamics, population genetics, succession, temporal differentiation**

Introduction

Population dynamics -- Animal populations fluctuate over time. Although various factors influence the dynamics of populations, they could be categorized into endogenous and exogenous factors (Pickens, 2007; Lima et al., 1999; Stenseth et al., 2003). Endogenous factors include the unique life-history traits of different species (e.g., age of 1st reproduction, litter size, and longevity), and the degree of tolerance among population members. They set up a baseline upon which population size fluctuates (Boonstra, 1994; Tkadlec et al., 1998).

On the other hand, exogenous factors such as weather, food availability, predators, and habitat succession would add to and/or interact with the endogenous factors to modify the dynamic patterns of populations further (Stenseth et al., 2003; Boonstra et al., 2006; Yarnell et al., 2007). Some exogenous factors of anthropogenic origins such as overexploitation, habitat loss and degradation, spread of competitive or predatory alien species are well known causes of population decline, or even local extinction (Jackson et al., 2008; Trites et al., 2007; Johnson et al., 1999; Hall-Martin, 1992). The change in population sizes by those forces is usually dramatic. Unlike anthropogenic effects, natural succession of habitats would affect population fluctuations over a longer period of time. Both the strengths of endogenous and exogenous factors and their interactions could change with time. Thus, few animal populations fluctuate with easily identifiable patterns. The most famous case is probably the multi-annual (10-year) cycles of the snowshoe hare and their predator, lynx (Stenseth et al., 1998; Royama, 1992).

The strengths of endogenous and exogenous factors and their interactions change with space as well. For a given species, suitable habitats often intersperse in a sea of unsuitable habitats. Thus, members of a population usually do not distribute uniformly over space, and frequently form subpopulations. The suitability of habitats (determined by the endogenous and exogenous factors mentioned earlier) affects the birth, death, dispersal rates, and consequently population

sizes.

Population genetics -- As anthropogenic effects and/or natural succession alter the suitability of habitats, the genetic structures for animal populations change too. Generally, population genetic studies have focused on the effects of anthropogenic activities. Although many studies have investigated how climate change over an evolutionary time scale influence the evolution of species, few population genetic studies pay attention to how natural succession, an ecological time scale phenomena, changes genetic structure for animal populations. Natural succession changes the amount of suitable habitat, thus indirectly the sizes and genetic structure of populations. Furthermore, natural succession often changes the dispersion of suitable habitat patches. Thus, the heterogeneity of the habitat changes, and the formation of subpopulation changes.

Harvest mice – The harvest mouse (*Micromys minutus*) is widely distributed in Eurasia. With a reddish/brownish coat color, the species is one of the smallest (6-8 grams) rodents in the world. The species inhabits grasslands or bushes (Churchfield, 1997), and makes ball-shaped nests among vegetation with shredded leave blades. Their prehensile tails and toe pads allow them to move through vegetation easily.

Trout (1976) suggested that populations of the harvest mouse fluctuate dramatically both within and between years. Population sizes could increase or decline in magnitude of orders within a very short period of time. Such a dynamic pattern seemed to exhibit periodicity in some areas. The species inhabits grasslands or bushes (Churchfield, 1997), habitats that are in early successional stage. The vegetative community changes in a fast pace in such ecosystems, and offer a unique system to study the effects of habitat succession on population dynamics and population genetics.

A harvest mouse population inhabits the salt marsh in the Guandu Nature Park at suburb Taipei, an important stopover for migratory waterfowls. Consistent with the description by

Trout (1976), Lee (2001) found that the harvest mouse population in Guandu exhibited a dramatic fluctuation within year. Population size dropped to near zero during summer. Lee (2001) also demonstrated that the species preferred dense and avoided sparse vegetation, consistent with an early study (Bence, 2003). From the observation of aerial photos from 2000-2004, I found that the Guandu salt marsh underwent rapid succession. The composition and spatial structure of vegetative community has noticeable changes over 4 years. Particularly, suitable habitats (dense vegetation) seemed to have declined, and become fragmented. Such changes would affect not only the population sizes, but also genetic structure of the local harvest mouse population.

Research question – The current study attempted to investigate the effects of habitat succession on the population fluctuation and population genetics of the harvest mouse. I tested several hypotheses: (1) habitat succession reduced suitable habitats, and consequently reduced population sizes; (2) habitat succession fragmented suitable habitats, and consequently altered population genetic structure (see conceptual map, Fig. 1).

Materials & methods

Study site

The study site was a salt marsh in the Guandu Nature Reserve (25° 07'N, 121° 28'E) located in northern Taiwan at the confluence of Danshui and Jilong Rivers (Fig. 2). The landscape consists of a mosaic of freshwater and brackish ponds, mudflats, marshes, rice paddies, and woodlands, in which a rich variety of organisms inhabits. The reserve has been designated as an Important Bird Area by the Birdlife International, and the Guandu Nature Park within the reserve has undergone intensive waterfowl habitat management since early 1990s. Seven small mammal species has been recorded in the Guandu Nature Park, including harvest mouse (*Miomys minutus*), *Rattus losea*, *Apodemus agrarius*, *Mus caroli*, *Crocidura shantungensis* and common shrew, weasel. Major vegetation included *Alternanthera philoxeroides* (Moq.) Griseb., *Ipomoea cairica* (L.) Sweet, *Commelina diffusa* Burm. f., *Brachiaria mutica* (Forsk.) Stapf, *Panicum repens* L., *Paspalum distichum* L., *Phragmites communis* (L.) Trin., *Typha angustifolia* L..

Trapping:

A 210 X 65m (13650m²) rectangular grid with 14 trap lines, containing 301 stations in 5 m spacing was established in the Guandu Nature Park in March 2005 (Fig. 3). I conducted the study from March 2005 to March 2006. Trapping was conducted monthly, with the odd- (152 stations) and even-number (149 stations) lines serviced every other month. I placed one Ugglan live trap (Ugglan special #2, LxWxH = 25 x 8 x 6.5 cm, Grahnb, Hillerstorp, Sweden) at each station during each trapping session. Traps were placed at station on the ground and locked open for 4 consecutive days for pre-baiting purpose. Traps were set on the 5th evening, baited with roll oats mixed in peanut butter, and checked the following morning for 3 consecutive days. I placed traps on vegetation during wet seasons to avoid flooding. Small mammals were marked by a unique toe-clipping upon first capture. Upon each capture of animals, I recorded trap station, individual identification, gender, reproductive condition, body weight, and body condition such as amount of parasite and wounding. All animals were subsequently released at the point of capture. Clipped toes were preserved in 80% alcohol in the field and later stored in -20°C in the laboratory for subsequent molecular analyses.

