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關渡草澤地之巢鼠族群生態及遺傳結構

Population Ecology and Genetic Structure of Harvest Mice *(Micromys minutus)* in Guandu Salt Marsh

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中文摘要

台北市立關渡自然公園提供了巢鼠(*Micromys minutus*)不同品質的棲地類型棲息。本研究目的為探討：(1) 棲地演替是否會影響巢鼠之族群遺傳結構 (2) 巢鼠族群波動如何影響巢鼠之族群遺傳結構。研究樣區區分為密生、疏生以及疏密混生的棲地類型，而巢鼠較偏好密生的棲地類型；然而在 2000 年至 2005 年間棲地的演替使得樣區內密生棲地的面積大幅下降，造成巢鼠偏好棲息的棲地面積減少且分佈破碎化。雖然密生棲地的分布在 2000 年至 2005 年間造成巢鼠分佈破碎化，但其遺傳結構在空間上並無明顯的分化。而在 2000 及 2005 年皆由 M-ratio 偵測到族群曾歷經瓶頸效應，而 mode-shift 及異型合子偏高此兩方法皆未偵測到瓶頸效應，顯示瓶頸效應並非發生於近期，而是更早的歷史事件造成此兩年皆偵測到瓶頸效應。在年內則由於夏季的族群低點，造成 2005 年的夏季前後的族群有顯著的遺傳上的分化。綜合以上結果，關渡草澤地的棲地演替會在短時間內造成巢鼠族群在空間上以及時間上的遺傳結構變化。

關鍵字：瓶頸效應、棲地異質性、*Micromys minutus*、微隨體基因座、族群波動、族群遺傳、演替

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 site 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 year, 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, *Micromys minutus*, microsatellite, population dynamics, population genetics, succession**

Introduction

Animal populations fluctuate over time. Although various factors influence the dynamics of populations, they could be categorized into endogenous and exogenous factors (Lima et al., 1999; Stenseth et al., 2003; Pickens, 2007). 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 and Zejda, 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; 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 (Hall-Martin, 1992; Johnson et al., 1999; Trites et al., 2007; Jackson et al., 2008). The change in population sizes by those forces is usually dramatic.

Unlike anthropogenic effects, natural succession of habitats often 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 fixed patterns. The most famous case is probably the multi-annual (10-year) cycles of the snowshoe hare and their predator, lynx (Royama, 1992; Stenseth et al., 1998). Most populations fluctuate without easily identifiable patterns.

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.

As anthropogenic effects and/or natural succession alter the suitability of habitats, the genetic structures for animal populations change as well. Generally, many studies focused on the relationship between succession and population ecology (e.g., Masters, 1993; Haim and Izhaki, 1994; Layme et al., 2004; Kearney et al., 2007; Yarnell et al., 2007; Janova et al., 2008). Few studies pay attention to how natural succession, an ecological time scale phenomena, changes genetic structure for animal populations (but see Tallmon et al., 2002). Natural succession changes the amount of suitable habitats, thus could indirectly alter the sizes and genetic structure of populations. Furthermore, natural succession often changes the dispersion of

suitable habitat patches, thus, the heterogeneity of the habitat and the formation of subpopulation would changes (Layme et al., 2004; Kearney et al., 2007).

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 (Ishiwaka and Mori, 1999).

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 England and Russia (Sleptsov, 1947; Piechocki, 1958; Migula et al., 1970; Kaikusalo, 1972; Trout, 1976). 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 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 during 2000-2004, I found that the Guandu salt marsh underwent rapid succession. The composition and spatial structure of vegetative community had 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.

The study of small mammal community in 2000 by Lee Yi-Chuan (Lee 2000) offered me a great opportunity to compare the population fluctuation and population genetics of the harvest between 2000 and 2005. It allowed me to investigate the effects of habitat succession over the 5 years. I proposed a conceptual map (Fig.1) indicating factors that might influence the genetic. structure of the harvest mouse population in the Guandu Marsh. I tested two hypotheses: (1) habitat succession reduced suitable habitats, and consequently reduced population sizes; (2) habitat succession fragmented suitable habitats, and consequently altered population genetic structure.

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 Jilon 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 Guegetation included *Alternanthera philoxeroides* (Moq.) Griseb., *Ipomoea cairica* (L.) Sweet, *Commelina diffusa* Burm. f., *Brachiaria mutica* (Forsk.) Stapf, *Panicu*andu Nature Park, including harvest mouse (*Miromys minutus*), *Rattus losea, Apodemus agrarius, Mus caroli*, *Crocidura shantungnensis* and weasel (*Mustella sibrica*) (Lee, 2001). Major v*m repens* L., *Paspalum distichum* L., *Phragmites communis* (L.) Trin., *Typha angustifolia* L. (Lee, 2001).

Trapping

A 215 X 65m (13975 m^2) 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). The grid was surrounded by natural and artificial boundaries on nearly all sides, and was separated from other habitats suitable for the harvest mouse in the surrounding area. The channelized Jhong-Gang river flowed along the north side of the grid; two ponds and bareland, avoided by the harvest mice, inlayed the south side. The east side of the grid was bordered by common reed, also avoided by harvest mice. An irrigation ditch ran parallely to the east side lied behind the common reed. The distance between the ditch and east boundary of the grid was about 30 meters. At the west side, the salt marsh extended for another 50m beyond the grid, narrowed by a pond lied at the south-west of the grid, and ended with the Guandu stable. The study site largely overlapped with the grid of Lee (2001). 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, Grahnab, 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 with peanut butter, and checked the following morning and late afternoon 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 immediately released at the point of capture after processing. Clipped toes were preserved in 80% alcohol in the field and later stored in -20℃ in the laboratory for subsequent molecular analyses.

Habitat patch types

According to the spatial distribution of dominant plant species and the preference of harvest mice (Bence et al., 2003; Kuroe et al., 2007), 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 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, both dense and sparse species appeared. The category of each patch was shown in Table.1. 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^{TM} 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℃ 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℃ with a solution of 2X SSC, 0.1% SDS, and then twice, each for 15 min at 65℃ 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 Mg^{2+}, 0.2 mM dNTP, 10X buffer (20mM of Tris-HCl (pH8.8), 10mM KCl, 10mM $(NH_4)_2SO_4$, 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℃ 5 min, followed by 40 cycles of 94℃ 30 s, optimal annealing temperature for 30 s, 72℃ for 30 s, and a final extension step at 72℃ 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

Data from 2000-2001 were provided by Ms. Yi-Chuan Lee. Data included the capture-mark-recapture data of small mammals in Guandu salt marsh, toe-clip tissue of harvest mice and vegetative cover at each trapping station from 2000-Mar to 2001-Feb. Because the trapping grid of Lee (2000) was larger than mine, I only used the data or samples of harvest mice and plant cover from the overlapped trapping station of the two trapping grids for further population ecology and population genetics analyses.

Analyses of population ecology

Succession was detected by comparing the cover of the major plant species in the study site between 2000 and 2005. Though the sampling methods of cover were slightly different between the two years, both presented detail vegetative composition of the study site. I used the same criteria for both years to classify the habitats into three patch types: dense patch, sparse patch and mix patch (Table 1). The difference in the coverage of each patch type between the two years was calculated by G-test to determine if succession occurred. The preference of harvest mice to each patch type was tested with chi-square tests (test of goodness of fit).

I estimated population size with the minimum number known alive (MKNA) method. I used chi-square tests to examine if the distribution of population sizes

among seasons were different. I used G-tests to test if sex ratios were significantly deviated from zero within years, seasons and patches.

For each trapping session I estimated the number of resuits, defined as the individuals that were caught the 1st time in the traps. They could be new borns from the study population or immigrants from outside the study population. I defined disappearance as individuals disappeared caught at least once but never showed up again which could be caused by death or emigration. The number of disappearance was also calculated for each trapping session. The immigration or emigration of harvest mice from the study site should have been minimal since the study population was more or less surrounded and isolated by landscape features hostile to the harvest mouse as I described earlier..

The differences in the number of recruits or disappearance among seasons and patches between two years were tested with chi-square tests (test of homogeneity). The difference between years was tested with Wilcoxon signed rank test. The distribution of recruits or disappearance among patches was tested with chi-square (test of goodness of fit) to see if the distribution was random.

Reproductive success was defined as the number of new juveniles divided by the number of adult females. The reproductive success of adult females between dense and mix patches was compared with Fisher's exact test. The sample size of

reproductive success for the sparse patches was too small to make meaningful comparison.

Analyses of population genetics

I used FSTAT 2.9.3 to obtain observed and expected heterozygosities, allele counts and size ranges (Goudet, 1995). Then CERVUS 3.0 was used to test each locus for Hardy-Weinberg equilibrium and linkage disequilibrium (Kalinowski, 2007). The nominal significance level of 0.05 was corrected with the sequential Bonferroni procedure when testing linkage disequilibrium (Holm, 1979). Null alleles was detected with CERVUS 3.0. Large allele drop out and error due to stutter band was detected with MICROCHECKER (Van Oosterhout et al., 2004).

I used two approaches to investigate the population structure through spatially and temporally perspectives. First, I used a Bayesian approach to conduct clustering analyses without predefined population in study site. Second, I used a traditional population differentiation approach based on F_{ST} analysis.

In spatial population structure, I used STRUCTURE 2.1 (Pritchard et al., 2000) to conduct clustering analyses. In admixture model, analyses were run in length of burn-in period for 50000, numbers of MCMC after burn-in for 5000, iterating K=1,2 and 3 five times and with spring or autumn(winter) populations. I also used

Arlequin 3.01 (Excoffier et al., 2005) to calculate pair-wise estimates of F_{ST} between predefined subpopulation, and these estimators were statistically tested with ten 100 permutations. I chose to calculate F_{ST} values rather than R_{ST} values because of the better performance of F_{ST} estimates when divergence among samples was expected to be low (Balloux and Goudet, 2002).

