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Causes and Consequences of Roost Switching by the Bat Myotis formosus (V espertilionidae)

Ying-Yi Ho
Western University

Causes and Consequences of Roost Switching by the Bat *Myotis formosus* (Vespertilionidae)

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Ying-Yi Ho

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School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

ABSTRACT

Group living is widespread among animals. Theory predicts that animals should aggregate if the benefits associated with group living outweigh the costs. Benefits associated with group living could arise passively (e.g. aggregate independently to exploit a patchily distributed resource) or actively (e.g. cooperation).

Some forest-dwelling bat species switch roosts frequently, but maintain an association among specific individuals, and therefore are fission-fusion societies, the temporary splitting of colonies into several subgroups. Normally, movements between alternative roosts are costly. Fission-fusion societies might overcome the costs by providing benefits associated with large subgroup size or cooperation. In addition, sociality could influence the movements of individuals within and between social groups and affect the gene flow. However, our understanding about the underlying causes and effects of fission-fusion behaviour is still lacking.

I used both direct behavioural observation and indirect molecular analysis to study the roosting behaviour and sociality of *Myotis formosus*. In my study area, *M. formosus* used two different types of day roosts in summer, houses and foliage, which were extremely different in availability, permanency, and space. I found the roost type affected roosting behaviour of bats. In general, roost switching frequency of foliage-roosting *Myotis formosus* was high and associated with intrinsic and environmental factors. *Myotis formosus* in large subgroups switched roosts less frequently than those living in smaller subgroups. Bats were faithful to specific roosting areas, and the nonrandom association between individuals indicated that they live in a fission-fusion society and switched roosts to enhance social interaction. Reproductive status also

affected the association pattern and roost switching frequency of foliage-roosting *M. formosus* while the similarity in genetic relatedness or matrilineal relationship did not facilitate association between individuals. Benefits associated with cooperation or clustering as well as female philopatry are possible reasons for the fission-fusion society of *M. formosus*. The molecular data based on the contrasted pattern found from the biparentally inherited nuclear microsatellite markers and maternally inherited mitochondrial DNA markers also suggest that gene flow between colonies is male-mediated, while females appeared to show high fidelity to a small roosting area. Behaviourally, the male-mediated gene flow among bat colonies might occur by male dispersal and/or mating outside the colony, including swarming sites, hibernacula, or on the migration route. However, the details about the mating of this species are still unknown.

Keywords: roosting behaviour, roost switching, roost fidelity, nonrandom association, fission-fusion, population structure, gene flow, female philopatry.

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Chapter 1. Introduction

Benefits associated with group living

Many animals live in groups which vary in size, stability and composition (Krause & Ruxton 2002). Animals should aggregate to form a group if the benefits associated with group living outweigh the costs (Alexander 1974). Benefits and formations of group living can arise passively or actively. For example, in passive aggregations, individuals may group independently to exploit a patchily distributed resource, with individuals also deriving benefits from their association within the aggregation, such as reduced risk of predation, access to mates, thermoregulation, or increased foraging efficiency (Lee 1994). Individuals can also aggregate actively to benefit from interactions with specific individuals, or cooperation (Pusey & Packer 1997). Cooperative interactions can evolve between individuals that are genetically related (Emlen 1997), ultimately increasing the inclusive fitness of the group member (Hamilton 1964), or between non-kin, although this typically requires long-term associations between individuals to overcome the assumed initial short-term costs (Lee 1994). Distinguishing the benefits associated with group formation is important to understanding the evolutionary cause(s) of sociality of animals.

Group living is widespread among bats, which live in a diversity of social situations, including colonial living for part of the year (Bradbury 1977; McCracken & Wilkinson 2000). Association between group members can vary in space and time and among species. For example, in *Tadarida brasiliennsis*, aggregation size can reach 20 million individuals within a single cave (Davis *et al.* 1962), whereas many foliage-

roosting species roost alone or in small groups (Kunz 1982). Group sizes in many non-tropical species can also vary seasonally. During the summer months, females gather in maternity colonies during pregnancy, whereas both sexes gather in hibernacula during the winter when they may roost alone or in direct contact with others (Altringham 1996).

Bats can derive both passive and active benefits from group living. Although most bats do not build roosts, much of their activities are focused in roosts, which can be patchily distributed and vary in abundance according to roost type (Kunz 1982; Kunz & Lumsden 2003). One passive benefit of aggregation is incurred from simply clustering together, which reduces the cost of thermoregulation (Racey & Swift 1981; Roverud & Chappell 1991; Wilde et al., 1995; Willis & Brigham 2007). Colonial living also may reduce the risk of predation from increased vigilance and dilution effects (Fenton et al. 1994; Speakman et al. 1999). When aggregated individuals are genetically related or in long-term associations, they can gain additional active benefits through cooperation. Various forms of active benefits due to cooperation have been documented in bats (Wilkinson 1987), including information transfer that may improve foraging efficiency by reducing foraging costs when food availability and distribution is ephemeral (Wilkinson 1992; Wilkinson 1995). Group living may also lower the cost of finding suitable roosts (Kerth & Reckardt 2003), and reciprocal food sharing between kin and non-kin individuals could increase the chance of survival of the group members (Wilkinson 1984). Communal nursing may benefit lactating females that simultaneously rid themselves of excess milk and increase the overall survival of juveniles within the maternity colony (McCracken 1984; Wilkinson 1992b). When individuals aggregate for passive benefits, group composition might not be as important as group size. For

example, the cost of thermoregulation is reduced as the group size increases (Roverud & Chappell 1991). In contrast, if individuals aggregate for active benefits that occur through cooperation, group composition might be more important than group size.

Fission-fusion society

Some bats do not use the same roost every day, especially tree-roosting species whose roost fidelity appears related to roost abundance and stability (Lewis 1995; Kunz & Lumsden 2003). Although some forest-dwelling bat species switch roosts every few days, they still maintain an association with specific individuals, and therefore are fission-fusion societies (Kerth & König 1999; O'Donnell 2000; Willis & Brigham 2004; Rhodes 2007; Popa-Lisseanu et al. 2008), previously described in primates and cetaceans (Terborgh & Janson 1986; van Schaik 1999; Connor et al. 2000). For bat species, a fission-fusion society was defined as "the temporary splitting of colonies into several subgroups" (Kerth 2008) or "members frequently coalesce to form a group (fusion), but composition of that group is in perpetual flux, with individuals frequently departing to be solitary or to form smaller groups (fission) for a variable time before returning to the main unit. Individuals often preferentially associate with some members of the larger group and may even avoid associating with other members" (Barclay & Kurta 2007). Repeated fission and fusion of subgroups allows bats to interact with members of other subgroups, and enjoy the benefits of large group size even when roost space is limited. Thus, bats in a subgroup could maintain stable social groups which are larger than the number of individuals at any given roost (Willis & Brigham 2004). Group composition should be important for group formation in species showing fission-fusion societies and involve some active benefits associated with cooperation. The best example of this may

be the food sharing demonstrated by roosting aggregations of vampire bats (*Desmodus rotundus*) (Wilkinson 1984; Wilkinson 1985). A second example of the importance of group composition has been found in the information transfer about roosts among *Myotis bechsteinii* (Kerth & Reckardt 2003). In addition, kinship influences social interactions of bats even during foraging. For example, mothers and daughters of *M. bechsteinii* and *Rhinolophus ferrumequinum* overlapped more in their foraging sites than other individuals, which may have occurred through a mechanism of maternal inheritance of preferred foraging sites (Kerth et al. 2001; Rossiter et al. 2002). Bats living in a fission-fusion society might be more likely to seek the company of certain individuals even if roost-sites did not differ in environmental conditions. In such cases, kin-biased associations are likely to occur and there should be a positive correlation between relatedness of subgroup members and the frequency of association due to the additional indirect benefits (increasing inclusive fitness) individuals gain from the shared genetic material of kinship.

Roost fidelity

Roost fidelity often varies among bat species. In general, cave-dwelling bats show high roost fidelity and may use the same roost for several years. In contrast, bats roosting in trees (foliage or hollows) often switch roosts frequently and show a high fidelity to a small roosting area rather than to specific roost-sites (Lewis 1995; Kunz & Lumsden 2003). However, the reasons for roost switching remain unclear (Barclay & Kurta 2007). Movements between alternative roosts are assumed to be costly due to increased risk of predation, reduced familiarity with foraging opportunities, disrupting social bonds, and energy costs associated with finding new roosts (Alcock 1989).

However, it is also assumed that benefits associated with roost switching exist, and that such benefits outweigh the costs. It is important to understand the underlying causes of roost switching because such behaviour can affect sociality by disrupting the social group (Lewis 1995), and also may reflect other conditions, such as environmental change or different stages in life history (Barclay & Kurta 2007), that elicit the movement. The fission-fusion model is one hypothesis that may explain roost switching behaviour: both the frequency of roost switching and high fidelity to a small area could improve the interactions between individuals and maintain a large social group (O'Donnell 2000), especially when the physical capacity of a roost is limited. Willis & Brigham (2004) further suggested that for tree-roosting species, a group of trees rather than a single tree could be considered to be the functional roost.

Other hypotheses that have been suggested to explain why bats switch roosts frequently include: 1) reducing predation risks; 2) lowering ectoparasite loads, 3) responding to disturbance, 4) decreasing commuting costs to foraging areas, 5) responding to unfavorable changes in microclimatic or structural conditions in the roost (Lewis 1995; Kunz & Lumsden 2003; Barclay & Kurta 2007). These factors are not mutually exclusive and several of them could simultaneously affect the roost switching behaviour of a species (e.g. Lewis 1996; Russo et al. 2005).

Age, sex or reproductive condition in bats may translate into different environmental requirements. Reproductive females experience higher energy demands than non-reproductive females and males (Racey 1982), and therefore may prefer roosts that provide more thermal benefits to enhance the development of young (pre- and post partum). Lactating females would be expected to show higher roost fidelity than

pregnant females because juveniles would be much heavier than the fetus and therefore more costly to transport before they are volant. In addition, the energetic demands of lactation are significantly higher than those of pregnancy. These differences would presumably lead to different association patterns among different reproductive or age classes.