Habitat patch types

According to the spatial distribution of dominant plant species, I categorized the habitat into three patch types: dense patch, sparse patch, and mix patch (Hallet et al.,1983; Lee, 2001). The trapping grid was divided into 5-m x 5-m cells. Each cell was assigned to a patch type. In dense patches, the dominant species (> 70% cover) were para grass (*Brachiaria mutica*), Torpedograss (*Panicum repens*) and Climbing dayflower (*Commelina diffusa*). In spare patches, the dominant species was reed (*Phragmites communis*). In mix patches, none of the above-mentioned species had cover > 70 %. The measurement of vegetative cover was performed in March and August (to represent dry and wet season, respectively) during 2000 and 2005. Data from the two months were averaged, and mean values were used to assign each cell to a patch type.

Isolation of microsatellite

The harvest mouse DNA was extracted from an adult harvest mouse captured in central Taiwan according to the standard phenol-chlorophorm extraction procedures described in Sambrook et al. (1989). Genomic DNA was digested with *Sau3AI* and fractioned on a 1% agarose gel. DNA of size range 300-1200bp was eluted, purified with GFX™ Band Purification Kit (Amersham) and ligated into plasmids PUC118/*BamHI*/*BAP* (TaKaRa) according to manufacturer's protocols. Ligated plasmids were transformed into the competent ECOS 101 cells (Yeastern Biotech). Recombinant clones containing inserts were transferred to Hybond-N⁺ nylon membranes (Amersham), which were hybridized to a set of oligonucleotide probes, including (AC)₁₅, (AT)₁₅, (AG)₁₅, (AAT)₁₀, (AAG)₁₀, and (GATA)₆. Probes were labeled with Digoxigenin (DIG) Oligonucleotide 3'-End Labeling Kit (Roche). Hybridization was performed at 50-53°C for 16 hours in a standard hybridization buffer, consisting of 5X SSC, 0.1% Sodium N-lauroylsarcosine, 0.02% SDS, and 1% Blocking Reagent (Roche). The membranes were washed twice, each for 5 min at 45°C with a solution of 2X SSC, 0.1% SDS, and then twice, each for 15 min at 65°C with a solution of 0.1X SSC, 0.1% SDS. Chemiluminescent detection was performed with DIG Luminescent Detection Kit (Roche). A total of 64 positive clones were sequenced using a MegaBACE 500 automated sequencer. Twenty-five clones containing repeat motifs with more than 6 repeats and sufficient flanking region were selected to design primers. About 4% of screened clones yielded positives clones, which was higher than the average of 2-3% in many other taxa (Zane et al. 2002).

Primers were designed with the on-line program Primer 3.0 (Rozen and Skaletsky, 2000) and FastPCR 1.2 (Kalendar, 2007), a free software. Polymerase chain reaction (PCR) conditions were optimized for each primer pair. Each PCR reaction mixture (10µL) contained 50-100 ng template DNA, 0.5 units of *Taq* DNA polymerase (Bioman, Taipei, Taiwan), 2.0 mM of Mg²⁺, 0.2 mM dNTP, 10X buffer (20mM of Tris-HCl (pH8.8), 10mM KCl, 10mM (NH₄)₂SO₄, and 0.1% Triton X-100, Bioman), and 0.25µM primer, with the forward or reverse primer being end-labeled with fluorescent dyes. Amplification was carried out by the thermal profile: 94°C 5 min, followed by 40 cycles of 94°C 30 s, optimal annealing temperature for 30 s, 72°C for 30 s, and a final extension step at 72°C for 7 min. PCR products were run on linear polyacrylamide

(LPA) gels with a MegaBACE 500 automated sequencer. ET-400 Size Standard (Amersham) was used as a size marker to determine allele sizes. Individual genotypes were determined and individuals with ambiguous genotypes or homozygote were amplified and scored at least twice to determine the allele sizes.

Statistical analyses

Analyses of population genetics

Test	software
Microsatellite characteristics	
Hardy-Weinberg equilibrium	CERVUS 3.0
Null allele	CERVUS 3.0
Linkage disequilibrium	FSTAT 2.9.3
Large allele drop out	MICROCHECKER
Error due to stutter	MICROCHECKER
Calculating genetic diversity	
Number of alleles	FSTAT 2.9.3
Genetic diversity	FSTAT 2.9.3
Allelic richness	FSTAT 2.9.3
Detecting bottleneck	
Mode-shift	BOTTLENECK
Heterozygote excess	BOTTLENECK
M-ratio	AGARst 3.3

Results

Population size and sex ratio

Population size, estimated as the minimum number known alive (MNKA), fluctuated within

years (Table 1). In 2000, the population size reached low (1-2 individuals / month) during summer. In 2005, the population size remained low from summer to winter (Table 1). The population sizes reached the high points in 2000 winter (December, 42 individuals) and 2005 spring (March, 35 individuals). The distribution of population sizes among seasons was significantly different between years ($\chi^2 = 101.53$, $d.f. = 3$, $P < 0.001$).

There were ? males and ? females in 2000; ? males and ? females in 2005. Sex ratios were male-biased in both years, however, only the bias in 2000 was significant (G -test, $G = 6.08$, $d.f. = 1$, $P < 0.05$). Within each year, there were more males than females in all seasons, however, only the bias of 2000 spring ($G = 5.01$, $d.f. = 1$, $P < 0.05$) and 2005 summer ($G = 6.931$, $d.f. = 1$, $P < 0.01$) were significant.

There were more males than females in each patch type except sparse patch probably because there were too few individuals lived in sparse patches. Male/female sex ratios ranged from 1.54 to 1.87 each patch. However, only the dense patch population in 2000 deviated from 1:1 significantly (G -test, $G = 4.818$, $d.f. = 1$, $p < 0.05$).