For the analyses of temporal population structure, I used STRUCTURE 2.1 (Pritchard et al., 2000) to detect temporal population structure without prior population information. The setting was similar to the description above. I also used Arlequin 3.01 (Excoffier et al., 2005) to calculate pair-wise estimates of F_{ST} between pre-summer and post-summer populations, and these estimators were statistically tested with ten 100 permutations.

I used three methods to investigate population bottleneck effect in 2000 and 2005. First, allele frequency data was tested for evidence of a "heterozygosity excess" (Cornuet and Luikart, 1996; Luikart and Cornuet, 1998) using the program BOTTLENECK (Piry et al., 1999). Calculations were performed using the two-phase model of mutation (TPM) which is intermediate to the IAM and SMM model and best fit the mutation of microsatellite (Dirienzo et al., 1994). Three statistical tests (sign test, standardized differences test, and Wilcoxon signed-ranks test) were conducted in order to determine whether there was significant heterozygosity excess, which may

indicate that a recent bottleneck has occurred. Second, a qualitative descriptor of allele frequency distribution (the mode-shift indicator), which discriminated between bottleneck and stable populations (Luikart et al., 1998), was conducted with the software BOTTLENECK. Third, I also tested for bottlenecks using the method purported by Garza and Williamson (2001). The method was based on estimating *M,* the ratio of the number of alleles to the range in allele size, which was expected to be reduced after a bottleneck. M ratio was calculated with the program AGARst 3.3 (Harley, 2003). The critical value of *M (Mc)* was generated with the program critical M (Garza and Williamson, 2001). The significance of the observed values is determined by comparing the mean M over all loci with a distribution on M values calculated from theoretical populations in mutation-drift equilibrium. *Mc* is set at the lower 5% tail of this distribution. I assumed a two-phase mutation model (di Rienzo et al., 1994) with 10% larger mutations with an average 3.5 repeat units as recommended by Garza and Williamson (2001) and $\theta = 4Ne\mu = 1$, 4 or 10 (where μ= mutation rate = 10^{-3} $locus^{-1}$ $generation^{-1}$ and *Ne* = 250, 1000 and 2500, respectively, is the pre-bottleneck effective population size).

I tested all three methods rather than any one of them because heterozygosity excess and mode-shift can detect very recent bottlenecks. As M-ratio can detect recent bottleneck, but also allow to detect ancient bottlenecks hundreds of generations ago

that may be difficult to observe with the heterozygosity excess or mode-shift approaches (Zenger et al., 2003; Abdelkrim et al., 2005; Spear et al., 2006).

Results

Population ecology

Population size and sex ratio

Population size, estimated as the minimum number known alive (MNKA), fluctuated within years (Table 2). In 2000, the population size reached low (1-2 individuals / month) during summer. In 2005, the population size remained low from summer into winter (Table 2). The population sizes reached 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 2000 and 2005 ($\chi^2 = 101.53$, $d.f. = 3$, $P < 0.001$).

There were 74 males and 47 females in 2000; 37 males and 24 females in 2005. Sex ratios were male-biased in both years, however, only the bias in 2000 was significant (*G*-test, $G = 6.08$, $d.f. = 1$, $P=0.0137$). Within each year, there were more males than females in all seasons, however, only the bias of 2000 spring ($G = 5.01$, $d.f. = 1$, $P=0.0252$) and 2005 summer ($G = 6.931$, $d.f. = 1$, $P=0.0085$) 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. In 2000, sex ratios were 1.54 and 1.71 in dense and mix patch respectively. In 2005, sex ratios were

1.55 and 1.87 in dense and mix patch respectively. However, only the dense patch population in 2000 deviated from 1:1 significantly (G-test, G = 4.818, d.f. = 1, p=0.0282<0.05).

Recruitment and Disappearance

There was no recruitment in summer both years. In other seasons, more recruits appeared in 2000 than 2005 (Table 3). The distribution of recruits among seasons was not significantly different between years (χ^2 = 6.15, *d.f.* = 3, *P*=0.1045), though the amount of recruits were significantly larger in 2000 than 2005 (Wilcoxon signed rank test, W+ = 36, W- = 0, N=8, *P* =0.008). There were few disappearances in summer both years. However the disappearances made up 25 % and 33.33 % of summer population in 2000 and 2005, respectively. The distribution of disappearances among seasons between years was significantly different (χ^2 =54.78, *d.f.* = 3, *P*<0.001), but the amount of disappearances were not different between two years (Wilcoxon signed rank test, W+ = 36.50, W- = 18.50, N = 10, *P* =0.16). (Table 4)

The distribution of recruits among patches was not significantly different between years (χ^2 = 2.715, d.f. = 2, *P*=0.2573>0.05). The recruits among patches distributed randomly in 2000 (χ^2 =4.3471, d.f. = 2, *P*=0.1138>0.05), but the

distribution was not random (χ^2 =8.2251, d.f. = 2, P=0.0164<0.05) in 2005. The distribution of disappearances among patches was not significantly different between years (χ^2 = 3.521127, d.f. = 2, P=0.17). The disappearances distributed randomly among patches in 2000 (χ^2 = 3.857303, d.f. = 2, P=0.15), but the distribution was not random in 2005 (χ^2 = 14.25556, d.f. = 2, P=0.0008<0.001).

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 increased from 2.48 % to 8.31 % (Table 5). 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 for the dense patch type by the harvest mouse was significant in 2005 (χ^2 = 15.10, *d.f.* = 2, P=0.0005), but not in 2000 (χ^2 = 5.80, *d.f.* = 2, P =0.055). 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 as juveniles (Fig.7). The reproductive success of harvest mice within each patch type in 2000 and 2005 were shown in Figure.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=0.433). 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=0.292).

Genetic structure of the harvest mouse population

Variation of microsatellite

Six microsatellite loci were cloned from traditional method. The characteristics of 6 polymorphic loci show in table.6. 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 7). Comparing the genetic diversity between 2000 and 2005, the number of alleles per locus (Wilcoxon signed rank, W+ = 12.50, W- = 2.50, N = 5, P=0.18), allelic richness

(Wilcoxon signed rank, W+ = 16.50, W- = 4.50, N = 6, P=0.22) and Ho (Wilcoxon signed rank, W+ = 15, W- = 6, N = 6, P=0.44) are not significantly different. No loci showed departure from the Hardy-Weinberg equilibrium. I detected linkage disequilibrium in 3 pairs of loci in the population of 2000 (Table 8). The disequilibrium in 2000 population may be caused by genetic drift or demographic change rather than physical linkage of these loci (Ohta, 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 values were below the critical value under θ = 1, 4 or 10, and indicated historical bottleneck events (Table 9). The M-ratio is a more powerful method than the other two (Zanger et al., 2003; Abdelkrim et al., 2005; Spear et al., 2006). 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 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. 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 2.1 (Pritchard et al., 2000; Falush et al., 2003) showed that the population was not temporally structured (K=1). On the other hand, although F_{ST} values indicated that populations did not differentiate between season in 2000 (F_{ST} = 0.00852, permutation times=10100, P=0.336), the F_{ST} value supported that the population was not temporally structured in 2005 (F_{ST} = 0.012, permutation times=10100, P=0.018).

Spatial distribution and genetic differentiation

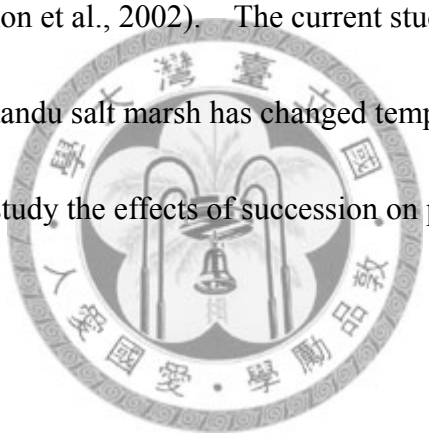
Harvest mice distributed mostly in dense patches. In 2000, dense patches were more or less contiguous, yet were separated by sparse patches in 2005 (Fig. 4 and 5). Thus, the population tended to aggregate on the east and west sides of the study site (Fig.5). The gene flow between the two sides was low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

two sides in 2005, and only one disperser dispersed 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. 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, permutation times=10100, P=0.73) and 2000 (F_{ST} = 0.007, permutation times=10100, P=0.101).

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 communities have been studied a lot (Briani et al., 2004; Yarnell et al., 2007; Zhang et al., 2007), 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.4), and offered an 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; Kuroe et al., 2007), as supported by a previous study in the same site (Lee, 2001). The highest densities of harvest mice occurred in dense patches in both year 2000 and 2005 (Fig.6), and the preference was statistically significant in 2005 and marginally in 2000 (P=0.0553). The succession in Guandu salt marsh not only changed the distribution of different patches, but also

the proportions among them (Table 5). The area of dense patches favored by *M. 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 factors which were independent of succession. It was not clear which of those factors contribute more than others to the changes observed in the harvest mouse population.

The reproductive success was highest in dense patches in 2005. In 2000, the averaged reproductive success was not different between dense and mix patch (Fig.8). This pattern could be explained by the ideal free distribution (Fretwell and Lucas, 1970). 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 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; Migula et al., 1970; Kaikusalo, 1972; 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.