Gene flow

Individual animals are usually distributed patchily according to the geographical distribution of habitats. Behaviour could influence the distribution of individuals and thus affect the distribution of genes, or population structure, defined as the spatial variation in density and genetic composition of individuals of a species (Hewitt & Butlin 1997). Gene flow among patchily distributed subpopulations could be achieved by immigration of individuals or gametes and the increase of gene flow among subpopulations would homogenize the genetic variation among subpopulations and decreased the level of population structure otherwise subject to allele loss by random genetic drift. Theoretically, gene diversity within populations is affected by the behaviour of animals, such as philopatry, dispersal, or breeding tactics (Chesser 1991a; Chesser 1991b). In mammals, sociality could lead to genetic differentiation among social groups, and behavioural traits such as mating system, dispersal and philopatry, and formation of new social groups would affect the degree of genetic subdivision (Storz 1999). Therefore, understanding population structure of a species could be used to infer social organization and its past and present behaviour processes.

In general, female philopatry and male dispersal appear to be common patterns in mammal species (Greenwood 1980). Such behaviours should promote genetic

differentiation more among groups of females than males (Storz 1999). Indeed, a higher level of genetic differentiation among female, and male-mediated gene flow seems to occur in many bat species (Petit & Mayer 1999; Castella et al. 2001; Kerth 2002; Arnold 2007; Safi 2007; Chen et al. 2008; Vonhof et al. 2008). Moreover, the level of genetic differentiation within bats is also affected by migratory behaviour, with migratory species were usually less genetically differentiated than non-migratory species because individuals are expected to be able to mate during migration (Burland & Worthington Wilmer 2001), while the gene flow of non-migratory species could be facilitated by the mating at the swarming site, which is usually associated with several adjacent colonies (Kerth et al. 2003; Veith et al. 2004; Rivers et al. 2005).

In mammals, female philopatry is expected to facilitate the cooperation and formation of social groups among females (Greenwood 1980), as well as the cohesiveness of the social group which might exclude immigrants if local resources are limited or cooperation is costly (Kerth et al. 2002b). *Myotis bechsteinii* exhibited extreme female philopatry and male dispersal and a strong colony structure among maternity colonies based on maternal mitochondrial DNA (Kerth et al. 2002a). The data suggested that the gene flow of nuclear DNA among colonies was mediated by males, and exchanges of females among colonies were nearly absent even though these colonies were in close proximity and there was no obvious physical barrier. *Myotis bechsteinii* appeared to form a fission-fusion society, switching roosts very frequently within the roosting area. The confrontation test also showed that females were aggressive to individuals from other colonies (Kerth et al. 2002b) indicating that social interaction might be a barrier for gene flow among colonies. Similar social boundaries between

groups of bats living in close proximity have been documented from behavioural observations (e.g. *Chalinolobus tuberculatus*, O'Donnell 2000; *Thyroptera tricolor*, Vonhof et al. 2004; *Eptesicus fuscus*, Willis & Brigham 2004; *Nyctalus lasiopterus*, Popa-Lisseanu et al. 2008). This implies that the social barrier might be common in bat species living in fission-fusion societies and the absence of exchange of females among colonies could increase genetic differentiation among maternity colonies regardless of geographical proximity. Racey and Entwistle (2003) suggested this social barrier might have profound implications for conservation because a loss of members from one colony may not be compensated for by the addition of conspecifics from other colonies or roosting areas. Therefore, the decrease of group members due to human disturbance may be more severe than previously expected and the conservation efforts should be addressed at the level of individual colonies.

The use of molecular data to measure gene flow in bats has increased rapidly in recent years (Burland & Worthington Wilmer 2001) while data from direct observations (e.g., mark-recapture) were generally limited (but see Entwistle et al. 2000; McCracken & Wilkinson 2000) because of the difficulty of direct observation of bats. But molecular methods and direct observation might provide different information about gene flow and the combination of both approaches should provide more insight into the underlying process (Slatkin 1985; Slatkin 1987). For example, the banding study of *Plecotus auritus* indicated that both males and females were philopatric, possibly leading to a low level of gene flow among colonies (Entwistle et al. 2000). Genetic data showed weak genetic differentiation among colonies, in contrast to banding data suggesting that *P. auritus* mate outside the colony (Burland 1999).

Study Species

Myotis formosus has strikingly bright coloured fur (yellow), and is distributed from Afghanistan to Japan and Philippines (Nowak 1994). In Taiwan, the known colonies occur mainly on the Southwest plain area (Lin et al. 1997; Shen & Lee 2000; Chang et al. 2005; Chang 2007). This species is a medium-sized bat (11-17g) that is insectivorous and has been found to feed on Coleoptera, Diptera, and Lepidoptera (Yang 1996). Like most non-tropical bats, females aggregate in spring and summer in buildings or foliage to form maternity colonies where they rear their single pup (Yang, 1996; Shen & Lee, 2000). Peak of parturition occurs in June and most pups are weaned by the end of July (Yang, 1996; Shen 1996). After weaning, adult females and their young leave the maternity colony; it is not currently known where these bats live in winter, although a few individual bats have been observed in autumn or winter at high elevation (> 1500 m) locations in Taiwan. Females are philopatric and a small number of males also returned to their natal roosts in their second year (Shen 1996). Adult males are usually solitary, but sometimes roost with females (Shen 1996).

My research focused on three interrelated aspects of the roosting behavior of *M. formosus*. In part A, I investigated the pattern of roost switching of *M. formosus* and tested predictions arising from hypotheses about the causes of roost switching. I quantified the frequency of roost switching and investigated both intrinsic and environmental factors that might be associated with roost switching. In part B, I used three different association indices to measure the strength of association and discriminate between active association and passive association of dyads. I also investigated potential factors which might explain the nonrandom association between dyads. In part C, I used

the results from part A to further investigate the roost switching that occurred mostly within the range of individuals' roosting area to determine the genetic consequences of the bats' behaviour. In this study, I refer to a "roosting area" as a patchily and discrete range of area within which bats were commonly found. I used "colony" to refer the collection of bats from a roosting area. In addition, I use "roost-tree" or "roost-house" to identify the specific tree or house that bats roosted and "roost site" as the specific site on the roost-tree (or house) where the bats roosted.

Objectives

Part A. Causes of roost switching

At my study site, *M. formosus* used two different types of day roosts in summer: houses and foliage. My objectives were to provide basic information regarding their roost switching behaviour and to determine whether roost switching was associated with intrinsic and/or environmental factors by testing roost fidelity for correlations with these factors. The three intrinsic factors that were considered were sex, age and reproductive status. For example, pregnant and lactating females have much higher energy demands than males or post-lactating or non-reproductive females and lower mobility due to the increased loading from the weight of the fatal or infant. I predicted that lactating females would show the highest roost fidelity followed by pregnant females and then by other individuals.

Lewis (1995) concluded that availability and stability of roosts were usually associated with higher roost fidelity. However, these two factors are difficult to separate. By comparing conspecifics using two types of roost in the same area, I hoped to

distinguish underlying characteristics (e.g., availability, permanency, and space) of how different roost types affected roost switching by bats.

As previously discussed, roost switching might be influenced by several factors. As such, I focused on six of these: 1) to reduce probability of predation, 2) to lower ectoparasite loads, 3) to respond to disturbance, 4) to decrease commuting costs to foraging area, 5) to respond to the change of microclimate in the roost, and 6) to enhance social interaction (fission-fusion model).

1) Bats roosting in larger subgroups could benefit from lower predation risk due to the dilution effect or even the increased vigilance and predation confusion (Krause & Ruxton 2002). Fenton et al. (1994) reported that increasing the bat subgroup size would decrease the predation risk of each subgroup member. In the other hand, the conspicuousness of the bat subgroups was enhanced by increasing the subgroup size as well as the frequent use of specific roost-site or roost tree, which would increase the probability of detection and attack by predators. Therefore, roost switching to an unfamiliar area of the predator would be effective way for predator avoidance (Lewis 1995). If bats switched roosts to reduce probability of predation, I predicted that 1) individuals roosting in larger subgroups would switch roost less frequently than those in small subgroups, 2) after a roost switching, the size of the new subgroup would be similar or larger than the previous subgroup, and 3) most roost switching would be among roost-tree (or house) instead of within roost-tree (or house).

2) Ectoparasites could induce severe cost to their hosts. Many bat ectoparasites deposit eggs or pupae on the walls of the roost (Marshall 1982), and roost switching by bats is an effective way to interrupt the life cycle of ectoparasites (Reckardt & Kerth

2006; Bartonibka & Gaisler 2007). Ectoparasite loads positively correlates with roost switching frequency in some bat species (e.g. *Antrozous pallidus*, Lewis 1996; *M. bechsteinii*, Reckardt & Kerth 2007). If *M. formosus* switched roost to reduce the ectoparasite load, I predicted that 1) individuals with higher level of ectoparasite loads would switch roosts more frequently, 2) the roost switching frequency would increase as the subgroup size increase because the large subgroup size would increase the infection rate of its member, and 3) lactating female would switch roost more frequently to reduce the risk for hairless juvenile, which is prone to be infected.

3) Unpredicted factors including the rapid destruction, deterioration or unsuitable of the roost by nature or human force or the disturbance brought by the predators or competitors may cause the bat switch roost immediately (Barclay and Kurta 2007), and might also have an effect for the recurrent roost switching (Lewis 1995). The capture and handling of bats by researchers were a source of disturbance to bats. If the recurrent roost switching of *M. formosus* is a response to this disturbance, I predicted that newly caught individuals would switch roosts more frequently than those caught previously.

4) Bats might switch roosts to reduce commuting costs to foraging areas. I predicted that the roosting sites closer to foraging areas would show higher roost fidelity than those commuting greater distances to foraging. In this instance, moving distance between consecutive roosts would be in a comparable scale to the moving distance between roosting and foraging sites.

5) Microclimates in roosts should influence roost quality (Kunz 1982). Bats roosting in less protected settings would switch roosts more often than those using climatically stable roosts. Therefore, I predicted that bats roosting in the foliage would

be affected more by the rain (and switch roost more frequently) than those roosting at house because the foliage provided a less protection form the rain than the building.

6) The roost switching of *M. formosus* might serve to enhance the social interaction among colony members, if they live in a fission-fusion society. If *M. formosus* live in a fission-fusion society, I predicted that: 1) Individuals live in the large subgroup would switch roost less frequently than those lives in small subgroup because they demand less for increasing social interaction by roost switching. 2) Individuals would increase the social interaction by roost switching, and therefore the number of bats associated with a given individual would increase as its roost switching frequency increased. 3) A nonrandom association between individuals. 4) Bats would show fidelity to the roosting area while switching roost frequently, which could improve the social interaction between individuals and maintain a large social group. And 5) Individuals roosting in a foliage roost (limited space) would switch roost more frequently than those in the house (spacious roost).

The predictions for each hypothesis are summarized in Table 1.1.