Recruitment and Disappearance

There was no recruitment in summer both years. In other seasons, more recruits appeared in 2000 than 2005 (Table). The distribution of recruits among seasons was not significantly different between years ($\chi^2 = 6.15$, $d.f. = 3$, $P > 0.05$), though the amount of recruits were significantly larger in 2000 than 2005 (Wilcoxon signed rank test, $W+ = 36$, $W- = 0$, $N=8$, $p < 0.01$). There were few emigrants in summer both years. However the emigrants made up 25 % and 33.33 % of summer population in 2000 and 2005, respectively. The distribution of emigrants among seasons between years was significantly different ($\chi^2 = 54.78$, $d.f. = 3$, $P < 0.001$), but the amount of emigrants were not different between two years (Wilcoxon signed rank test, $W+ = 36.50$, $W- = 18.50$, $N = 10$, $p > 0.05$).

The distribution of recruits among patches was not significantly different between years (χ^2

= 2.715, $P > 0.05$). The recruits among patches distributed randomly in 2000 ($\chi^2 = 4.3471$, $P > 0.05$), but the distribution was not random ($\chi^2 = 8.2251$, $P < 0.05$) in 2005. The distribution of emigrants among patches was not significantly different between years ($\chi^2 = 3.521127$, $P > 0.05$). The emigrants distributed randomly among patches in 2000 ($\chi^2 = 3.857303$, $P > 0.05$), but the distribution was not random in 2005 ($\chi^2 = 14.25556$, $P < 0.05$).

Habitat succession and harvest mice distribution

Habitat succession occurred in Guandu salt marsh (Fig. 4). From 2000 to 2005, the dense patch decreased from 83.47 % to 53.49 %, mix patch increased from 14.05 % to 38.21 % and sparse patch increase from 2.48 % to 8.31 % (Table 2). The change in the percentage of each patch type was significant (*G-test*, $G_{adj} = 17.699$, $d.f. = 2$, $P < 0.0001$).

Furthermore, not only did the area but also the continuity of dense patch decrease from 2000 to 2005 (Fig. 4). The spatial distribution of harvest mice in the study site matched that of dense patches (Fig. 5). The preference of the dense patch type by the harvest mouse was significant in 2005 ($\chi^2 = 15.10$, $d.f. = 2$, $P < 0.01$), but not in 2000 ($\chi^2 = 5.80$, $d.f. = 2$, $P > 0.05$). The density of the harvest mouse was highest in dense patch and lowest in sparse patch in both years (Fig. 6).

Reproductive success

According to the weight distribution of the harvest mouse population, I defined individuals with body weights lower than 6g juveniles (Fig. 7). The reproductive success of harvest mice within each patch type in 2000 and 2005 were shown in Fig. 8. There was not any juvenile caught in sparse patch during both years. No significant difference in reproductive success between mice inhabited dense and mix patches was observed in 2000 (Fisher's exact test, $P > 0.05$). In 2005, reproductive success of mice inhabited mix patch tended to be higher than those in dense patches, though the difference was not statistically significant (Fisher's exact test, $P > 0.05$).

Genetic structure of the harvest mouse population

Variation of microsatellite

All six microsatellite loci are polymorphic in the harvest mouse populations from 2000 and 2005. The number of alleles per loci ranged 6-10 and 5-10 in 2000 and 2005, respectively. The observed heterozygote ranged 0.706-0.843, and 0.637-0.854 in 2000 and 2005, respectively (Table 3). Comparing the genetic diversity between 2000 and 2005, the number of alleles per locus, allelic richness and H_o are not significantly different (Wilcoxon signed rank test). No loci showed departure from the Hardy-Weinberg equilibrium. I detected linkage disequilibrium in 3 pairs of loci in the population of 2000 (Table 4). The disequilibrium in 2000 population may be caused by genetic drift rather than physical linkage of these loci (Tomoko, 1982). No evidence indicated null alleles, large allele drop out, or error due to stutter.

Bottleneck between years

The analyses by either mode shift or heterozygote excess didn't detect bottleneck effect in 2000 and 2005 populations. However, M-ratios were 0.669 in 2000 and 0.618 in 2005, both showed values indicated bottleneck (Garza and Williamson 2001). The M-ratio is a more powerful method than the other two (REF). The results revealed that both 2000 and 2005 populations have gone through bottlenecks, thus the reduction of population size that cause the bottleneck effect occurred before year 2000.

Bottleneck between seasons (temporal differentiation)

The harvest mice population sizes in the Guandu salt marsh went through a very low point during summer in both 2000 and 2005 (Table 1). Few individuals lived from spring to autumn. Only one out of 119 individuals in 2000, and 2 out of 62 individuals in 2005 lived from spring through summer to autumn. I analyzed the temporal differentiation between spring and autumn (winter) populations. Clustering analyses using the software STRUCTURE showed that only the population was not temporally structured ($K=1$). On the other hand, although F_{st} and R_{st} values indicated that populations did not differentiate between season in 2000, the F_{st} value did support that the population was not temporally structured in 2005 (Table 3, $F_{st} = 0.012$, $p < 0.05$).

Spatial distribution and genetic differentiation

Harvest mice distributed mostly in dense patches. In 2000, dense patches were more or less contiguous, yet were separated by sparse patches in 2005 (Fig. 4 and 5). Thus, the population tended to aggregate on the east and west sides of the study site (Fig. 5). The gene flow between the two sides was low in both years. The Capture-Mark-Recapture (CMR) data did not detect any dispersal event between the two sides in 2005, and only one dispersal occurred in 2000.

Two clustering methods were applied to detect spatial genetic structure of harvest mice. I divided both populations by spring and winter (autumn) groups in order to eliminate the temporal effect on population genetic structure. Both software TESS 1.1 and STRUCTURE 2.1 detected only one population in the study site in both years. The divergence between east and west subpopulations of harvest mice was not significant in 2005 ($F_{st} = -0.0047$, $P > 0.05$) and 2000 ($F_{st} = 0.007$, $P > 0.05$).

Discussion

Many studies have shown that geographical subdivision can affect population structure. Most focused on how artificial or natural geographical boundaries shaped the population structure of focal species (Johnson et al., 2003; Keller et al., 2004; Proctor et al., 2005). The relationship between succession and animal populations have been studied a lot (Yarnell et al., 2007; Zhang et al., 2007; Briani et al., 2004), however, few studies evaluate the effects of succession on the structure of animal populations (but see Tallmon et al., 2002). The current study found that the vegetative cover of the Guandu salt marsh has changed temporally (Fig. 1), and offered a great opportunity to study the effects of succession on population structure of small mammals.