Other than population fluctuation of harvest mice, environmental change can also affect the bottleneck detected here. Guandu plain collapsed and became the lake-bed of Taipei Lake in an earthquake in 1694. The water retreated around 1859, marshes appeared along river sides and inland was exploited by farming. So the population of harvest mice in Guandu salt marsh arrived in recent one hundred and fifty years. Farmland became the major landscape feature in the inland of Guandu plain and marsh distributed along the river sides until late 1900s. The building of the dike and broadening of the Guandu Notch facilitated the expanding of *Kandelia candel*(L.)Druce in the river side of the dike from 0.17ha in 1979 to 17.34ha in 1993

and all 10.60ha salt marsh disappeared from 1979 to 1989 (Lin, 1994). And the inland side of the dike, the salt marsh was occupied by waste dumps and buildings. Since harvest mice don't live in mangrove or artificial facilities, the habitat for them reduced in the recent several decades. In summary, the major fluctuation of harvest mice year to year, the founder effect of the harvest mouse population after the Taipei lake retreated, and the change and damage of suitable habitats could result in the detection of bottleneck effect in 2000s.

Seasonal dynamics of harvest mice and its effects on genetic structure

Both the field data in 2000 (Lee, 2001) and 2005 indicated that *M. minutus* population size fluctuated seasonally. In spring or winter, the population size reached the high point of the year (Table 2). 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 *M. 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 from Barn owl

pellets as well (Buckley, 1977). This suggested that harvest mouse population size did reduce in summer. Nevertheless, shift in habitat utilization between seasons might be a possibility that resulted in summer low in the population from Guandu salt marsh. The sparse patches composed of common reed (*Phragmites communis*) that 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 *P. communis* leaf area is too low to build nests. Estivation was also not likely to be the reason of summer reduction because from capture-mark-recapture data, few harvest mice lived in spring can remained after summer.

Probably due to the prolonged population reduction in summer, the F_{ST} value indicated populations between 2005 spring and autumn have been differentiated. However, 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 be more susceptible to genetic drift than higher density populations. Low density phase during population fluctuation would elevate the degree of genetic differentiation (Bowen, 1982; Berthie et al., 2006). 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 in 2000, until 2005 when several artificial ponds were established for wetland management in the Guandu Nature Park (Lee, 2001). The construction greatly reduced the harvest mice habitat and density of harvest mice and therefore reducing immigration from the unsampled area into study site (see Waser and Elliott, 1991). 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 and smaller population size. 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. There may be a positive relationship between timespan and temporal differentiation (Aars et al., 2006). Furthermore, this finding suggested the impact on population genetic structure could happen or be detected in a relatively fine temporal scale compared to other temporal

differentiation studies (Sinsch, 1992; Stewart et al., 1999; Aars et al., 2006; Schweizer et al., 2007). The temporal differentiation signature was stronger in low density phase (2005) rather than high density phase (2000) also implied the degree of population differentiation could be affected by the density of populations (Bowen, 1982; Berthier et al., 2006). It suggests that the variation of population differentiation could change along with the annual fluctuation of harvest mice. The temporal genetic structure would be more vulnerable during the low density phase than high density phase.

Conclusion

Succession offers another perspective for studying genetic structure. This kind of researches relied on relatively long term sampling and took a lot of effort in the field. *M. minutus* populations undergo great changes in density, both seasonally and from year to year. This research suggests succession could be an important force in shaping the genetic structure both spatially and temporally. The variation of temporal genetic differentiation of *M. minutus* would be varied from year to year and the seasonal population differentiation could happen while population density is low.

Both spatially and temporally within population perspective were studied in this research. Previous studies showed that among population spatial effects on genetic structure were stronger than within population temporal effects (Whitlock et al., 1992; Schweize et al., 2007). This case offered an example if the scale were within population both spatially and temporally, the temporal effect on genetic structure could be stronger than spatial effect.

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Table. 1. The three patch types in Guandu marsh.

	Commelina diffusa ***Brachiaria mutica*** ***Panicum repens***	***Phragmites communis***
Dense patch	> 90%	-
Sparse patch	<10 %	>90%
Mix patch	10% - 90%	<90%

Table. 2. 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
Total		25			2			39			75	
2005	35	20	19	5	5	2	4	0	1	2	5	14
Total		45			5			5			15	

Table. 3. The number of recruits by year, seasons, and month.

	spring			summer			autumn			winter		
	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
2000	0	11	10	0	0	0	4	17	17	25	17	16
Total		21			0			38			58	
2005	0	4	5	0	0	0	2	0	1	1	3	10
Total		9			0			3			14	

Table. 4. The number of individuals disappeared by year, seasons, and month.

	spring			summer			autumn			winter		
	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
2000	2	9	10	1	0	0	4	12	6	23	24	
Total		21			1			22			47	
2005	18	7	13	0	3	1	4	0	0	0	1	
Total		38			4			4			1	

Table. 5. The percentage of each patch type in dry and wet seasons in 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. 6. Characteristics of 6 polymorphic microsatellite loci in *Micromys minutus*. i, interrupted repeat motif; Ta: annealing temperature

locus	Repeat motif	Primer sequences	Ta(℃)	Allele size range(bp)	No. of alleles
MM-B03	$i(TG)_5GTA(TG)_{16}$	TCCCTTCTGCTTTCACATCA	63	159-187	10
		CCACAGAGTGTCTCTCTATTGCAG-HEX			
MM-C02	$i(TG)_{17}GTA(TG)_5$	GCCTCCCATTTTTCACAGTC-TAMRA	63	215-245	7
		AGGCTTCCTCGTTCAAGACA			
MM-D03	$(TG)_{17}$	CACACGGGCCTTTGTTCTACCTGC	60	304-352	9
		TCAGACTAACTCTCTGGGTCACTGC-FAM			
MM-E05	$(GT)_{21}$	CACTGTTAAGTTCATCTCTGTGGTTG	57.7	228-242	8
		TCTTTGCTGAGGAATGAGACTGGTCTGTGG-TAMRA			
MM-F03	$i(TG)_3C(TG)_{16}$	GCCAGTCCTGAGACCCTTTG-FAM	56.5	128-146	6
		TCTTTGCCATCAATGTAGAGCTTGCAGG			
MM-H04	$(TG)_{23}$	AGTCTTCATAATTCAACCTCATGGT-HEX	60	100-120	8
		AATCCTCAGTTATTAGTGCATGTGC			

Table. 7. 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	NS
C02	7	6.934	128	0.750	0.747	NS
D03	10	10	120	0.800	0.793	NS
E05	8	8	128	0.742	0.763	NS
F03	6	5.952	126	0.730	0.706	NS
H04	8	7.875	128	0.711	0.708	NS
			2005			
B03	10	10	74	0.757	0.854	NS
C02	6	5.972	72	0.736	0.761	NS
D03	7	7	70	0.814	0.748	NS
E05	6	6	74	0.608	0.637	NS
F03	6	6	73	0.726	0.732	NS
H04	5	5	73	0.740	0.709	NS

Table. 8. 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. 9. Test for population reductions in genetic variation in each temporal sample based on the M-ratio test

	Samples	
	2000-2001	**2005-2006**
M	0.669	0.618
Mc $(\theta = 4N_e\mu = 1)$	0.7505	0.7430
Mc $(\theta = 4N_e\mu = 4)$	0.7019	0.6858
Mc $(\theta = 4N_e\mu = 10)$	0.6843	0.6599

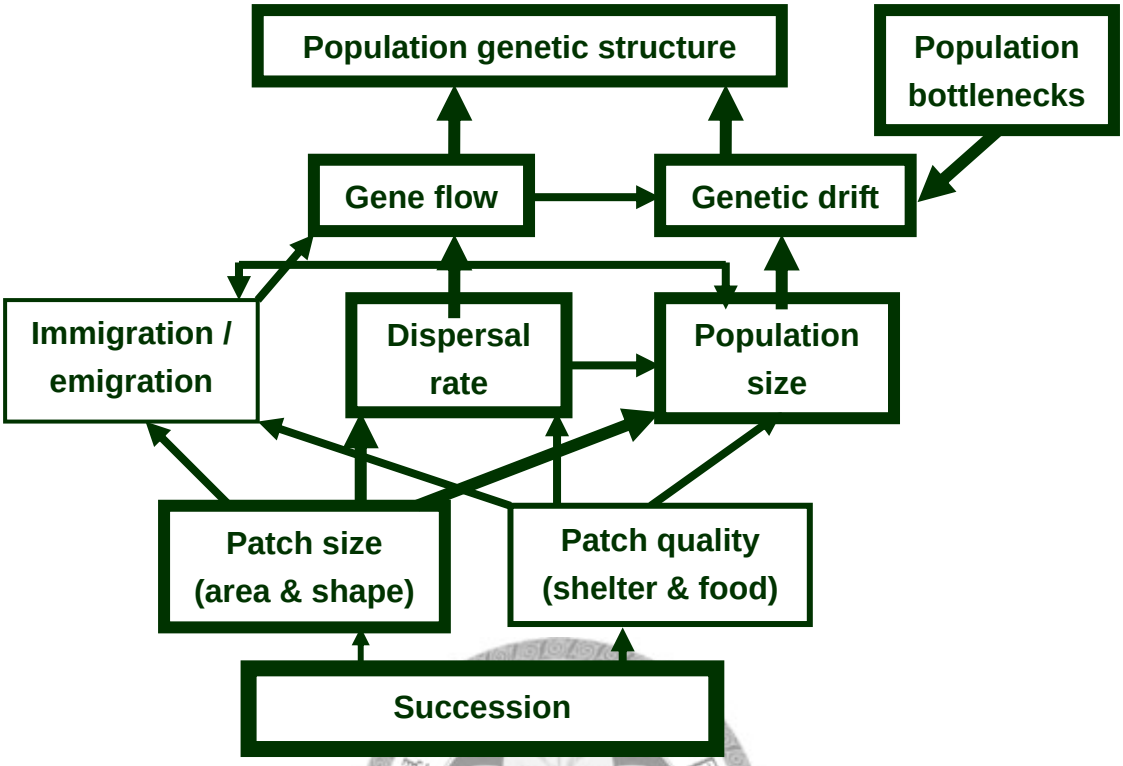


Figure. 1. The conceptual map of the current research.