Part B. Analysis of nonrandom association

In this section, I analyze the association pattern between dyads roosting in foliage and in a building. I predicted that bats roosting in foliage are constrained by subgroup size, and therefore should show a fission-fusion society which would increase the numbers of roost mates and overcome the small group size that is limited by the size of foliage roosts. In this active aggregation, bats, which roost on foliage and thus is prone to subject to the limited roost capacity, should show nonrandom association between dyads which should be independent from the attractiveness of the roost. Subgroup size for

individuals in the house roost (Roosting Area 2) whose maximum capacity for bats was far larger than the whole foliage colony was not expected to be similarly limited. In addition, individuals with different reproductive status or sex may be expected to have different requirements for group living. I predicted that pregnant and lactating females would show a higher association than individuals in the other reproductive classes. Benefits of association would increase the inclusive fitness if the group members were genetically related, and I predicted that genetically related individuals would associate with each other more often than non-related individuals.

Part C. Genetic subdivision among colonies

Having determined that individual *M. formosus* switched roosts frequently and that some individuals showed high fidelity to a roosting area both within and between years, I used both biparentally (nuclear microsatellite loci) and maternally (mitochondrial DNA sequence) inherited genetic markers to investigate genetic variability and gene flow between roosting areas, and to investigate patterns of dispersal.

If roost switching occurred mostly within the range of individuals' roosting areas and rarely between areas as shown by banding data in part A, the rate of exchange of mitochondrial DNA haplotypes between roosting areas would be low because mitochondrial DNA was maternally inherited and female consists most of the individuals in the maternity colonies while the gene flow of the nuclear microsatellite DNA could be mediated by males, e.g. immigration of males, philopatric females mating with males from other colonies in the swarming sites or migration route. And I predicted that there would be: 1) low variability in mitochondrial DNA sequence but diversity within nuclear microsatellite loci would be higher and 2) a stronger genetic structure from mitochondrial

DNA than from nuclear microsatellite loci despite the absence of a geographical barrier between roosting areas. I also tested if there was a bias in dispersal between sexes because from banding data both adult males and females returned to the roosting areas but only a small portion of subadults were observed returning the roosting area in their second years. Male-biased dispersal prevails in mammals (Greenwood 1980) and has been documented in several bat species (e.g. *Nyctalus noctula*, Petit & Mayer 1999; *Macroderma gigas*, Worthington Wilmer et al. 1999; *Myotis myotis*, Castella et al. 2001; *M. bechsteinii* Kerth et al. 2002a). I also tested if individuals that only appeared for a short period of time in the roosting area were transients, based on their genetic differences from resident individuals.

Chapter 2. Materials and Methods

Study sites

I conducted fieldwork in Yunlin and Tainan counties, on the Southwest plain of Taiwan from May to August in 2005 and 2006. This plain is the largest agriculture area in Taiwan where sugarcane and paddy rice were the most common crops. For part A and B, I conducted the behavioural observation for *Myotis formosus* at three summer roosting areas located in Shueilin and Beigang Township, respectively in Yunlin county (23° 34' N, 120° 17' E). First, a park affiliated with the Beigang sugar refinery (BS, or Roosting Area 1, 23° 34'8 N, 120° 17'49 E) in the Southwest side of the Beigang downtown, and the area of the park was about 4 ha., having > 150 tree species, and most were broad-leaved trees where *M. formosus* had used as maternity roosts in summer (Figure 2.1; Figure 2.3). Second, a house (SH, or Roosting Area 2, 23° 33'39 N, 120° 15'7 E; Figure 2.1; Figure 2.2) built in 1935, according to the house owner, where bats came to form maternity colony annually, roosting under the bean, since 1936. The peak colony size there was c.a. 200 - 250 during 1993-1995 (Shen 1996; Yang 1996), but decreased annually since then. In this case, the roost-house alone actually represented a roosting area because I did not find other roost-tree or houses in its surrounding area, and this is the only non-foliage roost in my study. Third, the Beigang Sports Park (BP, or Roosting Area 3, 23° 34'35 N, 120° 18'12 E), about 4 ha., one km away from the Roosting Area 1, and located at the Northeast side of the Beigang downtown (Figure 2.1). To the best of my knowledge, the three roosting areas contained the largest colonies of *M. formosus* in Taiwan.

In addition to the three roosting areas, I collected wing biopsies from six other roosting areas (Figure 2.1; Table 2.1).

Part A. Causes of roost switching

Capture, Marking and census

From May to early August 2005 I caught *M. formosus* in mist nets placed near roosting trees (Roosting Area 1 and Roosting Area 3) or house (Roosting Area 2) before dawn when foraging bats were returning to the roosts. To minimize disturbance, I conducted the mist netting no more than once a week at the same location. In addition, I also hand netted bats in their roost-sites after mid July when the most juveniles had been weaned and bats started to leave the roosting areas. At this time the lower density of bats made mist-netting less effective.

I banded bats with one two-colour-striped plastic band (size: 3.7mm, L & M Bird Leg Bands, Inc., San Bernardino, CA, U.S.A.) on one arm (female: left; male: right) and one numbered one-colour plastic band (size: 3.7mm, L & M Bird Leg Bands, Inc., San Bernardino, CA, U.S.A.) on the other arm, together giving each individual a specific colour combination plus a sample number, and the colour combination could be identified by visual observation, allowing me to conduct subsequent roost census without re-capture the bats. At Roosting Area 2, I included four adult females, not caught by in my experiment, with different source of recognizable markers as the sample for behavioral observation but not for genetic analysis. Two of them were adult females with one coloured band on each forearm banded in 1995-1996 and observed in 2005 but not 2006. The other two were observed in both 2005 and 2006. One was caught from a tree

at the vicinity of the Roosting Area 2 by an other researcher in 2004 and banded with one aluminum ring on right arm which made it the only *M. formosus* banded with aluminum ring at Roosting Area 2. The other had never been caught but had a remarkable partial albino in forehead and I recognized it as an adult female by sighting that it had a dependent young in summer 2006. At Roosting Area 1, I included two adult females, one of them lactating in the second year, caught and banded with recognizable color bands by other researcher in September 2005 for the census in 2006.

I measured the body mass, forearm length and recorded sex. Juveniles (young of the year) were distinguished from the adults by trans-illuminating the wings to identify the presence of the cartilaginous epiphyseal plates in finger bones (Anthony 1988). Reproductive status of adult females was determined by the following criteria. I determined the pregnancy of female by palpation on the abdomen, the lactation by the presence of enlarged nipples, the hairless skin around the nipples and the expression of the milk when gently squeezed the nipple, and the post-lactating by the retaining of the enlarged nipples, the hairless skin around the nipples but no excretion of the milk (Racey 1988). If none of the sign of reproduction were recognized from an adult female, then I assigned the individual as a non-reproductive female. I checked for the presence of ectoparasites by scanning the wing and tail membranes and looked through the furs of the bats.

From May to August 2005, I clipped the fur between the scapulae and glued radio transmitters, weighted 0.51 g, (BD-2N, Holohil Systems, Carp, ON, Canada) to some adult females with different reproductive status using Skin Bond cement (Smith and Nephew United, Largo, Fl., U.S.A.). The weight of the radio transmitter was controlled

to be lower than 5% of the bat's body mass (Aldridge & Brigham 1988). Then I released the bats within 1-2 h after capture at the site of capture. Captures were under the permit of the county government.

I used one SRX400 telemetry receiver (Lotek Engineering Inc., Newmarket, ON, Canada) and one R1000 telemetry receiver (Communications Specialists, Orange, CA, USA), both fitted with a three-element Yagi antenna or omni-directional antenna mounted on a vehicle roof to locate the day roost. I also used the balcony on the top of a 10-floor building (23° 34'31 N, 120° 17'29 E) in Northwest side of the Beigang downtown for monitoring, from where the receiver could detect a signal from up to four km away and all direction except the East side where the downtown located. I also followed the bats in the night to their foraging areas.

During radio-tracking, once I found a day roost used by the radio-tagged bat, I verified the presence of the radio-tagged bat by a 20X telescope or 8X binoculars. The subgroup size and the presence of other color banded bats as well as the presence of the dependant young were recorded. Since bats may roost closely but not always contact each other, and were capable to crawl a short distance along the leaves they hanged on but seldom flew during the daytime, I defined that individuals which roosted as far as within two wingspans distance (the maximum distance that two separate bats could have potential body contact without moving) and on the same branch as in the same subgroup. I also recorded the location of the roost-site on the tree by measuring the following three parameters, 1) the distance to the main trunk, 2) the relative angle (0-360°) to the main trunk, and 3) the height to the ground, from the roost-site. In 2006, I also recorded whether there was rain and the time of the day of the rain.

In addition to radio-tracking, I conducted a daily census based on the coloured banded bats in both years at Roosting Area 1 and 2. At Roosting Area 1, I chose and monitored 47 trees, most of which were along the walkways and have been found to be used by *M. formosus* in previous study (Chang 2007), in every census, and also checked an extra of about 50 trees in the vicinity less frequently and in irregular interval (Figure 2.3). I also conducted occasional censuses at Roosting Area 3 in both years to check if bats switch between different roosting areas. I searched bats by eyes with the aids of a 20X telescope or 8X binocular to identify the banded individual. This approach minimized disturbance to roosting bats. I used a digital camera equipped with a 300 mm lens to collect more detailed information about roosting bats which allow me to confirm individual identities and the position of the roost-site. Once I located a bat on the tree, I also recorded the subgroup size, the ID of the bats and the presence of the dependant young. The location of the roost was also measured and recorded following the same way for radio-telemetry. At Roosting Area 2, because the beam that bats roosted on was uniform, I divided it into five roost-sites as bat could crawl along the beam in the day but had to fly to reach different roost-sites and flying during the daytime was not usual to even in this house roost (Figure 2.2).

Besides identifying the reproductive status of adult females at capture, I also determined the reproductive status of adult females during censuses. Lactating females had dependent young (juvenile) underneath their abdomens. Because I could not check the presence of the dependent young every day especially when the mother was in an interior position in a large group or to know the exact date of parturition because newborns were small and usually covered by mother, I checked the roost-site at night or

before sunset, especially those occupied by a large group of bats to see the stage of development of young left there. This allowed me to estimate the date of parturition. If a lactating female had been observed with dependent young for more than four weeks, I only identified the first four weeks as lactating because juveniles started to fly after age four-week (Shen & Lee 2000). For those which had been recognized as lactating female, I defined them as pregnant female on those days that one week before the estimated date of parturition. I also defined individuals as post-lactating female if they were observed on the days six weeks after the estimated date of parturition because juveniles reached the same length of forage bout around 45-day-old (Shen & Lee 2000). If I never saw a female have juvenile through the summer, it was recorded as non-reproductive females. If I never saw a female associate with a juvenile nor stayed through June to mid July, the lactation period, it was assigned as unknown reproductive status.