Succession and population size

Micromys minutus preferred to live in dense patches which might be because of the suitability for nesting (Bence et al., 2003). A previous study in the same site also supported this point (Lee, 2001). The highest densities occurred in dense patches in both years (Fig.5), and the

preference was statistically significant in 2005 and marginally in 2000 ($P=0.0553$). Furthermore, the reproductive success was highest in dense patches in 2005(Fig.8). The succession in Guandu salt marsh not only changed the distribution of different patches (Fig.4), but also the proportions among them (Table 2). The area of dense patches favored by *Micromys minutus* decreased nearly 30% from 2000 to 2005. In the same time, the population size of harvest mice also decreased from 119 to 62 individuals.

Nevertheless, several other variables such as climate, food availability, predation, and interspecies competition could change along with succession. They may have changed due to or independent of succession. It was not clear which of those factors contribute more than others to the changes observed in the harvest mouse population.

In 2000, the averaged reproductive success was not different between dense and mix patch. This pattern could be explained by the ideal free distribution. The higher population density in 2000 might reduce the suitability of the favored dense patches, and force some individuals to use less-preferred patches. Thus, the succession of the salt marsh affected the population size of harvest mice, and would change the habitat utilization pattern.

Succession and spatial distribution

Habitat succession changed not only the spatial distribution of dense patches, but also the connectivity between patches (or subpopulations of harvest mice). Comparing the population distribution of harvest mice in 2000 and 2005, I found that the connectivity of population was lower in 2005. The 2005 population tended to be separated into two subpopulations (Fig.5). However, I did not detect genetic differentiation between the two subpopulations. The individuals dispersed between the two subpopulations have been limited probably relied on the corridor lied in the north side of the study site. Due to the expansion of sparse patch over the course of succession, if the succession continues, dispersal would be further reduced by the sparse patches. I believe succession have the potential to structure the genetic of harvest mouse further in Guandu salt marsh.

Bottleneck effect among years

I detected bottleneck effect in both 2000 and 2005 with M-ratio (Garza and Williamson, 2001) but not mode-shift and heterozygosity excess (Cornuet & Luikart, 1996; Luikart & Cornuet, 1998). M-ratio allows for the detection of ancient bottlenecks that may be difficult to be observed using heterozygosity excess or mode shift approaches (Zenger et al., 2003; Abdelkrim et al., 2005; Spear et al., 2006). The M-ratio test can also detect very recent bottlenecks; as with the heterozygosity excess and mode-shift tests, the power to detect a bottleneck should be strongest in the recovery period immediately following the population crash before rare alleles return via migration and/or mutation. Since only M-ratio detected bottleneck effect in the harvest mice population of 2000 and 2005, it implied the population reduction may happen long before 2000, and the bottleneck signature could be detected by mode-shift and heterozygosity excess may have become weak. The observed pattern of bottleneck could be associated with the dynamic patterns of harvest mouse population. Harvest mice populations undergo major fluctuations year to year (Sleptsov, 1947; Piechocki, 1958; Kaikusalo, 1972; Migula et al., 1970; Trout, 1976). Previous studies indicated the maximum population size between consecutive years could decrease 8-folds. This suggested harvest mice frequently suffered from bottleneck effects due to annual major fluctuation.

Seasonal dynamics of harvest mice and its effects on genetic structure

Both the field data in 2000 (Lee, 2001) and 2005 indicated that *Micromys minutus* population size fluctuated seasonally. In spring or winter, the population size reached the high point of the year (Table 1). The population sizes in the summers were very low. This pattern was similar to the reduction in summer recorded in England and Russia (Sleptov, 1947; Trout, 1976). Although Harris (1979) argued that *Micromys minutus* was not active on the ground level during summer, which might make them less likely to be trapped by traps placed on the ground, study on Barn owl pellets collected during the same study (Trout, 1976) from the same farms at 6-week intervals produced a curve of “apparent absence” of harvest mice remains (Trout, 1976). A similar “summer low” situation was found in Norfolk as well (Buckley, 1977). This suggested that harvest mouse population size did reduce in summers. Nevertheless, shift in habitat utilization between seasons might be a possibility that resulted in summer low. The

sparse patches composed of common reed (*Phragmites communis*) could grow to 2-3 m in height. If harvest mice utilized common reed and were active on the vegetation instead of ground level during summer, it would be difficult to trap them. This is unlikely, however. Kuroe (2007) found harvest mice avoid utilizing common reed for nesting, because the density of *Phragmites communis* leaf area is too low to building nests.

Probably due to the prolonged population reduction in summer, the F_{st} value indicated populations between 2005 spring and autumn have been differentiated. Although R_{st} detected no differentiation both years, it had been suggested that F_{st} had better performance in detecting population differentiation when the divergence among samples is expected to be low (Balloux and Goudet, 2002). Nevertheless, I did not detect temporal differentiation in 2000. It could be explained by several intra- and inter-population factors. First, the population size was smaller in 2005 than 2000. Smaller population size would render the 2005 population susceptible to genetic drift than in 2000. Secondly, the intensity of drift would be stronger in 2005 than in 2000, because the former had a longer reduction of population size than the latter. Furthermore, the landscaped surrounding the focal population had changed between 2000 and 2005, and could contribute to the temporal differentiation in 2005. First, individuals immigrated from the unsampled harvest mice population outside the study site could alleviate the effects of summer low. Especially at the east side of study site there was suitable habitat for harvest mice. Between year 2000 and 2005, several artificial ponds have been established for wetland management in the Guandu Nature Park. The construction greatly reduced the harvest mice habitat, and could reduce immigration from the unsampled area into study site. Thus, inter-populationally, gene flow between focal and unsampled population was higher in 2000 than 2005; intra-populationally, genetic drift was more intensive in 2005 than 2000 due to the extended summer low. Together they may enhance the population differentiation in 2005.

The differentiation between seasons implied the reduction in summer every year had the ability to affect the genetic structure of the harvest mouse. Thus, the impact on population genetic structure could happen in a relatively fine temporal scale compared to other temporal

differentiation studies (Sinsch, 1992).

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Table 1. Population sizes of the harvest mouse by season and month in 2000 and 2005.

	spring			summer			autumn			winter		
	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
2000	4	11	12	2	1	1	5	18	23	42	34	25
	27			4			46			101		
2005	35	20	19	5	5	2	4	0	1	2	5	14
	74			12			5			21		

Table 2. The percentage of each patch type in dry and wet seasons of 2000 and 2005.