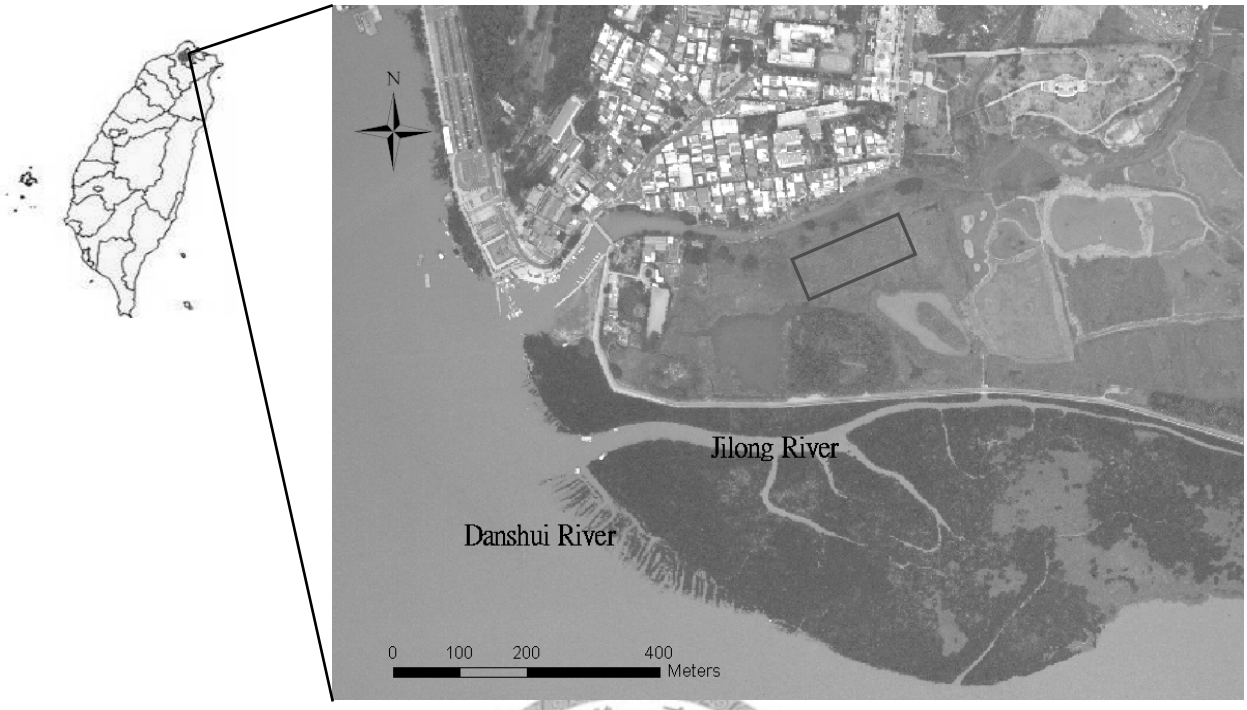


Figure. 2. An aerial view of the Guandu Nature Reserve in northern Taiwan.

The rectangular frame indicates the location of the trapping grid.

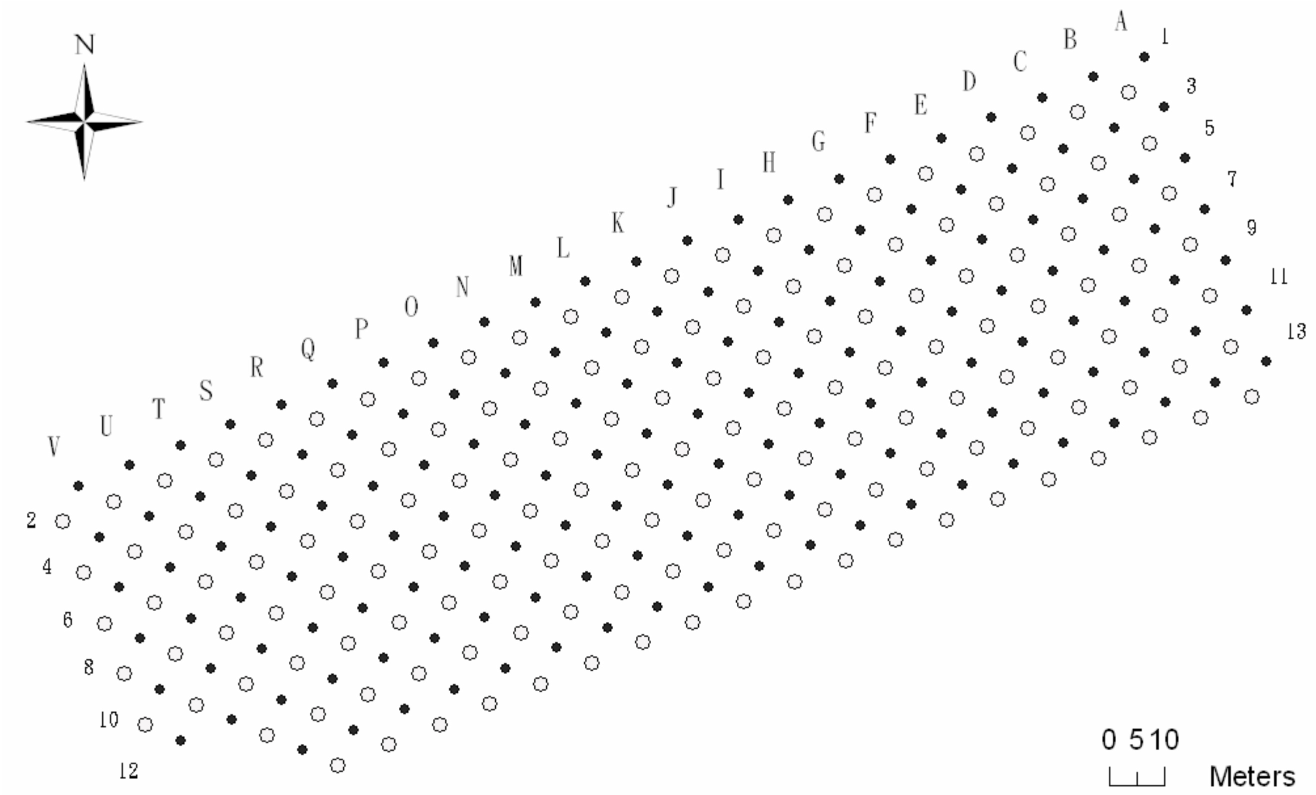


Figure. 3. The trapping grid at the study site. Solid and empty dots represent odd- and even-numbered transect lines, respectively. A total of 301 stations covered an area of 65 x 215 m^2.