I conducted the bat census daily in Roosting Area 1 and Roosting Area 2 from early May through late August in 2005 and 2006 respectively, and three to seven times a week at each roosting area (Table 2.2).

Data analysis

To compare roost switching behavior, I calculated indices for roost-site fidelity and roost-tree (or house) fidelity for banded bats. To calculate roost-site fidelity, I recorded when a bat returned to the same roost-site or moved to a different one on the next day. I used the same data to calculate the roost-tree (or house) fidelity when bats returned to the same roost-tree (or house) (for Roosting Area 2) or moved to a different roost-tree (or house) on the next day. I calculated the roost-site fidelity as:

Roost-site fidelity = roost-site stay/ (roost-site stay + roost-site switch)

,where roost-site stay was the number of days the bat returned to the roost-site used on previous day, and roost-site switch was the number of days the bat switched to a different roost-site on the next day. The roost-tree (or house) fidelity was calculated as:

Roost-tree (or house) fidelity = roost-tree stay/ (roost-tree stay + roost-tree switch)

, where roost-tree stay was the number of days the bat returned to the roost-tree (or house) used on previous day, and roost-tree switch was the number of days the bat switched to a different roost-tree or house. By definition, both indices range from 0 to 1, where 0 indicates that individual never used the same roost-site or roost-tree (or house) on consecutive days and 1 means the individual always returned the same roost-site or roost-tree (or house). I calculated the indices either across all observation days for a given individual or across all individuals for a given period of days.

To determine the effect of the roost type, sex, age and reproductive status on the roost fidelity, I used the indices of roost-site fidelity and roost-tree (or house) fidelity of individuals as dependent variable, and the roost type, sex, age and reproductive status as independent variable. I divided the roost type into foliage (Roosting Area 1) and house (Roosting Area 2), the sex and age into adult female (AF), adult male (AM) and juvenile (J), and the reproductive status into pregnant female (PF), lactating female (LF), post-lactating female (PLF) and non-reproductive female (NRF).

To determine the effect of disturbance, I compared roost fidelity of bats within ten days after capture (day 1 – day 10) with the roost fidelity of the same individuals one year later. If the same individuals appeared one year later, I sampled a ten-day period from one year ± 10 days (day 356 to day 375) after it was caught when applicable. Therefore only individuals that appeared at the same period of time in both years were compared.

To validate the data from visual census, I compared the percentage of days that radio-tagged bats were located on the selected trees at Roosting Area 1 to the percentage of days that non radio-tagged bats were located on the selected trees at Roosting Area 1.

I used randomizations (Manly 2007) to determine if the individual prone to be in a larger subgroup would also have higher roost fidelity. I calculated the correlation coefficient of the roost-site fidelity and the mean subgroup size obtained from the behavioural observation, and then randomly paired the roost-site fidelity with the mean subgroup size of different individuals and calculated the correlation coefficient after each randomly pairing. I repeated the randomizations 10 000 times using Excel add-in software PopTools v 3.0 (available at http://www.cse.csiro.au/poptools/) to obtain a distribution of the correlation coefficient, which represented a distribution of expected correlation coefficient. I then compared the correlation coefficient for the observed individuals with the distribution of expected correlation coefficient. If the original correlation coefficient was within the highest 5% of the expected distribution of the correlation coefficient, it was considered to be significant at 5% level. For the recordings that I could determine both whether a given individual returned its roost-site or switched roost-site on the second day and its subgroup size, I assigned these recordings into two

groups based on whether the bat switched roost-site. Then I compared the mean subgroup size between the two groups to determine if bats roosted in a large subgroup was less likely to switch roost. I also tested if roost switching or returning the roost was associated with subgroup size change. I calculated the mean subgroup size for all bat-days into four groups, which was before a roost switching, after a roost switching, before a returning the same roost or after a returning the same roost. I then compared the mean subgroup size before and after the roost switching as well as before and after a returning the same roost to determine if roost switching or returning associated with a increasing in subgroup size.

To determine the effect of rain on roost switching, I compared the overall frequency of roost switching and non-switching next day across the whole colony between the days that were rainy or rainy in the night and the days that were not rainy or rainy in the night.

To determine the relationship between roost fidelity and the number of individuals one bat associated with, I calculated the correlation coefficient of roost-site fidelity and the number of individuals associated, and then compared it with a distribution of the expected correlation coefficient, which was generated by randomly pairing the roost-site fidelity and the number of individuals associated 10 000 times. I ran the randomizations (Manly 2007) using Excel add-in software PopTools v 3.0 (available at http://www.cse.csiro.au/poptools/). If the correlation coefficient from the observed data were among the highest 5% of the distribution of randomly generated correlation coefficient, then it was considered to be significant at 5% level.

I only included individuals with a roost-site stay + roost-site switch ≥ 3 or roost-tree stay + roost-tree switch ≥ 3 for the calculation of indices, and groups that had a sample size ≥ 3 for the comparison between groups. I used two tailed *P*-values and a 0.05 level of significance. The Bonferroni correction ($\alpha = 0.05/ n$, n = total number of groups for pairwise comparison) were applied when the pairwise comparison were non-independent. Data are shown as mean ± SD. I performed all statistical analysis using SPSS v16 unless otherwise mentioned.

Part B. Analysis of nonrandom association

Association indices

I used the daily census and radio-tracking data from Roosting Area 1 and Roosting Area 2 to analyze the association pattern between colony members of *M. formosus*. First, I used three indices to measure associations between bat dyads: 1) simple ratio index (or SR-index, Cairns & Schwager 1987; Ginsberg & Young 1992), which measured how often two animals roosted together, 2) χ^2-index (Wilkinson 1985), which discriminated whether the associations of bat dyads were due to passive or active attraction to the roost-site, and 3) subgroup-size-index (SGI-index, Kerth & Konig 1999), which discriminated the associations which were significantly positive or negative to the subgroup size.

(1) I used the SR-index to measure how often each pair of individuals roost together, and it was calculated as

$$\text{SR-index} = X / (X+Y_{AB}+Y_A+Y_B)$$

, where X is the number of days that individual A and B were observed roosting together, Y_{AB} is the number of days that both individual A and B were observed but roosted in separate groups, Y_A was the number of days that only individual A was observed, and Y_B was the number of days that only individual B was observed. The simple ratio index is statistically unbiased (Ginsberg & Young 1992) and provide more appropriate estimate of the associations of individuals living in the same group in comparing to other association indices (Cairns & Schwager 1987).

(2) I used the χ^2-index (Wilkinson 1985) to discriminate the "active" associations from the "passive" associations that both individuals were associated just due to the sharing of the same roost preference. It was calculated as

$$\chi^2 = (X - \sum_{1}^{k} P_{AK}P_{BK}T)^2 / \sum_{1}^{k} P_{AK}P_{BK}T$$

,where T is the smaller of the total number of days that individual A and B were sighted, N_A and N_B, respectively, P_{AK} and P_{BK} were the proportion of days that individual A and B were observed at roost-site K, respectively, and thus $\sum P_{AK}P_{BK}T$ was the expected number of days that the two individuals roosted together. I did not calculate the χ^2-index when the dyads had no overlap in the roost-site due to it would cause the sum of the $P_{AK}P_{BK}T$ be zero. And alternatively, the dyads which had a χ^2-index meant that the pair of individuals had at least one roost-site overlap.

(3) I used the SGS-index (Kerth & Konig 1999) to correct the effects of associations due to subgroup size. Normally, the probability that two individuals roosted together would increase as their subgroup size increased. The expected probability that individual A and B observed in the same roost-site on a particular day, P_{AB}, was calculated as

$$P_{AB} = \sum_{1}^{k} (N_k / N_{total}) * [(N_k - 1) / (N_{total} - 1)]$$

,where N_k was the subgroup size in the roost-site k on a particular day and N_{total} was the total number of bats observed from all k roost-sites on that particular day. And the SGS-index was calculated in a form of χ^2-value which could be used to discriminate the significantly positive or negative associations.

$$\text{SGS-index} = (X - \sum_{1}^{d} P_{AB})^2 / \sum_{1}^{d} P_{AB} + (Y_{AB} - \sum_{1}^{d} Q_{AB})^2 / \sum_{1}^{d} Q_{AB})$$

,where d was the total days that both individual A and B were, and Q_{AB} equaled to 1 - P_{AB}, which was the number of days that individual A and B would have been observed in the different roost-site assuming random associations. I did not calculate the SGS-index for the dyads which had never been sighted on the same day through the course of the study due to the sum of the P_{AB} would equal to zero.

I calculated all three indices using MATLAB v7.4 (written by Dr. Mark Skowronski, Appendix A). I only calculated these indices only for the intervals that both individuals of a dyad were banded and observed, and only included the individuals that

had been sighted for ≥ 5 times and only analyzed the dyads which had an overlapping history ≥ 5 days.

Genetic relatedness

DNA extraction and amplification

I measured the genetic relatedness from microsatellite loci and from a sequence of mitochondrial DNA. I took a 3-mm in diameter wing membrane punch from each wing of captured bats (Worthington Wilmer & Barrat 1996), and stored the samples in 95% ethanol.

Mitochondrial DNA

I extracted DNA from sampled tissue using a GenEluteTM Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich, Oakville, Ontario, Canada). Using PCR primers L16517 5 –CATCTGGTTCTTACTTCAGG- 3 (Fumagalli et al. 1996) and sH651 5 – AAGGCTAGGACCAAACCT- 3 (Castella et al. 2001) a shorter version of the primer H00651 (Kocher et al. 1989) I amplified the second hypervariable domain (HVII) of the mitochondrial DNA. This is highly variable in other bat species (Castella et al. 2001; Metheny et al. 2007). I made amplifications in 50 μL reaction volumes, containing 2 μL of template DNA, 3.0 mM $MgCl_2$, 0.5 mM dNTPs, 1X PCR buffer (Invitrogen Life Technologies, Burlington, ON, Canada), 2 units of Taq polymerase (Invitrogen Life Technologies, Burlington, ON, Canada), and 0.15 μM of each primer. Amplifications were performed using the Biometra T1 Thermocycler (Whatman Biometra, Göttingen, Germany) by the following program: started with 3 min at 94°C; then followed by 29 cycles of 1 min at 94°C, 1 min 54°C, and 1.5 min at 72°C; and ended by a final extension

of 10 min at 72°C. Three μL of PCR products were run out in 1% agarose gel to verify the results, and the bands of HVII were about 800 bp in length. The rest of the samples were sent to the McGill University and Genome Quebec Innovation Centre, Montreal, Quebec (https://genomequebec.mcgill.ca) for sequencing on an ABI 3730XL sequencer according to manufacturer's protocol. The sequencing platform at McGill generated chromatograms and text files of sequence in FASTA format. L16517 was used as the forward primer for sequencing.