	2000-1 wet	2000-1 dry	2005-6 wet	2005-6 dry	2000-1(average)	2005-6(average)
Dense	85.12%	80.99%	54.49%	47.51%	101(83.47%)	161(53.49%)
Mix	13.22%	15.70%	34.55%	45.85%	17(14.05%)	115(38.21%)
Sparse	1.65%	3.31%	10.96%	6.64%	3(2.48%)	25(8.31%)
Total station	121	121	301	301	121	301

Table 3. The number of alleles (A), allelic richness, number of samples (N), observed heterozygosities (Ho) and expected heterozygosities (He) in the harvest mouse population in 2000 and 2005.

Locus	A	Allelic richness	N	Ho	He	Hardy-Weinberg equilibrium
2000						
B03	8	8	127	0.843	0.843	Yes
C02	7	6.934	128	0.750	0.747	Yes
D03	10	10	120	0.800	0.793	Yes
E05	8	8	128	0.742	0.763	Yes
F03	6	5.952	126	0.730	0.706	Yes
H04	8	7.875	128	0.711	0.708	Yes
2005						
B03	10	10	74	0.757	0.854	Yes
C02	6	5.972	72	0.736	0.761	Yes
D03	7	7	70	0.814	0.748	Yes
E05	6	6	74	0.608	0.637	Yes
F03	6	6	73	0.726	0.732	Yes

H04	5	5	73	0.740	0.709	Yes
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You need to explain the symbols in the footnote.

Table 4. The occurrence of linkage disequilibrium in 2000 and 2005. *: indicates significant values. P value (0.05) after Bonferroni correction = 0.003.

2000						
	B03	C02	D03	E05	F03	H04
B03	-	NS	*	*	NS	NS
C02		-	NS	*	NS	NS
D03			-	NS	NS	NS
E05				-	NS	NS
F03					-	NS
H04						-
2005						
	B03	C02	D03	E05	F03	H04
B03	-	NS	NS	NS	NS	NS
C02		-	NS	NS	NS	NS
D03			-	NS	NS	NS
E05				-	NS	NS
F03					-	NS

H04						-
-----	--	--	--	--	--	---

Table 5. The temporal differentiation of spring and autumn populations.

Index	Year 2000	Year 2005
Fst	0.00852	0.012*
Rst	0.0029	-0.0082

* indicates significant values.

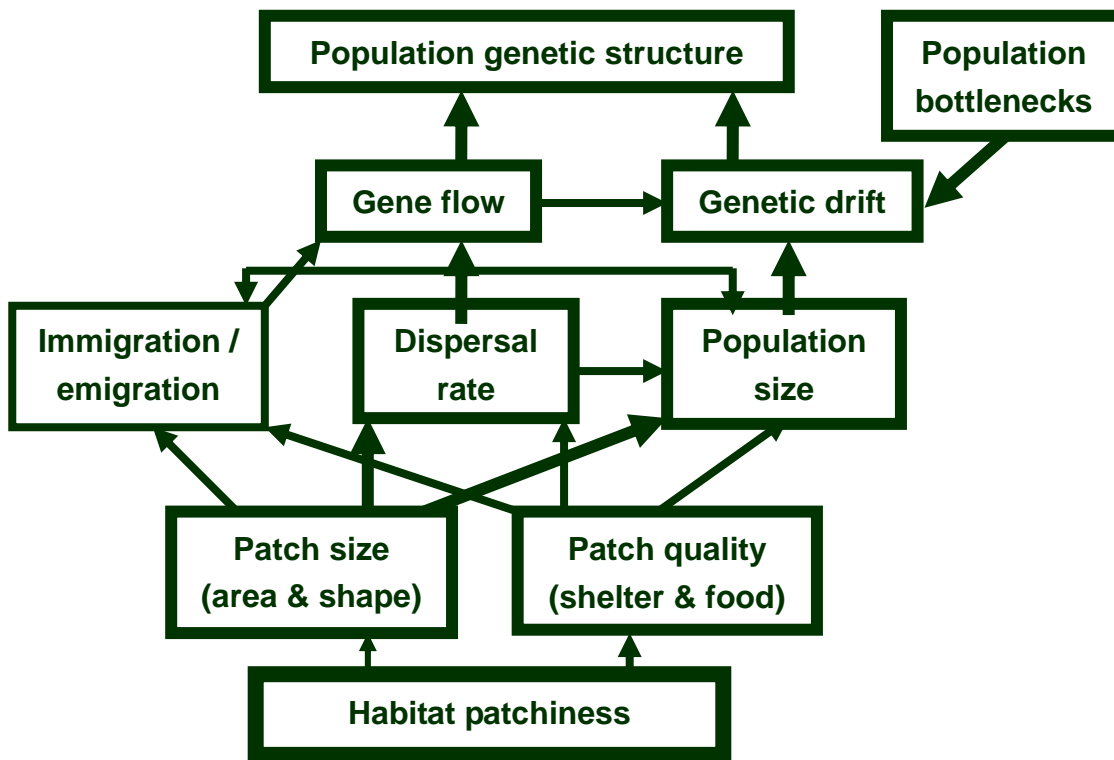


Figure 1. The conceptual map of the current research.