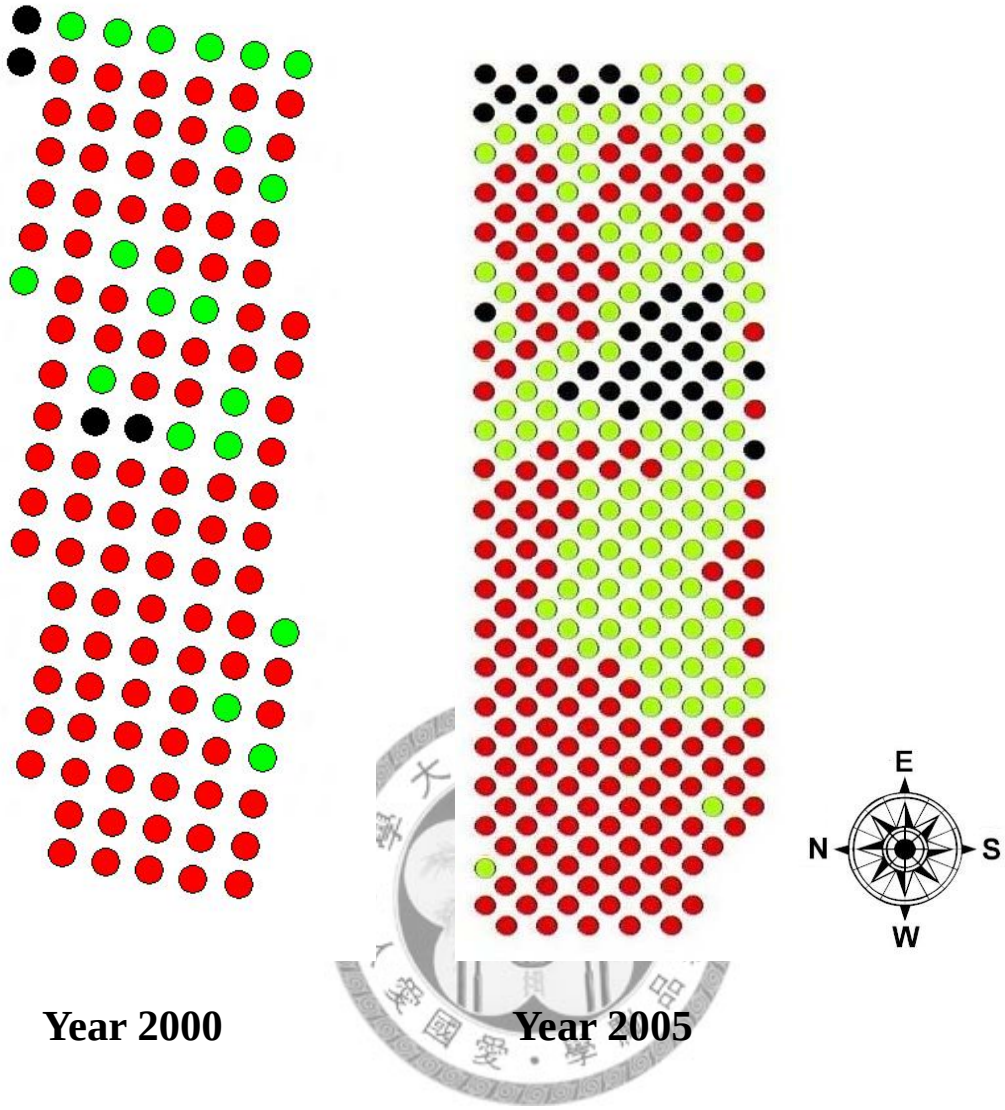
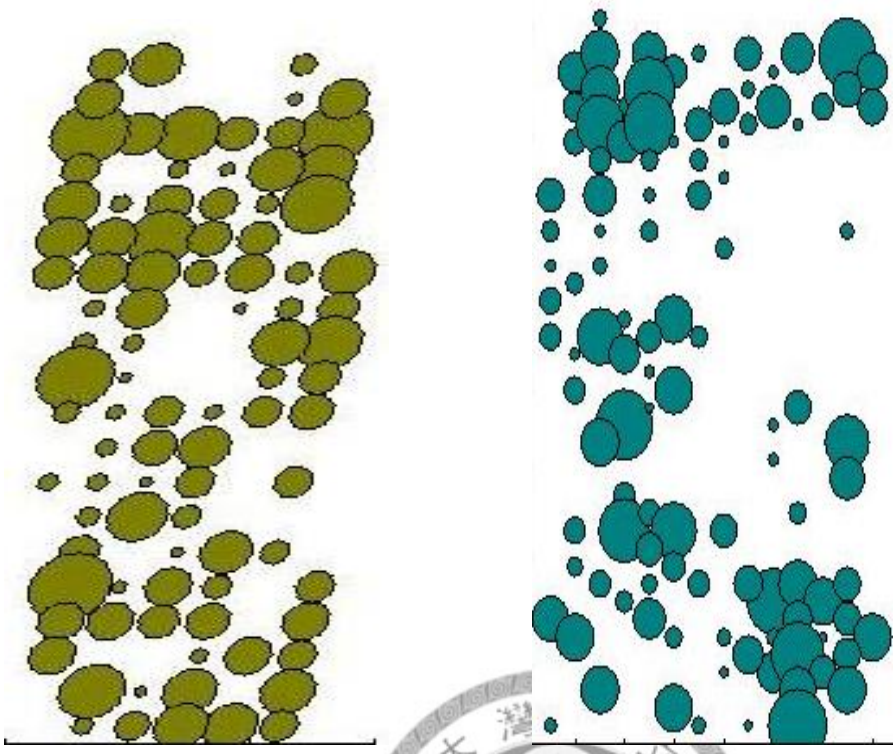


Figure. 4. Patch type at each trapping station in 2000 and 2005, red dots: dense patch; green dots: mix patch; black dots: sparse patch.



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.

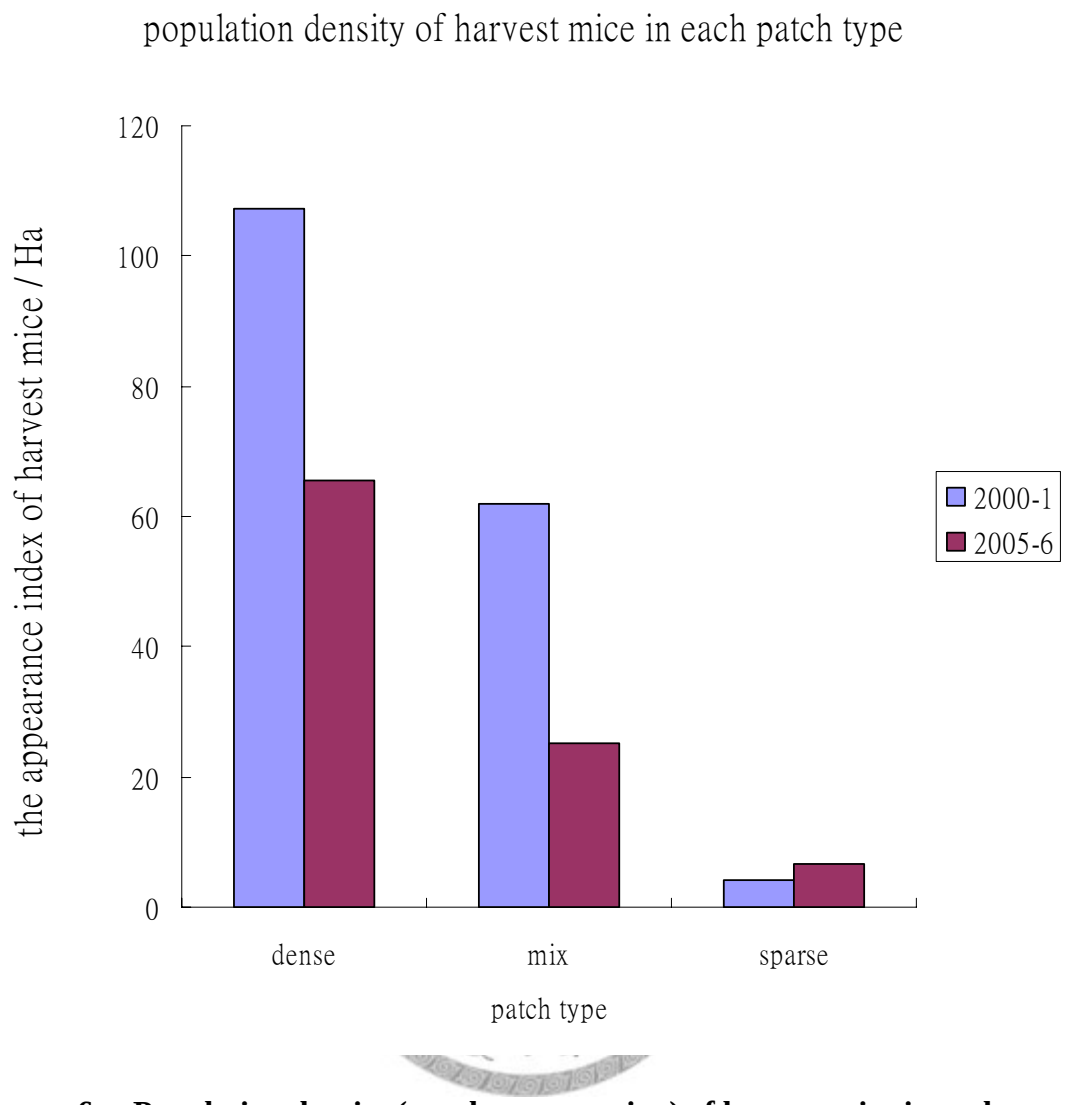
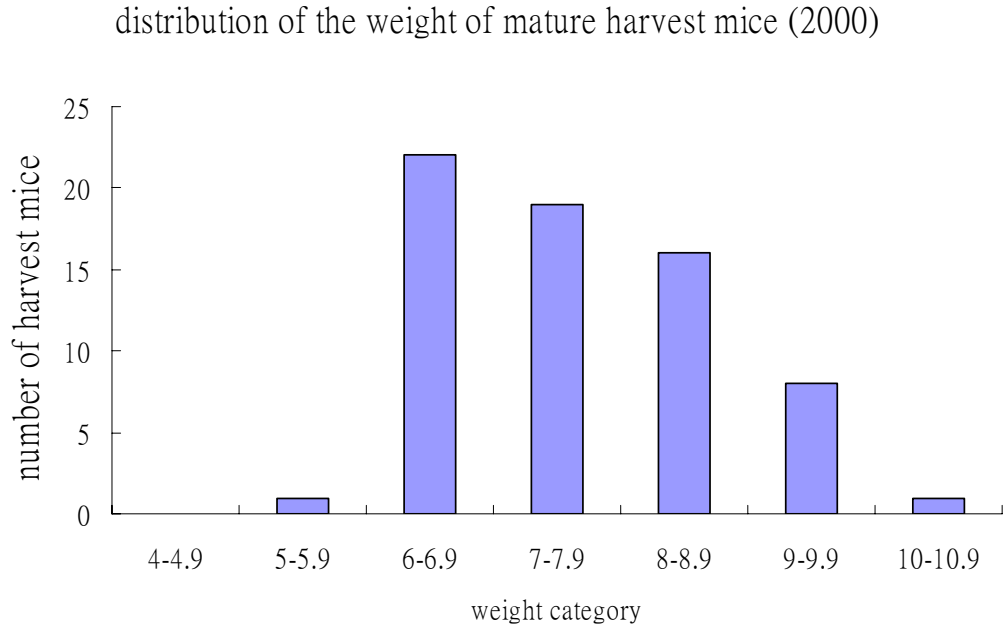
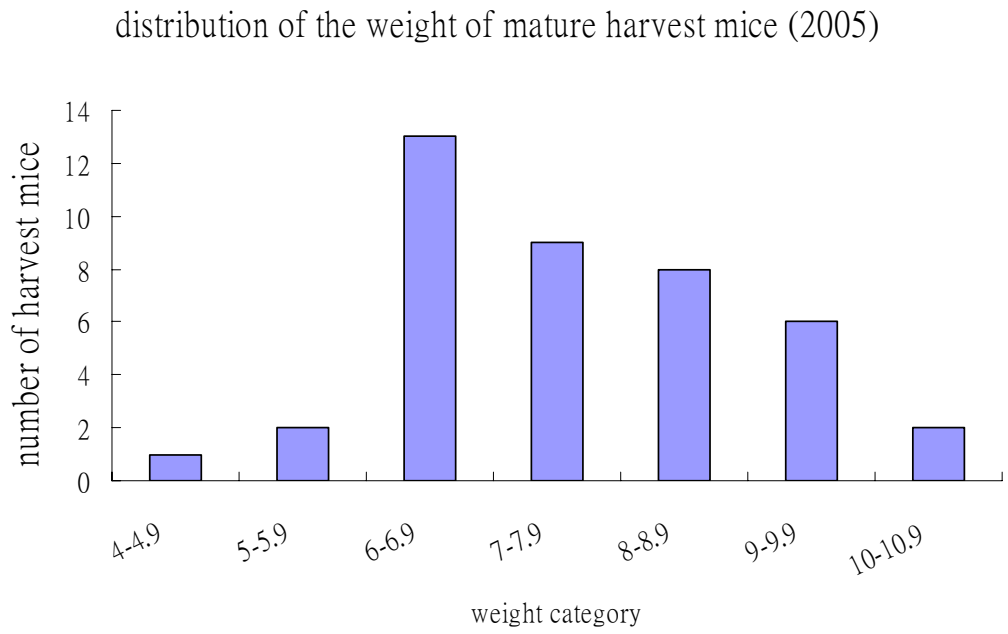