In the whole sequenced region, only 261 bp (from 91-351 bp region) were used for the analysis because of a largely 6-bp (TACGCA) repeating region after the first 345 bp (see Fumagalli et al. 1996; Castella et al. 2001). I edited chromatograms using BioEdit (Hall 1999) and aligned using the implemented feature ClustalW (Thompson et al. 1994). Any nucleotide differences that occurred only in one sequence were also sequenced by running the reverse primer (5 – GCGTATGCGTAAGCTTTTGTG- 3) which I designed myself to sequence the first 345 bp (including the unused, initial 91 bp) before the repeating region.

I classified individuals as from the same matriline if they shared the same mitochondrial DNA sequence (haplotype) whereas bats with different sequences (haplotypes) were classified as from different matrilines.

Microsatellite DNA

I used seven dinucleotide microsatellite loci in 10 μL PCR-amplification (Table 2.3). Each PCR solution contained 1-2 μL of extracted DNA, 1.5-3.0 mM $MgCl_2$ (Table 2.3), 0.25 mM dNTPs, 1X PCR buffer (Invitrogen Life Technologies, Burlington, ON, Canada), 0.5 units Taq polymerase (Invitrogen Life Technologies, Burlington, ON,

Canada), and 0.2-0.25 μM of each forward and reverse primer (Invitrogen Life Technologies, Burlington, ON, Canada) (Table 2.3). The forward primer was fluorescently labeled (Beckman Coulter, Mississauga, Ontario, Canada). I used the Biometra T1 Thermocycler (Whatman Biometra, Göttingen, Germany) to amplify the microsatellites by the following program: 10 min at 94°C; 35 cycles of 30 s at 94°C, 30 s at annealing temperature, and 45 s at 72°C; and an end extension of 10 min at 72°C. To determine the size of the allele, PCR products were analyzed by the CEQ 8000 Genetic Analysis System (Beckman Coulter, Mississauga, Ontario, Canada) following the protocol of the manufacture.

I calculated the mean number of alleles per locus (A), observed heterozygosity (H_o), and expected heterozygosity (H_e) within each colony using Microsatellite Analyzer (MSA; Dieringer & Schloetter 2003). I examined the genotypic linkage disequilibrium between each locus and potential deviation from Hardy-Weinberg equilibrium (HWE) for each loci and colony by GENEPOP web version of 3.4 (Raymond & Rousset 1995) using Markov chain method (1000 demonstration steps, 1000 batches and 10 000 iterations per batch). I also calculated the null allele frequency for each locus using CERVUS 3.0 (Kalinowski et al. 2007).

I calculated the pairwise relatedness for Roosting Area 1 and Roosting Area 2 using microsatellite genotype data and the program ML-relate (Kalinowski et al. 2006), which calculated the maximum likelihood estimates of relatedness. By definition, the value of the relatedness ranged from zero to one, with zero means dyads are unrelated, and one means the two individuals have the same genotype.

Data analysis

For SR-index, I used average cluster analysis (Manly 2005), implemented in the MATLAB v7.4 (written by Dr. Mark Skowronski, Appendix B), to visualize the patterns of associations within each roosting area. Following the methods of Vonhof and Fenton (2004), I used 0.1 as an arbitrary value, and if individuals were clustered above this value was considered to be a distinct subgroup.

I assessed whether the relatedness between dyads were correlated with the SR-index to test if the nuclear similarity would influence the association between dyads. I used a one-tailed Mantel test (Manly 2007) with 10 000 permutations of distance matrices consisting of SR-index value and pairwise relatedness of dyads with SR-index value using MATLAB v7.4 (written by Dr. Mark Skowronski, see Appendix C). To determine whether the matrilineal relationship would influence the association, I compared the value of SR-index and χ^2-index of dyads with the same haplotype with those of the dyads with different haplotypes using Mann-Whitney test. I also compared the number observed with the expected number of positive or negative significance of SGS-index value using the χ^2 test for goodness of fit (Sokal & Rohlf 1995) to determine the influence of matrilineal relationship. I conducted all statistical analyses using SPSS v16.0 unless otherwise mentioned.

Part C. Genetic subdivision among colonies

Sample

In addition to samples from Roosting Area 1, 2 and 3, I took wing biopsies from another six roosting areas (Roosting Area 4, 5, 6, 7, 8 and 9) in 2006. Bats sampled in

Roosting Area 1, 2, 3, 4, 5, 6 and 8 were caught from single roosts or from several roosts within a small area (i.e., < 200m in diameter). Bats caught from Roosting Area 7 and Roosting Area 9 that were at least 20 km from the other roosting areas (Figure 2.1; Table 2.1), roosted in trees in an areas about 1.5 km in diameter. To minimize disturbance to bats in nursery colonies, in 2006, I only hand netted bats after mid-July. I extracted the DNA and amplified both mitochondrial DNA and microsatellite DNA following the method above (see part B).

Because the bats were not all sampled from the same year and not all bats sampled from the same roosting area presented in both year, the individuals were analyzed and grouped by years and roosting areas.

Genetic diversity

For the genetic diversity of mitochondrial DNA in each colony, I calculated the number of different haplotypes and the number of unique haplotypes, the haplotype diversity (h), which is defined as the probability that two randomly chosen haplotypes were different in the sample, and nucleotide diversity (π), which was the probability that two randomly chosen nucleotide sites were different (Nei 1987), using Arlequin version 3.1 (Excoffier et al. 2005) for all the whole set. A relationship of different mitochondrial DNA haplotypes was constructed using TCS version 1.21 (Clement et al. 2000) based on the statistical parsimony (Templeton et al. 1992).

For microsatellite data set, mean number of alleles per locus (A), observed heterozygosity (H_o), and expected heterozygosity (H_e) within each colony were calculated. The genotypic linkage disequilibrium between each locus and potential deviation from Hardy-Weinberg equilibrium (HWE) for each loci and colony were

examined by GENEPOP web version of 3.4 (Raymond & Rousset 1995) using Markov chain method (1000 demonstration steps, 1000 batches and 10 000 iterations per batch). The null allele frequency for each locus was also calculated using CERVUS 3.0 (Kalinowski et al. 2007).

Genetic subdivision

I calculated the pairwise Φ_{ST} (Weir & Cockerham 1984) for mitochondrial DNA and tested for statistical significance using Arlequin version 3.1 (Excoffier et al. 2005). The pairwise F_{ST} (Weir & Cockerham 1984) for microsatellite data set was calculated and tested for statistical significance using Microsatellite Analyzer (Dieringer & Schlötterer 2003). The pattern of the isolation-by-distance was tested by comparing the correlation between the linearized genetic distance, $\Phi_{ST}/ (1-\Phi_{ST})$ for mitochondrial DNA and $F_{ST} / (1- F_{ST})$ for microsatellite data, and the geographic distance (Rousset 1997), and the significance was examined by the Mantel procedure (10 000 permutation) implemented in the software Isolation-by-Distance version 1.52 (Bohonak 2002). Colonies in 2005 were excluded in this analysis due to the small number of location sampled.

The hierarchical components of variation based on each genetic marker among and within colonies were also examined using the analysis of molecular variation (AMOVA) (Excoffier et al. 1992). Besides comparing the colonies of all individuals within the same year, females from different colonies were also compared (it was not applicable to males due to the small sample size). For individuals from Roosting Area 1, 2 and 3, where census were made for two years, additional comparisons were performed

for individuals appeared in both years. Colonies which contained a sample size less than five were excluded from the F statistics based analysis.

In addition, I also used a Bayesian clustering approach (Pitchard et al. 2000; Falush et al. 2003), implemented in the STRUCTURE version 2.1 to identify the number of genetic distinct populations or clusters (K). Ten independent runs of value of K, between 1-18, with a burn-in period of 50 000 and 500 000 Markov chain Monte Carlo (MCMC) iterations using an ancestry model incorporating admixture without prior population information were performed. The posterior probability for each K was then calculated using the mean estimated log-likelihood of K to choose the optimal K.

Parentage

I assessed the presence of the parent-offspring relationship using the likelihood-based approach, for microsatellite loci only, implemented in the program CERVUS 3.0 (Kalinowski et al. 2007). In this study, the parameter I used for the simulation was: 95 adult females and 17 adult males as candidate parents, the typing error rate of 0.02, and the proportion of loci typed of 0.99. By this way, I could also prevent the high mutation rate of microsatellite loci, ranging around 10^{-3} or 10^{-4} per generation (Avise 2004), excluding the true parent-offspring relationship due to genotype mismatched due to mutation. The proportion of candidates sampled was set at 0.3, and the significance was set at > 80% confidence. After identifying the parent-offspring by CERVUS, I also compared the mtDNA haplotype of the potential mother-daughter and mother-son dyad, and exclude the possibility of maternal relationship if the mtDNA haplotype was not matched.

Sex-biased dispersal and transients

I tested two predictions arising from the hypothesis that 1) dispersal was sex-biased and 2) individuals that only observed for a short period of time in their roosting area were transients. First, concerning dispersal of male *M. formosus*, and therefore the males and females observed in the same roosting area would have different genetic makeup since originated form different roosting areas. And second concerning the source of transient individuals, if the individuals that only observed for a short period of time in the experimental roosting areas were transients and eventually philopatric to the roosting area elsewhere, their genetic makeup was expected to be different from those spending most of time in that roosting area (residents). I used the sex-biased dispersal test implemented in FSTAT (Goudet 2002) designed to identify biases in dispersal between two a priori defined groups of individuals. The model assumed that individuals were sampled after dispersal so only adult individuals were included. For the first prediction I treated males as the dispersing sex (immigrants), and females as the non-immigrants (residents), including only colonies sampled in 2006 with ≥ 5 adult individuals. For the second prediction I used adult samples from Roosting Area 1, 2 and 3 where I had two years of census data. I grouped and compared the individuals in two different ways. First, I assumed and grouped individuals only observed in first year were transients (immigrants), and those sampled in both years as residents. Second, I assumed and grouped individuals never observed for more than three days in both year as transients (immigrants), and those I observed for more than three days at both years as residents. I chose Weir and Cockerham's (1984) F_{ST} as the statistical descriptors for the comparison of individuals' genotype because it has the highest power for detecting biased

dispersal over a variety of sampling schemes (Goudet et al. 2002). F_{ST} for the immigrants were expected to be lower in comparing to the residents, and the significance was tested by 10 000 permutation.