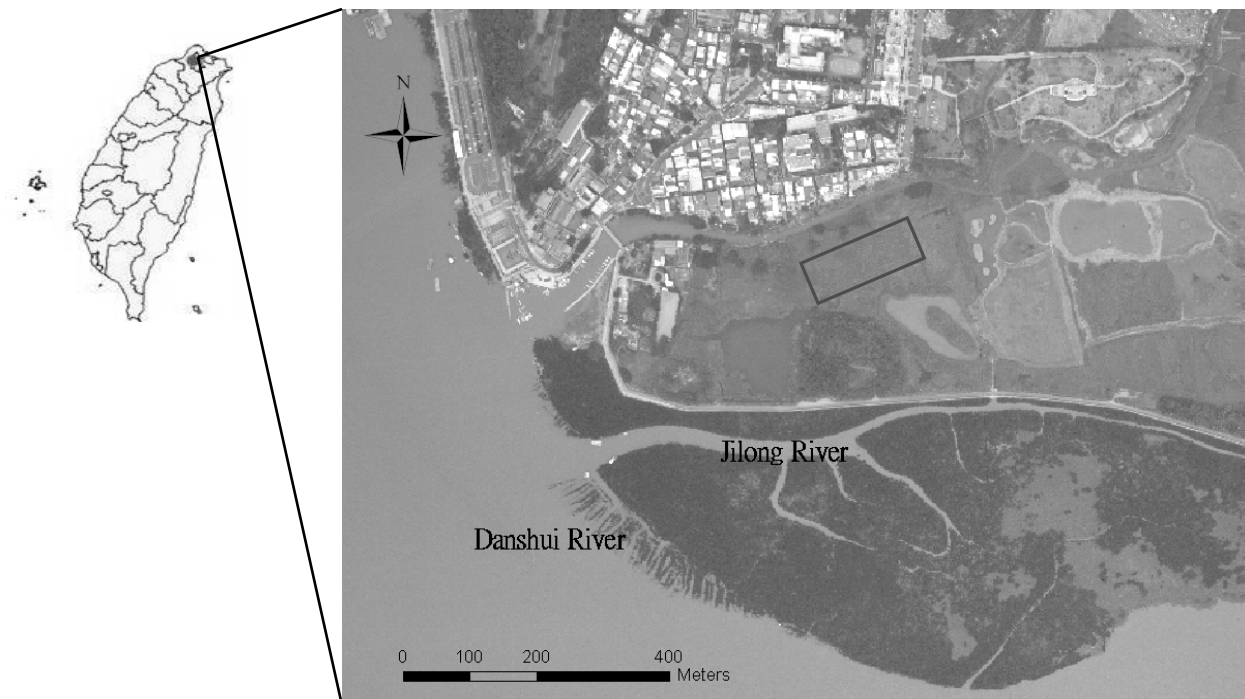


Figure 2. An aerial view of the Guandu Nature Reserve in northern Taiwan. The rectangular frame indicates the study site.

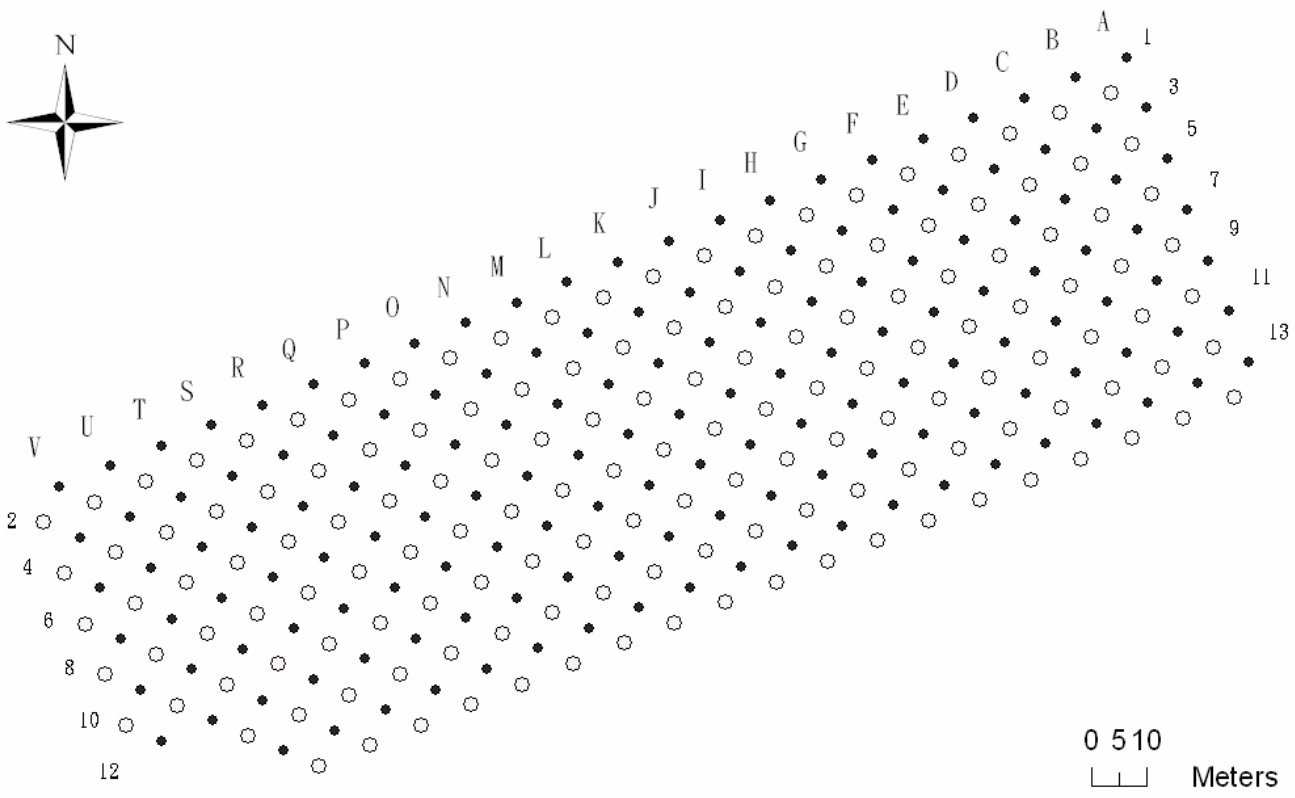
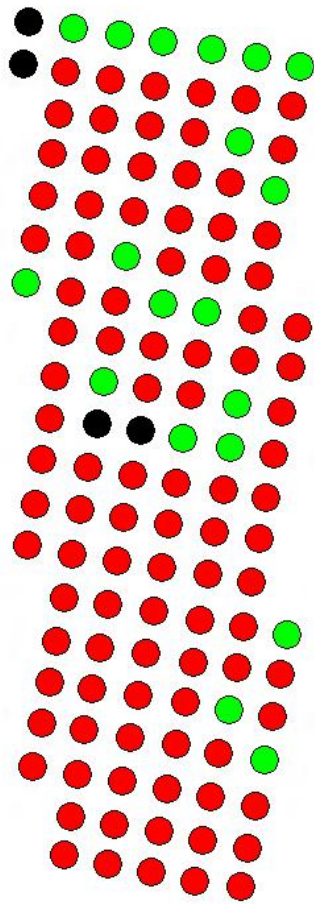
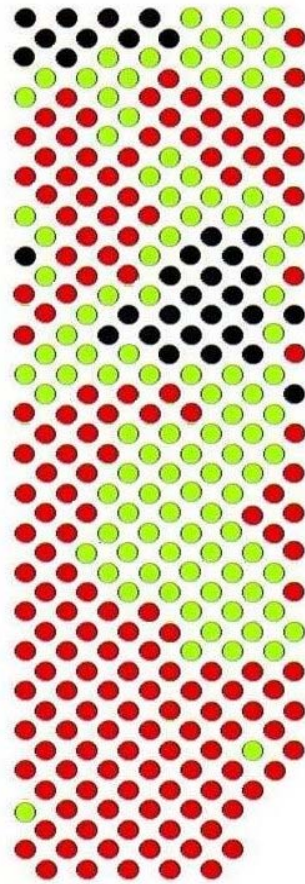


Figure 3. The trapping grid at the study site. Solid and empty dots represent odd- and even-numbered transect lines, respectively. A total of 301 stations covered an area of 65 x 215 m².