Figure. 6. Population density (number per station) of harvest mice in each patch type in 2000 and 2005.



(a) 2000



(b) 2005

Figure. 7. Weight distribution of sexually mature adult in (a) 2000, and (b) 2005.

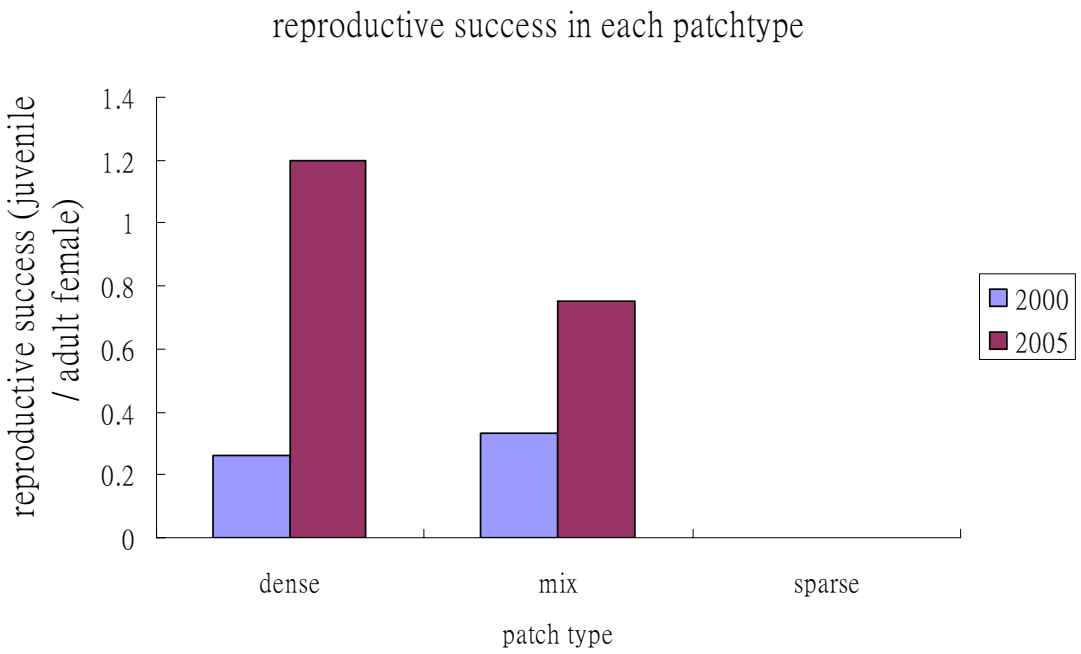
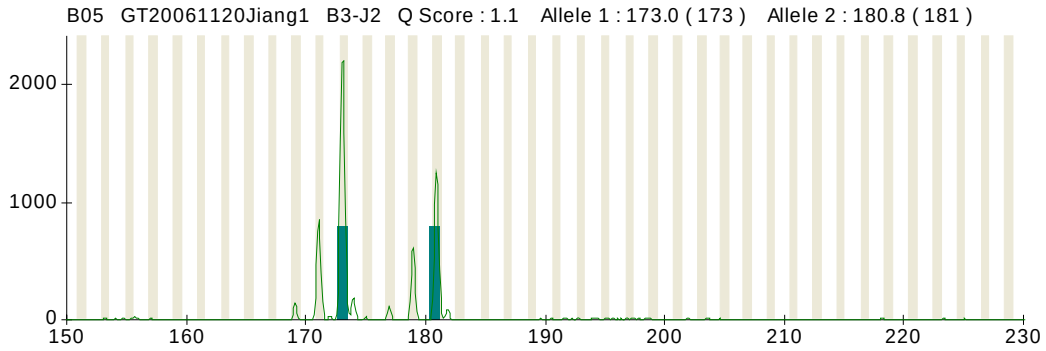


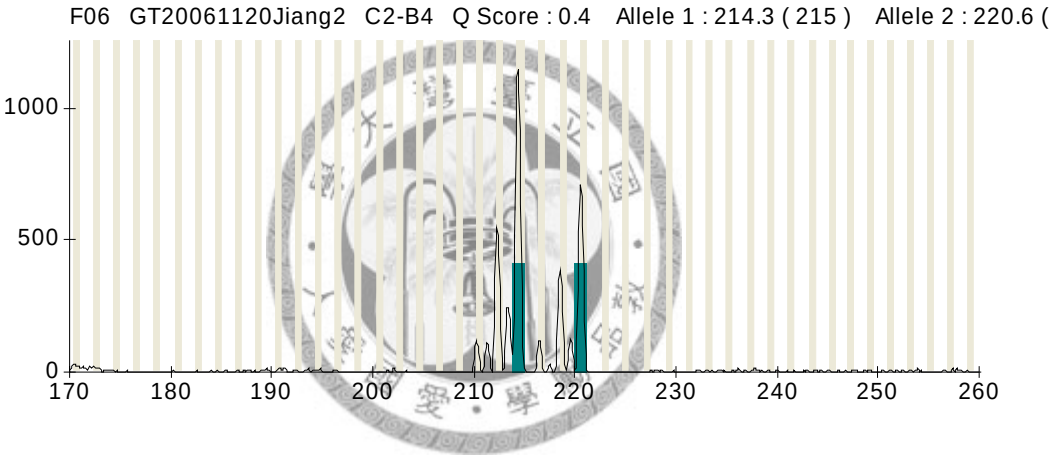
Figure. 8. Reproductive success (number of juveniles per adult females) of the harvest mouse in each patch type.

Appendix A. Samples of scoring genotyping peaks at each microsatellite lous.