Chapter 3. Results

Part A. Causes of roost switching

Colony size

The total number of bats in both sites fluctuated in both years (Figure 3.1), indicating that not all bats returned their roosts every day. At Roosting Area 2, the highest number of roosting bats occurred in May, after which the number gradually decreased and reached its lowest number in late August at which point the census stopped. The same pattern occurred in the Roosting Area 1 in 2006, but not in 2005, when the highest number occurred in mid July when most young became volant. Some events contributed to the fluctuations of the colony size. In 2005, close-up shots of parturition behaviour of *M. formosus* at Roosting Area 2 by a TV team (on June 10 and 11) occurred before most individuals left for foraging and the numbers of roosting bats dropped sharply from 122 (June 8) to 78 (June 12). Numbers of bats in the roost did not rebound until July. Typhoons that had passed or affected the study area (2 in 2005, and 4 in 2006) directly affected colony size. For example, the category five typhoon Haitang directly hit the study area on 18 July 2005 and caused varying damage to some roost-trees in Roosting Area 1. This damage led to a sharp drop in colony size at both roosting area (Figure 3.1) although no structural damage at Roosting Area 2 was recorded. In addition, some slight but still obvious drops of colony size at Roosting Area 1 around mid May and mid July 2006 (see Figure 3.1) were also immediately following the passage of a typhoon.

Changes in colony size varied between 2005 and 2006 (Table 3.1). From 2005 to 2006, the colony size decreased significantly at both roosting areas (Mann-Whitney, at Roosting Area 1: $z = -4.083$, $P < 0.001$; at Roosting Area 2: $z = -6.239$, $P < 0.001$), as well as the number of subgroups found (Mann-Whitney, at Roosting Area 1, $z = -3.404$, $P < 0.05$; at Roosting Area 2, $z = -5.907$, $P < 0.001$). Subgroup sizes did not differ significantly between years at the two roosting areas (Mann-Whitney, at Roosting Area 1, $z = -1.45$, NS; at Roosting Area 2, $z = -0.527$, NS). The colony size at Roosting Area 2 was significantly larger than Roosting Area 1 for both years (Mann-Whitney test, in 2005, $z = -6.015$, $P < 0.001$; in 2006, $z = -5.079$, $P < 0.001$), but the number of subgroups were fewer (Mann-Whitney, in 2005, $z = -8.712$, $P < 0.001$; in 2006, $z = -10.989$, $P < 0.001$). The subgroup size was not different between the two roosting areas in both years (Mann-Whitney, in 2005, $z = -1.845$, NS; in 2006, $z = -1.553$, NS).

Sample size and annual fidelity

In total, I caught and colour-banded 105 bats at the three roosting areas (Table 3.2). One non-volant male juvenile was collected from the ground underneath a roosting group from Roosting Area 1 and was not banded. Of the 70 bats caught and marked in Roosting Area 1, 11 adults (8 females, 3 males) and seven juveniles (2 females, 5 males) were never seen again after release. Seven adults (6 females, 1 male) and one female juvenile had disappeared after release in 2005, but returned in 2006. Twelve adults (10 females, 2 male) and seven juveniles (1 female, 6 males) were only seen in 2005. Of the bats caught at Roosting Area 2 in 2005, one adult female and two males were never seen again after release. Of the five adult females observed in 2005, four were seen in 2006. Of the three bats caught at Roosting Area 2 in 2006, only one of the females stayed and

the other two (a mother with her dependent young) were never seen again after release. One adult female and three juveniles (1 female, 2 males) caught at Roosting Area 3 in 2005 were never seen after release. Of 15 adult females observed in 2005, 7 were observed again in 2006. Two individuals from Roosting Area 3 that disappeared after release in 2005 returned in 2006, including one female. The number of bats observed at each roosting area is summarized in Table 3.3. At both roosting areas more than 60% (62.2% at Roosting Area 1 and 66.7% at Roosting Area 2) of the individuals observed in 2005 returned to the same roosting area in 2006. This annual fidelity did not differ significantly between the two roosting areas (χ^2-test, $\chi^2 = 0.014$, d.f. = 1, NS).

The total number of times that bats were observed and the times that those records could be identified as a switch or stay event to the roost-site or roost-tree (or house) for roosting areas and years are summarized in Table 3.4. Data for Roosting Area 3 were included but not analyzed because it was census much less frequently (Table 3.4). More than 90% of the sightings could be identified as a switch or stay , and there were no significant differences in the identification rates between the Roosting Area 1 and Roosting Area 2 in both years (χ^2-test, in 2005, $\chi^2 = 0.10$, d.f .= 1, NS; in 2006, $\chi^2 =$ 3.806, d.f .= 1, NS). However, the identification rate of roost-tree (or house) switch or stay at Roosting Area 1 was significantly lower than Roosting Area 2 in both years (χ^2-test, in 2005, $\chi^2 = 136$, $P < 0.001$; in 2006, $\chi^2 = 84.993$, $P < 0.001$). This is likely because the bats that appeared to disappear from Roosting Area 1 may have been hidden in the foliage, but still present. Therefore, roost-tree fidelity in Roosting Area 1 is overestimated.

A higher trapping effort in 2005 (to complete banding) resulted in the average time each bat was observed at Roosting Area 1 being significantly higher in 2006 than in 2005 (Table 3.4; Mann-Whitney test, $z = -2.929$, $P < 0.01$). At Roosting Area 2, the average number of times each bat was observed did not differ between years (Table 3.4; Mann-Whitney test, $z = -0.266$, NS). On average, I observed more banded bats per day in 2006 than in 2005 at Roosting Area 1, but observed more in 2005 than in 2006 at Roosting Area 2 (Table 3.4; Mann-Whitney test, at Roosting Area 1, $z = -5.123$, $P < 0.001$; at Roosting Area 2, $z = -8.601$, $P < 0.001$). During the census period, each individual bat, was observed an average of 90% of the time at Roosting Area 2, but only 60% of the time at Roosting Area 1 (Table 3.4; Mann-Whitney test, in 2005, $z = -3.935$, $P < 0.001$; in 2006, $z = -3.523$, $P < 0.001$). There was no significant difference in individual bat re-sightings between years at the same roosting area (Table 3.4; Mann-Whitney test, in 2005, $z = -1.051$, NS; in 2006, $z = -2.170$, NS).

Radio-tracking

In total, I radio tagged and tracked 13 adult females for 110 bat-days (Table 3.5). Two bats were followed for two sessions. All bats that I followed roosted exclusively in foliage, except for one that roosted exclusively in a house at Roosting Area 2. The signals of all radio-transmitters were not detected every day (28/110 bat-days were not detected), and I considered undetected bats to be roosting away from the roosting areas. The incidence of radio-tagged bats roosting in Roosting Area 1 did not differ significantly from roost use by banded but not radio-tagged bats (Table 3.4; Kruskal-Wallis test, $H = 2.897$, NS). These data suggest that visual census did not underestimate the presence of

bats in the roosting areas. Therefore I combined the radio-tracking data with the non-radio-tracking for the subsequent analysis of roost fidelity.

Type of roost

In Roosting Area 1, bats switched roost-site frequently (every 2.1 ± 0.9 days) and used a number of roost-sites and roost-trees in both years (in 2005, 9.2 ± 5.6 roost sites from 3.5 ± 2.4 roost trees; in 2006, 16.6 ± 8.2 roost sites from 3.6 ± 1.9 roost trees) while at Roosting Area 2 bats switched roost site every 13.9 ± 8.7 days, and only used 2.67 ± 0.87 (in 2005) and 1.43 ± 0.53 (in 2006) roost sites. The positions where bats were found at Roosting Area 2 were very constant, and of 91.2% of the incidence, banded bats at Roosting Area 2 were observed at site B (see Figure 2.2).

Overall, roost-site fidelity was significantly higher at Roosting Area 2 than Roosting Area 1 in both years (Table 3.6; Mann-Whitney, in 2005, $z = -4.553$, $P < 0.001$; in 2006, $z = -4.165$, $P < 0.001$). There was no significant change in roost-site fidelity between years in Roosting Area 1 (Mann-Whitney test, $z = -0.498$, NS), but at Roosting Area 2 the roost-site fidelity was significantly higher in 2006 than 2005 (Mann-Whitney test, $z = -3.017$, $P < 0.01$). Fidelity to the roost-tree (or house) was significant higher than fidelity to the roost-site in most of the roosting area-year combinations, except at Roosting Area 2 in 2006 (Table 3.6), but there was no significant difference between the roost-tree (or house) fidelity of the two roosting areas in either year (Kruskal-Wallis test, $H = 2.3$, NS).

Sex and age

At Roosting Area 1, where the different sex-age classes were available for comparison, the roost-site fidelity was significant lower in adult males than adult females and juveniles (or subadults) in 2005 (Table 3.6; Kruskal-Wallis test, $H = 6.409$, $P < 0.05$; Mann-Whitney test, AF vs. AM, $z = -2.451$, $P < 0.05$; AM vs. J, $z = -2.045$, NS), but the difference was not significant in 2006 (Table 3.6; Mann-Whitney test, $z = -0.379$, NS). There was no difference in fidelity to roost-site or roost-tree between adult females and juveniles in 2005 (Table 3.6; Mann-Whitney test, roost-site, $z = -0.576$, NS; roost-tree, $z = -1.906$, NS).

Reproductive status

Roost-site and roost-tree (or house) fidelity of adult males, pregnant females, lactating females, post-lactating females and non-reproductive females is summarized in Table 3.7. Roost-site fidelity differed significantly among pregnant, lactating and post-lactating females in Roosting Area 1 in both years, Roosting Area 2 in 2005, and almost in 2006 (Table 3.8). Fidelity to roost-tree (or house) differed significantly among the sex-reproductive status groups at both roosting areas in 2006, but not 2005 (Table 3.8). Male and non-reproductive bats did not show any significant difference with any other groups, except in Roosting Area 1 in 2005 when the male roosting fidelity was significantly lower than lactating female (Table 3.9).