Year 2000



Year 2005

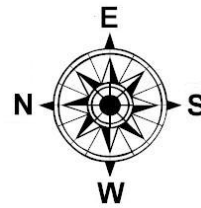
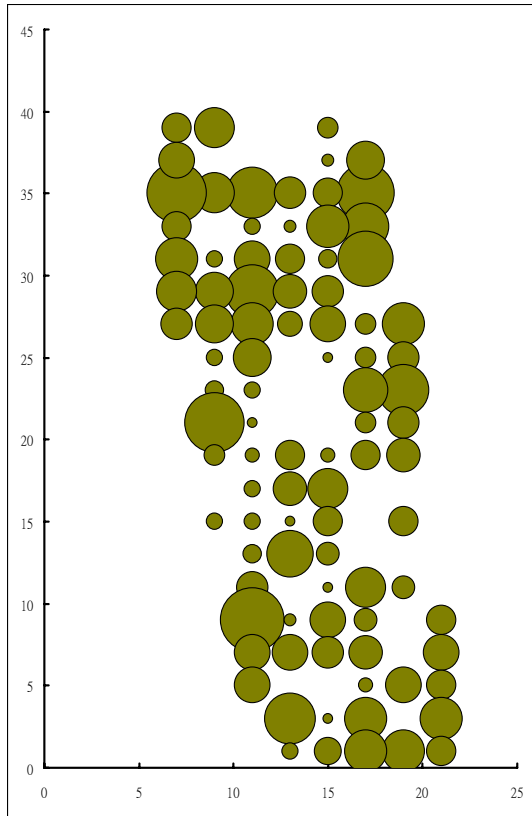
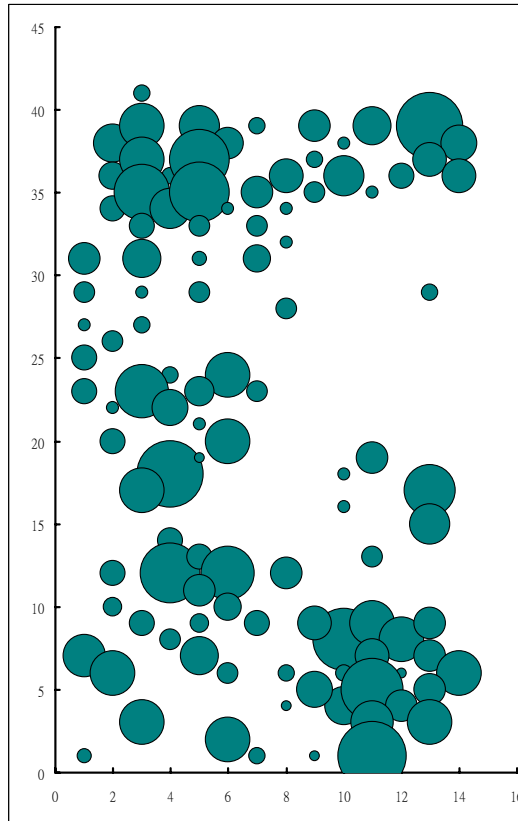


Figure 4. Patch type distribution in 2000 and 2005, red dots: dense patch; green dots: mix patch; black dots: sparse patch.



Year 2000



Year 2005

Figure 5. Spatial distribution of the harvest mouse at the study site. The area of each circle was proportional to the number of harvest mice at each station.

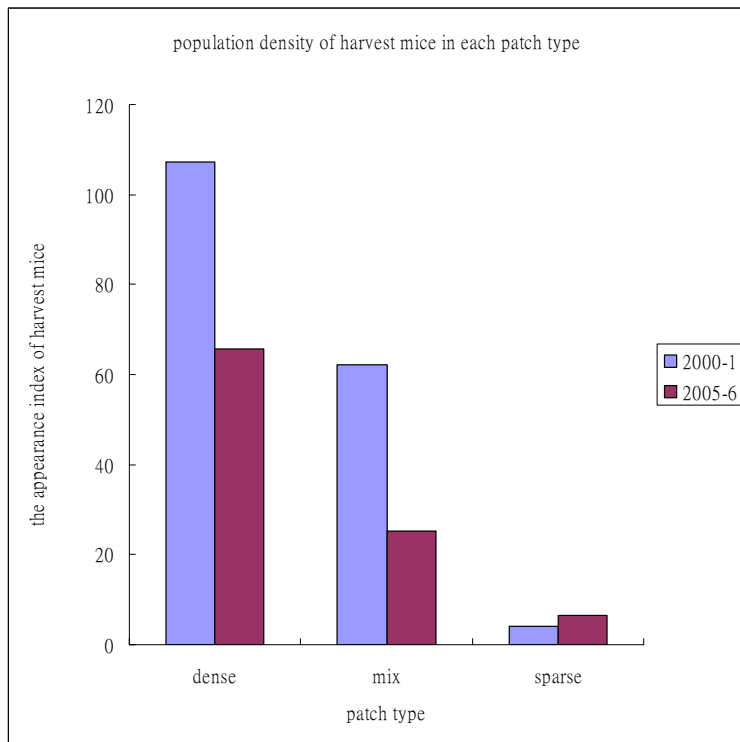


Figure 6. Population density (number per station) of harvest mice in each patch type in 2000 and 2005.