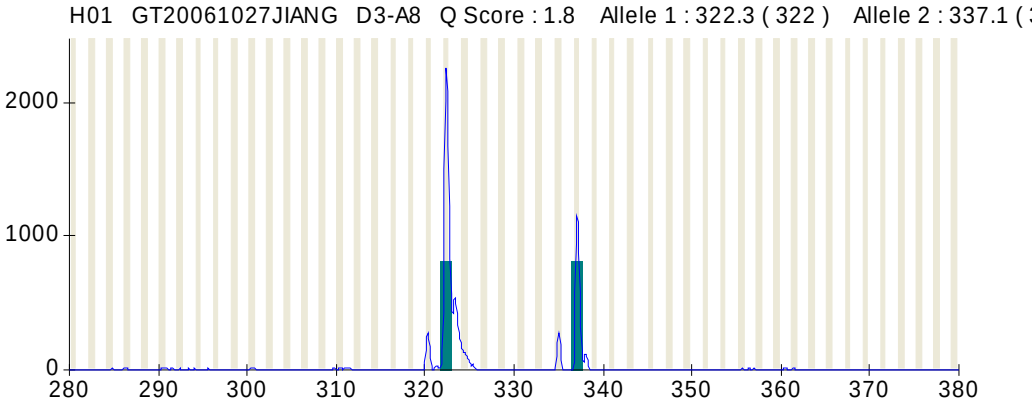
MM-B03



MM-C02

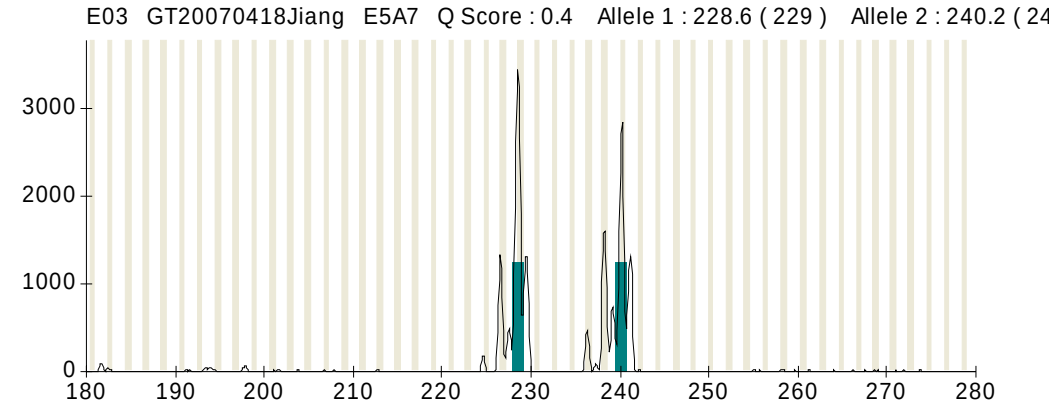


MM-D03

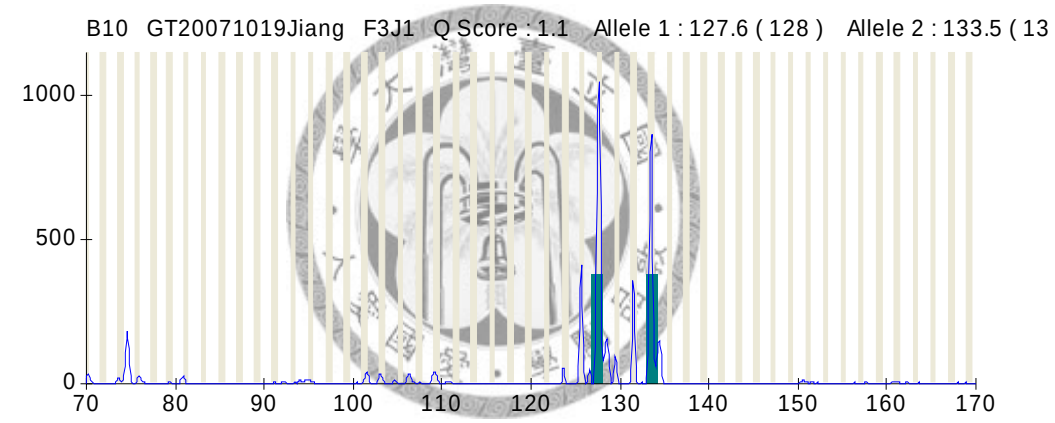


Appendix A. (continue) Samples of scoring genotyping peaks at each microsatellite lous.

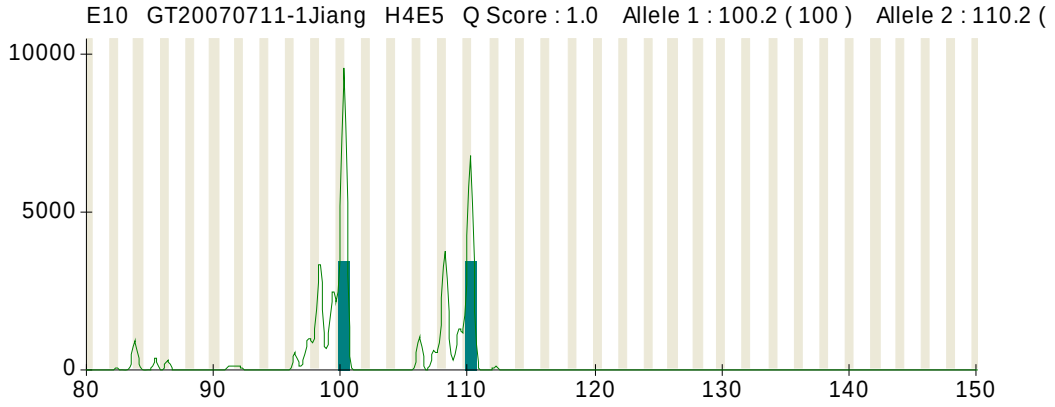
MM-E05



MM-F03



MM-H04



Appendix B. The size of the amplification products (bp.) of each sample in 6 loci.

ID	year	B03		C02		D03		E05		F03		H04	
E3	2001	181	187	221	233	324	324	228	236	128	146	106	110
E4	2001	171	181	221	225	322	345	228	230	146	146	110	120
E5	2001	171	177	221	221	324	337	230	236	128	146	100	110
E6	2001	171	173	233	233	322	345	234	236	134	146	120	120
E7	2001	177	181	225	225	324	345	228	234	134	146	106	110
E8	2001	181	181	225	233	324	324	228	228	128	134	110	120
E9	2001	173	181	225	225	322	337	228	236	134	146	110	110
E10	2001	171	177	221	233	324	337	236	236	136	146	110	110
F1	2001	177	181	221	233	324	345	228	236	136	146	120	120
F2	2001	179	179	214	221	337	337	234	236	134	138	106	110
F3	2001	187	187	225	233	322	352	228	234	128	146	110	110
F4	2001	185	187	233	233	324	337	234	236	134	138	118	120
F5	2001	177	177	221	225	324	324	232	236	128	136	106	118
F6	2001	171	179	221	225	0	0	230	232	134	136	110	110
F7	2001	177	185	225	225	322	324	228	234	134	146	110	114
F8	2001	173	177	221	221	322	345	228	236	128	146	110	118
F9	2001	173	185	233	233	322	337	228	242	134	146	110	120
F10	2001	177	181	221	233	0	0	228	236	146	146	110	120
G1	2001	171	185	221	225	324	337	230	236	146	146	100	120
G2	2001	173	185	221	221	322	324	228	236	128	128	118	120
G3	2001	177	185	221	233	322	324	228	228	128	136	118	120
G4	2001	173	187	214	233	337	345	234	236	134	146	106	120
G5	2001	173	175	214	233	324	337	228	240	128	128	120	120
G6	2001	171	185	221	225	322	333	228	236	128	146	110	120
G7	2001	181	181	233	246	342	345	230	230	128	146	110	120
G8	2001	175	187	214	233	324	337	234	240	134	134	102	120
G9	2001	173	177	221	225	322	324	228	236	128	146	110	110
G10	2001	173	179	225	225	322	333	228	230	134	146	110	120
H1	2001	175	181	214	233	324	337	236	242	128	146	106	120
H2	2001	177	181	214	221	322	345	238	242	146	146	110	118
H3	2001	181	185	221	225	337	345	236	238	128	128	110	120
H4	2001	187	187	214	214	0	0	230	238	128	146	110	120
H5	2001	177	177	225	233	337	345	230	238	128	146	120	120
H6	2001	177	177	221	233	322	337	238	238	128	146	110	120
H7	2001	177	185	225	233	322	322	230	242	146	146	118	118

Appendix B. (continue) The size of the amplification products (bp.) of each sample in 6 loci.

ID	year	B03		C02		D03		E05		F03		H04	
H8	2001	173	185	214	233	337	337	230	242	128	128	120	120
H9	2001	181	181	233	233	322	324	236	236	128	146	110	118
H10	2001	177	181	221	225	324	345	234	234	128	134	106	110
I1	2001	181	187	221	221	324	345	228	228	128	134	110	110
I2	2001	173	187	214	225	322	337	232	236	128	146	110	120
I3	2001	177	181	221	225	324	337	228	234	0	0	110	120
I4	2001	177	181	221	233	322	337	236	236	134	146	110	120
I5	2001	181	181	225	233	324	324	234	236	128	134	120	120
I6	2001	173	181	221	233	333	337	228	234	128	146	106	120
I7	2001	185	187	225	225	322	352	234	234	128	146	106	120
I8	2001	181	185	225	225	333	337	234	240	128	134	100	120
I9	2001	171	187	221	225	322	345	228	236	128	146	110	120
I10	2001	177	181	221	225	337	345	228	230	134	146	106	110
J1	2001	177	177	225	233	322	352	228	236	128	134	120	120
J2	2001	173	181	233	233	324	352	236	236	128	128	118	120
J3	2001	173	185	221	233	322	345	228	236	134	146	118	120
J4	2001	177	177	225	233	322	337	230	236	134	146	110	120
J5	2001	185	187	214	221	322	345	228	236	128	146	120	120
J6	2001	171	171	214	221	322	322	228	236	134	136	118	120
J7	2001	173	187	225	233	322	345	228	228	134	134	120	120
J8	2001	171	181	225	233	322	345	228	234	128	146	106	120
J9	2001	177	181	221	233	333	352	228	230	128	146	118	120
J10	2001	181	185	225	225	322	324	234	236	128	128	110	120
K1	2001	171	177	221	225	333	345	228	234	128	146	106	110
K2	2001	177	181	221	233	345	345	228	228	128	128	106	118
K3	2001	173	181	214	233	337	345	234	240	134	146	118	120
K4	2001	181	185	225	225	322	322	230	236	128	134	110	120
K5	2001	181	181	214	225	324	324	228	228	128	146	106	120
K6	2001	171	185	221	225	322	322	234	236	134	146	110	120
K7	2001	175	181	221	225	324	337	228	234	128	128	118	120
K8	2001	177	181	221	225	0	0	228	234	128	146	110	120
K9	2001	181	185	225	233	324	337	234	236	128	146	110	110
K10	2001	173	177	233	233	322	337	230	234	128	128	118	120
L1	2001	171	173	225	233	322	345	228	234	128	128	118	118
L2	2001	177	177	221	225	333	352	228	236	136	146	110	120

Appendix B. (continue) The size of the amplification products (bp.) of each sample in 6 loci.