Comparisons between females of the same reproductive status between areas showed that bats from Roosting Area 2 always showed a significantly higher fidelity to roost sites (Table 3.10 & Figure 3.2), but there was no significant difference between fidelity to roost-tree (or house) (Table 3.10).

Disturbance

Fidelity to roost-site and roost-tree (or house) within ten days after capture in 2005 was compared with the fidelity to roost-site and roost-tree (or house) exactly one year later. Roost-site fidelity after capture was slightly lower at Roosting Area 1 and much lower at Roosting Area 2, but the differences were not significant (Table 3.11).

Subgroup size

At Roosting Area 1, roost-site fidelity and the mean subgroup size joined by individual bats was significantly correlated in both years (in 2005, $r = 0.569$, $P < 0.001$; in 2006, $r = 0.288$, $P < 0.05$). There was no correlation at Roosting Area 2 in 2005 ($r = 0.569$, $P = 0.058$) or 2006 ($r = -0.098$, NS). When all roosting areas and years were combined, the overall correlation was significant ($r = 0.743$, $P < 0.001$; Figure 3.3). At both roosting areas and years, the average subgroup size of bats that stayed at the same roost-site or roost-tree (or house) for >1 day was significantly larger than for bats that made a switch the next day (Table 3.12 & Figure 3.4). On days when bats remained in the same roost-site, their subgroup size the next day increased (in 2005) or remained the same (in 2006) at both roosting areas (Table 3.13). When bats switched roost-sites, the size of the subgroup they newly joined was not significantly different from the subgroup the bats were in on the previous day (Table 3.13).

Rain

In 2006, I recorded 32 days with rain regardless of the time of the day. Fourteen days had rain at night and 16 nights had rain only during the day. On two days I did not record the time of rain. Bats at Roosting Area 1 significantly increased frequency of

roost-site switches after rain, but the frequency of roost-tree switches did not differ significantly (Table 3.14 & Figure 3.5). Rain showed no significant effects on bat roost switching frequency at Roosting Area 2 (Table 3.14 & Figure 3.5).

Ectoparasites

I found no ectoparasites on any of the 105 sampled bats. Therefore ectoparasite load was not correlated with the roost switching.

Moving distance

My data from visual censuses indicated that two individuals switched between roosting areas. One was a male subadult originally caught at Roosting Area 1 on 25 June 2005, then observed occasionally at Roosting Area 1 until 24 July. He was found at Roosting Area 2 from 25 to 27 July and then never observed again. An adult female caught when pregnant on 1 May 2005, was observed at Roosting Area 3 in both years and was last observed on 31 May 2006 at Roosting Area 1. Distances between Roosting Area 1 and Roosting Area 2 were 1.01 km, 4.72 km between Roosting Area 1 and Roosting Area 3.

Radiotracking data indicate that the mean distance between consecutive roosts (when roost switching occurred) for a given bat and the mean for all bats are shown in Table 3.5. Of 41 recorded roost switches, I did not locate 11 of the consecutive roost-sites. Known moves involved distances of from 1 to 3877 m but 43.3 % of the moves were <10m, 76.7% were ~100 m and only 10% were >1000 m. Lactating females moved less (79.9 ± 45.3 m, n = 6) than pregnant females (346.7 ± 416.2 m, n = 6) or post-lactating females (521.7 ±436.8 m, n = 3) but these differences were not significant

(Kruskal-Wallis test, $H = 1.442$, NS). In total, only on 4 of the 110 bat-days, all from post-lactating females, the bats roosted in the foraging sites.

I tracked 10 radio-tagged bats to their foraging sites on a total of 37 bat-nights from 24 different nights. The mean distance between roost-site and the farthest foraging site on a given night was 3.09 ±1.81 km (range: 0.7 - 5.9 km). Of the 37 bat-nights, only 13 switched roost-sites the next day and distances between roost-sites (range: 2 – 3877 m), only 8 of the 13 were able to be measured, were 18.98% of the distances between roost-site and forage site (range: 1450 – 4376 m). Roost tree switches were identified on 9 of 37 bat-nights. Ten records were unidentifiable as switch or stay.

Number of associates

In average, the bats in Roosting Area 1 associated with 8.1 ± 7.2 and 21.0 ± 9.8 other banded bats in 2005 and 2006, respectively. Individuals with higher roost fidelity usually had more associates than individuals with low roost fidelity (Figure 3.6; in 2005, $r = 0.606$, $P < 0.001$; in 2006, $r = 0.297$, $P < 0.05$). At Roosting Area 2, the numbers of associates were the same for all individuals. These bats averaged eight associates (not including G22, the juvenile male which was from Roosting Area 1) in 2005 and six in 2006. These values were the maximum association numbers for each bat and thus there was obviously no correlation between number of associates and roost fidelity at Roosting Area 2.

Part B. Analysis of nonrandom association

Association

Individual samples used in this analysis are the same as those used in part A, except three adults (two female, one male) from Roosting Area 1 in 2005, who were excluded because I observed them < 5 times (Table 3.15). At Roosting Area 1 in 2005 banded bats roosted in 157 roost-sites in 43 different trees. In 2006 banded bats used 239 roost-sites in 28 trees.

At Roosting Area 2, all individuals had an overlap history ≥ 5 days and were associated with each other so all of the dyads were included in the analysis for the three indices. However, at Roosting Area 1, not all dyads were included in the analysis. First, 19.51% (168/861) of the dyads in 2005 and 1.71% (12/703) of the dyads in 2006 had shared an overlapping history of < 5 days and were not included in the analysis of association index. Second, 495 dyads for χ2-index and 30 dyads for SGS-index in 2005, and 249 dyads for χ2-index and 16 dyads for SGS-index in Roosting Area 1 in 2006 were not calculated due to no overlapping in roost-site (for χ2-index) or never been observed on the same day (for SGS-index).

The degrees of the three pairwise associations among bats were highly variable (Table 3.16). At Roosting Area 1, the SR-index from 74.31% of the dyads (515/693) in 2005 was zero which indicated that those individuals had never associated during that year. The percentage decreased to 42.26% (292/691) in 2006. Considering the overlap history in the roost, about one seventh (12.70% in 2005, 15.77% in 2006) of the dyads had never been associated but had been using the same trees as roosts. This indicates that not all of the non-associations between individuals were due to spatial isolation. Cluster

analysis of the SR-index revealed that bats at Roosting Area 1 formed several subgroups, and that some of the subgroup members seldom interacted with each other. In 2005, six subgroups contained multi-individuals while 15 subgroups contained only one individual within each. In 2006, three subgroups contained multi-individuals while eight subgroups contained only one individual within each (Figure 3.7 a, b). At Roosting Area 2, all bats had a high SR-index value and were within the same subgroup (Figure 3.7 c, d).

Although the sample size was larger at Roosting Area 1 in 2005 than in 2006, both the duration that each banded bat had been included in the census (Figure 3.8; mean duration in 2005, 46.57 ± 27.31 days; in 2006, 66.84 ± 23.99 days) and the number of associates per bat (Figure 3.9; in 2005, 8.48 ± 7.15 bats; in 2006, 21.00 ± 9.77 bats) were significantly lower in 2005 than 2006 (Mann-Whitney test, duration, $z = -3.253$, $P < 0.01$; associates, $z = -5.228$, $P < 0.001$), resulting in a lower proportion of associated dyads. For example, of the eight bats that had never associated with other banded bats in 2005 (Figure 3.9), five came back in 2006, except one adult male remained not associated with any other bats, and associated with an average of 13.75 ± 9.18 bats in 2006.

Non-random association between sexes and roost types

Association patterns were significantly different between sexes. At Roosting Area 1, adult males were usually solitary, the mean subgroup size was 1.68 ± 1.19 in 2005 and 1.34 ± 0.34 in 2006, and had never associated nor had overlap in the roost-site with other adult males (Table 3.16), making the mean number of associates per male extremely low (0.67 ± 1.16 in 2005, 1 ± 1 in 2006). This only accounted for 1.6% and 2.7% of sampled bats in the year, respectively. Consequently, the SR-index and χ^2-index of the dyads which involved adult male were extremely low. Most were significantly

different from other sex-age dyad combinations (Table 3.17), and the number of significant positive associations was also few.

The adult female subgroups at Roosting Area 1 (15.04 ± 10.80 in 2005 and 15.18 ± 6.57 in 2006) was significantly larger than the adult male subgroups in both years (Mann-Whitney test, in 2005, $z = -2.641$, $P < 0.01$; in 2006, $z = -2.707$, $P < 0.01$), and the numbers of associates per adult female were significantly higher than for males (10.13 ± 7.09 bats in 2005, 22.71 ± 8.10 bats in 2006, Mann-Whitney test, in 2005, $z = -2.224$, $P < 0.05$; in 2006, $z = -2.726$, $P < 0.01$). In 2006 (when all sampled individuals were banded before census started), adult females associated with an average of 66.6% of the other banded females. Only 25.7% of the adult females associated with less than the average through the course of the experiment. These data indicated that most of the adult females interacted with one another.

The SR-index and χ^2-index of dyads that included two adult females were consistently higher than all the other sex-age combination and in some cases, were significantly different from other sex-age combinations (Table 3.16 & 3.17). This indicates that a closer and more active association existed between adult females. In addition, of the SGS-index value of the dyads consisting of two adult females, about 15% (in 2005) and 22% (in 2006) of the dyads were significantly positive and 10% (in 2005) and 15% (in 2006) were significantly negative indicating some non-random associations among adult females (Table 3.16). Dyads of two adult females that had a significantly negative value also had a lower value in SR-index than those of which had a significantly positive value (Mann-Whitney test, in 2005, $z = -8.825$, $P < 0.001$; in 2006, $z = -13.328$, $P < 0.001$; Figure 3.10), and this pattern was the same as for χ^2-index but only significant

in 2006 (Mann-Whitney test, in 2005, $z = -1.938$, $P = 0.053$; in 2006, $z = -5.353$, $P < 0.001$; Figure 3.10).

Bats at Roosting Area 2, where only adult females were observed, showed a significantly higher SR-index and lower $\chi 2$-index than adult females at Roosting Area 1 during the same year (Table 3.16 & 3.17). About one fourth of the value in the SGS-index were significant and exclusively positive.

Overall, most adult females significantly associated with some individuals in the roosting area. However, at Roosting Area 1, some had significant avoidance (negative) to other individuals as well (Table 3.18).