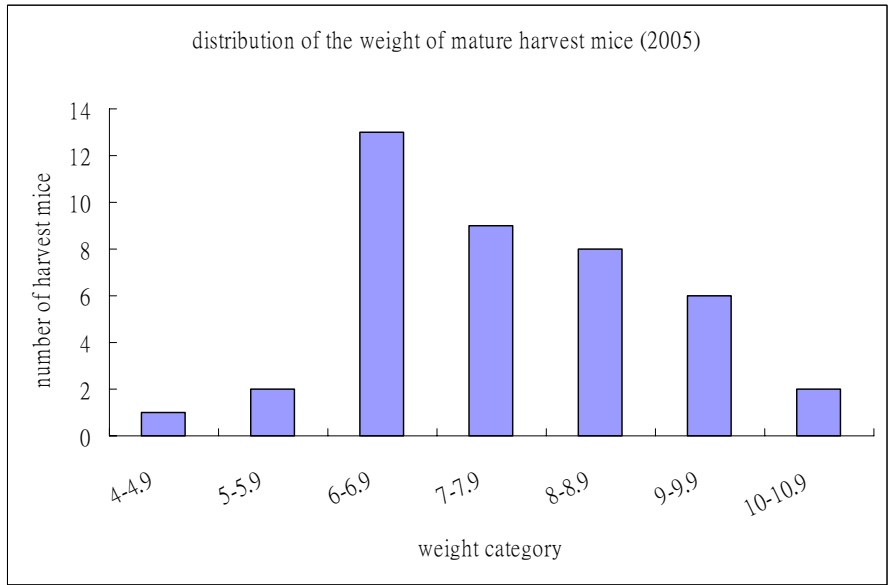
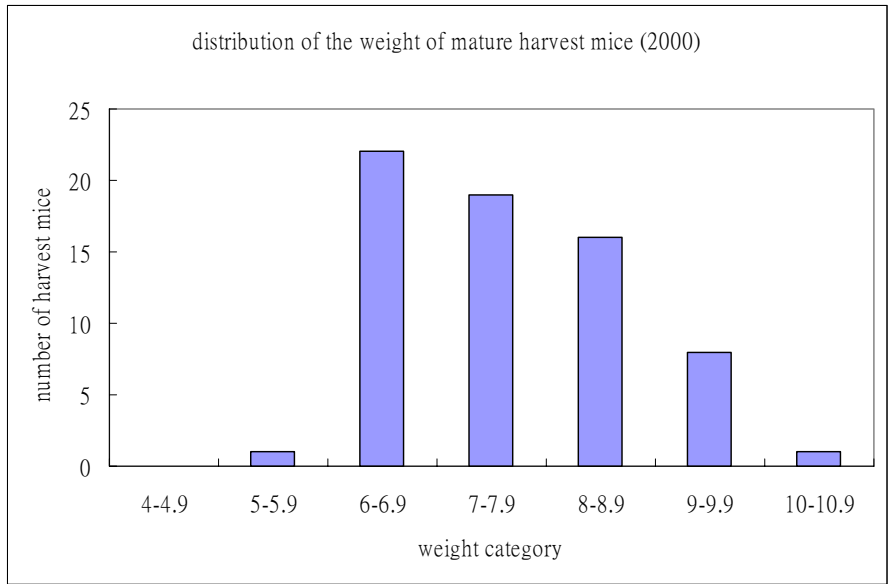


Figure 7. Body weight distribution male and female harvest mice.

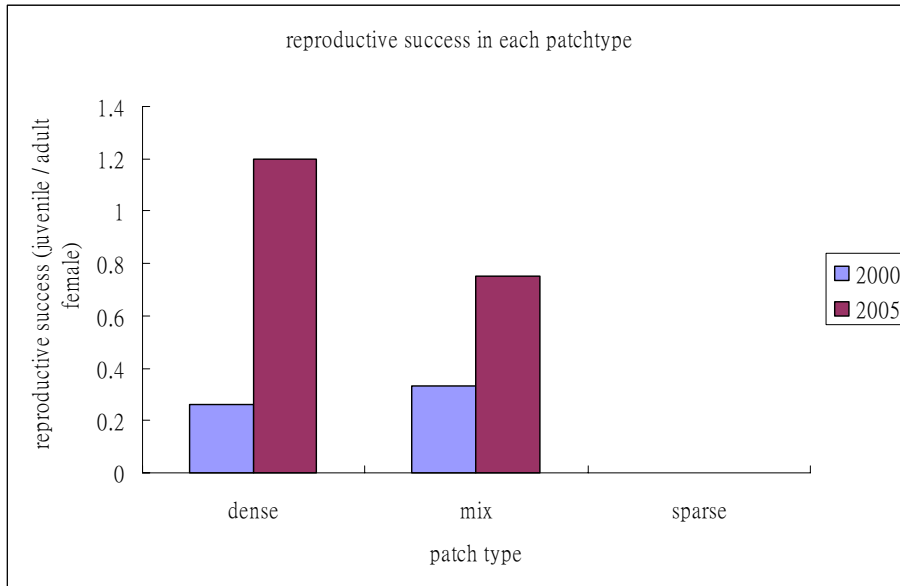


Figure 8. The reproductive success, numbers of recruits per adult female, of the harvest mouse population in each patch type.

以下論文將在美國哺乳動物學會年會(Ecological Society of America)發表。將在美國 Milwaukee 市(Wisconsin, USA, August 3-8, 2008)舉行。由於尚未成行，故未附會議心得報告。

A comparison of visual vs. odorous cues of predators on the life-history of the Latouchi's frog tadpoles

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An assumption on the study of amphibian life-history states that environmental risk is greater for larval than adult stage. Other things being equal, individuals with shorter larval stages should expect greater fitness. The threat of predation is a major environmental risk for tadpoles develop in the ponds. We presented the Latouchi's frog (*Rana latouchii*) tadpoles with predator cues (from water scorpions) to investigate their effects on the growth and development of tadpoles, and the jumping ability of froglets. Four types of cues were presented: visual, odor of predators, odor of predators consuming tadpole, & none. We tested the hypothesis that different cues had differential effects on the fitness of the Latouchi's frog. The results showed that visual cues elicited the greatest declined in fitness compared to the control (no predator cue). The mortality was high, and the body growth rate and developmental rates were both low for the visual cue group. The 2 groups that encountered odorous cues had similar fitness. Yet both had lower body growth rate and developmental rates than the control group. None of the effects on tadpoles was reflected on the jumping ability of froglets. The results indicated that visual cues had greater effects on the Latouchi's frog tadpoles. However, tadpoles facing environmental risks did not responded with accelerated developmental rates.