ID	year	B03		C02		D03		E05		F03		H04	
L3	2001	175	181	214	233	333	337	228	240	128	136	110	120
L4	2001	171	171	221	225	324	345	228	232	136	138	100	118
L5	2001	173	185	221	233	337	337	228	240	128	128	120	120
L6	2001	181	187	214	221	337	345	234	236	128	134	118	120
L7	2001	177	187	225	233	322	324	232	234	146	146	110	110
L8	2001	171	185	225	225	337	345	228	240	136	146	120	120
L9	2001	181	181	214	225	345	345	230	234	128	146	106	110
L10	2001	171	181	214	221	322	324	228	228	128	128	106	110
M1	2001	185	187	221	225	324	337	236	238	134	146	100	120
M2	2001	175	177	221	233	333	337	228	228	134	146	110	110
M3	2001	177	181	214	225	322	345	228	236	146	146	110	110
M4	2001	171	173	214	221	324	324	228	228	128	146	110	120
M5	2001	177	181	221	225	337	345	228	230	134	146	106	110
M6	2001	181	181	221	233	324	324	230	234	128	128	110	110
M7	2001	185	187	225	225	322	345	228	236	128	136	110	120
M8	2001	171	181	221	225	333	345	228	234	134	146	106	118
M9	2001	177	181	221	233	324	324	228	236	146	146	110	110
M10	2001	179	181	221	233	322	322	228	236	128	146	102	120
N1	2001	173	181	221	225	345	345	228	236	128	134	120	120
N2	2001	177	181	231	246	322	324	228	240	128	146	110	110
N3	2001	177	179	233	233	337	345	232	236	128	134	110	120
N4	2001	185	187	233	233	322	324	236	240	134	134	118	120
N5	2001	173	181	225	233	333	345	228	236	136	146	110	120
N6	2001	171	181	214	233	322	322	236	240	128	146	110	118
N7	2001	179	185	221	225	322	345	234	236	128	146	110	120
N8	2001	171	175	221	233	324	333	228	234	128	146	110	120
N9	2001	177	179	233	233	337	345	232	236	128	134	110	120
N10	2001	173	181	225	233	333	345	228	236	136	146	110	120
O1	2001	179	185	221	225	322	345	234	236	128	146	110	120
O2	2001	171	179	233	233	304	322	228	228	134	146	102	102
O3	2001	181	185	214	225	322	324	236	236	128	146	110	120
O4	2001	185	187	221	221	322	345	234	236	128	146	110	120
O5	2001	171	173	225	233	322	345	228	234	128	128	118	118
O6	2001	173	187	221	233	322	345	228	228	134	146	120	120
O7	2001	173	181	214	221	322	324	234	236	128	138	106	110

Appendix B. (continue) The size of the amplification products (bp.) of each sample in 6 loci.

ID	year	B03		C02		D03		E05		F03		H04	
O8	2001	177	181	225	246	324	333	236	236	128	128	110	120
O9	2001	177	187	221	246	324	331	228	228	136	146	118	118
O10	2001	173	185	225	233	322	324	236	236	132	138	120	120
P1	2001	173	177	225	225	324	324	232	236	136	136	118	120
P2	2001	173	185	214	225	322	324	236	236	128	136	118	120
P3	2001	173	185	214	221	322	337	228	236	128	146	110	120
P4	2001	173	185	221	233	322	345	228	236	128	134	118	120
P5	2001	171	173	214	233	322	333	234	234	146	146	110	120
P6	2001	173	181	225	225	0	0	236	236	128	134	110	120
P7	2001	171	181	214	233	322	322	236	240	128	146	110	118
P8	2001	173	173	214	225	0	0	236	236	128	138	110	120
P9	2001	0	0	227	227	0	0	228	228	0	0	108	120
P10	2001	171	177	225	233	322	337	228	228	134	146	102	120
Q1	2001	177	181	221	225	333	345	228	234	146	146	106	118
Q2	2001	181	187	214	221	0	0	228	236	128	134	118	120
Q3	2001	173	179	214	233	322	333	228	232	128	128	118	120
Q4	2001	173	185	225	225	322	324	236	236	128	136	120	120
Q5	2001	173	185	214	225	322	322	236	236	128	136	120	120
Q6	2001	185	185	225	246	333	337	228	228	128	128	120	120
Q7	2001	171	181	233	233	322	324	228	228	128	128	106	120
Q8	2001	175	181	225	233	322	322	228	234	146	146	110	110
Q9	2001	171	185	221	233	324	345	228	234	128	128	120	120
Q10	2001	181	187	233	233	322	324	228	236	134	146	102	118
A1	2005	177	185	233	233	324	333	228	230	134	136	106	118
A2	2005	179	185	221	233	322	324	236	236	134	138	110	120
A3	2005	159	181	214	225	322	337	236	240	128	128	110	120
A4	2005	177	179	214	233	322	324	228	228	134	134	110	110
A5	2005	173	183	214	233	322	337	228	228	134	146	120	120
A6	2005	177	187	225	233	322	333	228	230	134	134	106	118
A7	2005	177	183	225	233	333	345	228	240	128	134	118	118
A8	2005	171	177	221	246	322	337	234	236	146	146	106	120
A9	2005	171	177	221	225	337	345	230	234	134	136	120	120
A10	2005	177	177	221	221	322	322	228	236	146	146	106	120
B1	2005	175	185	214	225	322	337	228	234	128	136	110	120
B2	2005	171	171	221	225	337	342	228	236	136	146	106	120

Appendix B. (continue) The sizes of the amplification products (bp.) of each sample in 6 loci.

ID	year	B03		C02		D03		E05		F03		H04	
B3	2005	183	185	221	225	322	337	228	228	146	146	120	120
B4	2005	171	181	214	221	322	337	228	230	128	136	110	120
B5	2005	181	185	225	233	333	337	228	230	134	146	110	118
B6	2005	183	185	221	225	333	337	228	234	134	136	120	120
B7	2005	179	187	225	225	337	342	228	228	146	146	106	120
B8	2005	171	181	225	225	322	337	228	236	128	138	118	118
B9	2005	171	185	221	225	322	345	228	236	128	138	118	120
B10	2005	185	185	225	246	322	337	228	228	128	146	106	120
C1	2005	177	177	221	221	322	337	236	236	134	146	106	120
C2	2005	181	185	214	214	345	345	228	234	128	128	106	120
C3	2005	171	185	221	225	322	345	228	236	128	138	106	118
C4	2005	173	185	233	233	324	342	230	236	134	138	106	110
C5	2005	173	181	214	221	324	333	234	240	134	134	106	120
C6	2005	173	185	225	225	322	322	228	230	146	146	120	120
C7	2005	177	183	225	233	322	337	228	234	146	146	106	120
C8	2005	181	181	221	225	331	345	228	228	128	146	118	120
C9	2005	185	185	225	233	322	333	228	230	146	146	110	120
C10	2005	177	185	225	225	322	345	228	228	134	134	106	120
D1	2005	185	187	225	225	337	337	228	228	128	134	120	120
D2	2005	179	181	221	233	322	337	228	236	128	146	106	120
D3	2005	175	187	225	246	333	345	234	236	134	146	106	120
D4	2005	171	183	233	246	322	337	234	234	146	146	106	106
D5	2005	173	185	233	246	337	345	228	230	136	146	106	120
D6	2005	175	187	225	246	337	345	228	228	132	134	106	120
D7	2005	173	181	214	233	345	345	228	234	128	134	118	120
D8	2005	179	185	225	233	322	337	228	228	128	146	110	120
D9	2005	171	177	214	225	337	345	232	234	128	134	118	118
D10	2005	187	187	221	246	322	333	228	228	134	136	106	118
E1	2005	175	177	214	233	322	322	228	228	132	134	110	120
E2	2005	181	185	225	225	337	345	228	228	128	146	110	120
R1	2005	175	175	225	225	322	337	228	234	136	146	106	120
R2	2005	173	181	225	225	324	345	228	230	132	146	110	120
R3	2005	159	185	221	246	322	337	228	228	128	134	106	120
R4	2005	179	181	221	233	322	337	228	230	128	146	110	120
R5	2005	185	185	221	246	337	337	228	234	128	146	118	120

Appendix B. (continue) The sizes of the amplification products (bp.) of each sample in 6 loci.

ID	year	B03		C02		D03		E05		F03		H04	
R6	2005	179	185	221	233	322	337	228	228	128	134	118	120
R7	2005	173	185	214	214	333	337	234	236	128	146	110	118
R8	2005	159	173	221	246	337	337	228	228	134	146	120	120
R9	2005	181	181	225	225	337	345	228	236	146	146	110	114
R10	2005	159	185	221	225	322	337	228	228	128	146	118	120
S1	2005	177	181	221	225	345	345	228	228	146	146	114	114
S2	2005	185	185	221	225	322	322	228	236	128	134	110	110
S3	2005	175	175	221	221	337	342	228	234	128	146	120	120
S4	2005	185	185	221	225	322	333	234	240	134	134	118	120
S5	2005	185	185	221	225	322	337	236	236	128	134	110	114
S6	2005	159	185	225	225	337	345	228	228	134	146	110	120
S7	2005	173	181	231	233	322	337	228	228	134	146	106	110
S8	2005	173	181	225	233	322	345	228	232	134	146	118	120
S9	2005	173	175	214	246	322	337	234	236	128	146	110	120
S10	2005	185	185	225	246	324	345	228	228	128	146	120	120
T1	2005	173	175	221	233	322	337	228	228	128	146	110	120
T2	2005	159	175	225	246	322	345	228	234	128	128	120	120
T3	2005	185	185	225	233	322	345	228	232	128	146	120	120
T4	2005	185	185	225	225	337	337	228	236	128	146	110	114
T5	2005	177	185	221	246	0	0	228	232	146	146	110	120
T6	2005	159	175	225	246	322	322	228	234	128	146	110	120
T7	2005	171	175	221	246	0	0	236	236	132	146	110	120
T8	2005	175	177	0	0	0	0	228	236	146	146	106	110
T9	2005	159	181	0	0	0	0	228	228	0	0	0	0
T10	2005	185	185	214	221	322	322	228	228	128	134	120	120
U1	2005	175	185	221	225	322	337	228	240	128	146	110	120
U2	2005	185	187	221	221	322	345	228	240	128	146	106	120