Non-random associations among bats with differing reproductive status

At Roosting Area 1 the highest SR-index value always occurred between pregnant females, then lactating females, and the lowest were always between post-lactating females (Figure 3.11a; Kruskal-Wallis test, in 2005, $H = 45.735$, $P < 0.001$; in 2006, $H = 96.855$, $P < 0.001$). The post-lactating females also showed a significantly lower value in $\chi 2$-index in 2006, but not 2005 (Figure 3.11b; Kruskal-Wallis test, in 2005, $H = 5.601$, NS; in 2006, $H = 10.468$, $P < 0.01$). At Roosting Area 2, the SR-index value was high and the $\chi 2$-index was extremely low across bats with every reproductive status. However, the pregnant female in 2005 showed the lowest SR-index and highest $\chi 2$-index value (Figure 3.11; Kruskal-Wallis test, SR-index: in 2005, $H = 14.964$, $P < 0.001$; in 2006, $H = 3.238$, NS; $\chi 2$-index: in 2005, $H = 19.832$, $P < 0.001$; in 2006, $H = 3.020$, NS).

Dyads of pregnant females at Roosting Area 1 in both years also showed a higher positive association SGS-index than expected although the differences were not

significant (Table 3.19). A significantly higher frequency of negative association was detected in the dyads of pregnant females at Roosting Area 1 in 2006 but not in 2005 (Table 3.19). Only a few dyads showed a significantly positive association at Roosting Area 2, and there was no significantly negative association detected at Roosting Area 2 (Table 3.19).

Associations between years

At Roosting Area 1, of the 26 individuals included in the analysis for both years, the SR-index and SGS-index of dyads in 2005 were both significantly and positively correlated with the corresponding value in 2006 (Mantel –test, SR-index, $r = 0.499$, $P < 0.001$; SGS-index, $r = 0.188$, $P < 0.05$), and the value of $\chi 2$-index was also positively correlated between years although not significant (Mantel –test, $r = 0.223$, NS).

At Roosting Area 2 only six individuals were observed in both years. Individuals with higher values of SR-index and $\chi 2$-index in 2005 also had a significantly high value in 2006 (Mantel test, SR-index, $r = 0.709$, $P < 0.05$; $\chi 2$-index, $r = 0.475$, $P < 0.05$) and the SGS-index value was also positively correlated between years although not significantly (Mantel –test, $r = 0.364$, NS).

Genetic relatedness

Fifty-eight bats were genotyped for seven loci of the microsatellite DNA and fifty-seven bats were sequenced for mitochondrial DNA (Table 3.15). Only one individual (adult female) caught in this experiment was not sequenced for mitochondrial DNA. Individuals that not caught by this experiment (see Materials and Methods) were not included in the relatedness analysis.

Based on the 151 individuals sampled (see part C), the seven microsatellite loci were polymorphic with the number of alleles per locus ranging from 8 – 21. Each locus showed a moderate to high level of polymorphism with 8 – 21 alleles per locus. The observed heterozygosity (H_o) of each loci was 0.47-0.88, and the two lowest numbers were from D15 (H_o =0.57), which had the lowest number of alleles, and F19 (H_o =0.47) which showed a significant deviation from the HWE ($P < 0.001$) and thus was removed from the subsequent analysis (Table 3.20). Except F19, only 4 of the 54 locus-colony combinations showed significant deviation from HWE, but no consistent patterns occurred either in specific colonies or loci. None of the locus pairs showed significant linkage disequilibrium ($P > 0.22$).

Average pairwise relatedness of adults at Roosting Area 1 was 0.05 ± 0.002 and 0.06 ± 0.012 at Roosting Area 2. In the bats used for the association indices analysis, nine mitochondrial DNA haplotypes resulted from 11 polymorphic sites (Table 3.21 & 3.22).

Association vs. genetic relatedness

When all individuals at Roosting Area 1 were combined, I detected no significant differences in the degree of associations between dyads carrying the same versus different mitochondrial DNA haplotypes in either 2005 or 2006 (Figure 3.12 a, b; 2005: SR-index, z = -1.04, NS; χ^2-index, z = -0.13, NS; 2006: SR-index, z = -1.682, NS; χ^2-index, z = -0.812, NS). There were no significant differences in the degree of associations between dyads at either Roosting Area 1 or Roosting Area 2 when only adult females were included (Figure 3.12 c, d; Roosting Area 1 in 2005: SR-index, z = -1.365, NS; χ^2-index, z = -0.653, NS; Roosting Area 1 in 2006: SR-index, z = -1.101, NS; χ^2-

index, z = -0.837, NS; Roosting Area 2 in 2006; SR-index, z = -0.426, NS; χ^2-index, z = 0, NS).

The observed distribution of significant positive and negative associations based on the SGS-index did not significantly differ from the chance expected frequencies in dyads of all individuals with the same or different mitochondrial DNA haplotypes at Roosting Area 1 in 2005 and 2006 (Table 3.23). When only adult females are considered, the observed distribution of significant positive and negative associations based on SGS-index did not significantly differ from the expected frequencies by chance in dyads with the same versus different mitochondrial DNA haplotypes at either Roosting Area 1 or Roosting Area 2 (Table3.23).

The SR-index values compared with genetic relatedness of the dyads based on microsatellite DNA showed no significant correlation when all individuals were included (Mantel test: Roosting Area 1 in 2005, r = 0.043, NS; Roosting Area 1 in 2006, r = -0.045, NS) nor when only adult females were included (Mantel -test: Roosting Area 1 in 2005, r = 0.017, NS; Roosting Area 1 in 2006, r = -0.042, NS; Roosting Area 2 in 2005, r = 0.122, NS; Roosting Area 2 in 2006, r = -0.164, NS).

Part C. Genetic subdivision among colonies

Samples

I obtained tissue samples from 151 individuals from nine colonies, including 111 females and 40 males (Table 3.24). Sample sizes from each colony ranged from 2-71. Banding data revealed that not all bats roosting at Roosting Area 1 or Roosting Area 2 returned to the roost in the second year (see part A), and this might be reflected in the

genetic variation between years. Therefore I grouped individuals by years and colonies. The sample sizes for each roosting area and year are summarized in Table 3.24.

Genetic diversity

Mitochondrial DNA

I obtained HVII sequences from 147 bats. One adult female and one juvenile male from Roosting Area 1, one juvenile male from Roosting Area 7 and one juvenile from Roosting Area 9 failed to sequence (Table 3.24). I identified 22 haplotypes with 21 polymorphic sites (Table 3.25). The empirical transition/ transversion ratio was 9.5.

For a sample size > 3, haplotype diversity was high (range: 0.718-0.9, Table 3.27). Nine of the haplotypes were unique to single individual colonies where each was represented by only one or two copies. When adult males were removed, 10 haplotypes were unique to single colonies and haplotype H1, occur in 18 non-adult males, was unique to Roosting Area 1. Here 91.8% of the individuals shared the haplotypes with other individuals in the same colony. All haplotypes occurred in at least one adult female, except for one haplotype, which occurred only in one adult male from Roosting Area 2 (H23) and two male juveniles from Roosting Area 3 (H16). Within Roosting Area 1, 2 and 3 (where I obtained the most samples, 107/147), I found 19 haplotypes, accounting for 86.4% of all haplotypes found. Seven were found only in one year at the three roosting areas (Table 3.26). I identified two distinct clades from the relationship of different mitochondrial DNA haplotypes based on 21 of the 22 haplotypes identified (Figure 3.13). For colonies that had a total sample size > 3, all contained haplotypes from both clades, except the most distant colony from Roosting Area 9, which only had

haplotypes from clade B and haplotype H61. Haplotype H61 is the intermediate between clade A and B. At Roosting Area 1, from which most individuals were sampled, individuals of haplotype H1, H7 and H8 consisted of more than 78% of the colony while H7 and H8 formed clade A and H1 was from clade B. When adult males were removed, all colonies with a sample size > 3 had haplotypes from both clades.

The mean nucleotide diversity over all individuals was 1.41 ± 0.79 %. With the exception of the samples from Roosting Area 5 where the three individuals had identical sequences, nucleotide diversity at each colony ranged from 0.6-1.6 % (Table 3.27).

Microsatellite DNA

One hundred forty-eight of 151 individuals were genotyped at seven microsatellite loci. The remaining three only had 6 loci amplified. I observed high levels of heterozygosity in each colony-year combination. The mean number of alleles per locus ranged from 3.7-13.8. Despite the three lowest numbers occurring in the colonies with a sample size <5, the mean number of alleles per locus in all the other colonies was > 7.5 (Table 3.27).

Genetic subdivision

Using all individuals together, the Bayesian clustering approach, STRUCTURE, detected no differentiation among colonies based on microsatellite markers. The proportions of individuals assigned to each cluster were symmetric and equal to $1/K$, as K increased from 1 to 18. All individuals had genotypes from all clusters.

Pairwise F_{ST} based on microsatellite loci suggested nearly no genetic subdivision between colonies. I found no significant differences between the three colonies in 2005

(Table 3.28), and four (out of 21) statistically significant pairwise F_{ST} were between Roosting Area 1 and Roosting Area 4, Roosting Area 1 and Roosting Area 7, Roosting Area 3 and Roosting Area 7, and Roosting Area 9 and Roosting Area 7 in 2006 when both sexes were combined (Table 3.29). There were no significant differences among any comparisons based on females only (Table 3.30, Table 3.31). Although the AMOVA based on microsatellite data showed that more than 99% of the variation was within colonies, the comparison based on both sexes combined in 2006 showed a slightly higher variation from among colonies (1.38%) and a significant F_{ST} (0.014) (Table 3.32). The F_{ST} value from separate AMOVAs based on both sex combined and female only in the three colonies (Roosting Area 1, 2 and 3) were also similar (in 2005, -0.003 and 0; in 2006 0.006 and 0.003, respectively)

In contrast, pairwise Φ_{ST} based on 2006 mitochondrial DNA showed a wider range between pairs based on both sex combined (range: -0.007 – 0.706; Table 3.29) and females only (range: -0.043 – 0.802; Table 3.31). I found significant differentiations in about half of those pairs. The AMOVA based on mitochondrial data showed that a very low variation (< 2%). This variation was attributed to differentiation between the three colonies based on both sexes combined or females in 2005 and was not significant (Table 3.28, Table 3.30, Table 3.32). However, the AMOVA showed increasing variation among same three colonies in 2006 (both sexes combined, 10.38%; females only, 11.91%) translating to a significant differentiation (both sexes combined, $\Phi_{ST} = 0.104$, $P<0.001$; female only, $\Phi_{ST} = 0.119$, $P<0.001$). When I included the other colonies sampled in 2006, the AMOVA showed the highest variation among colonies (both sexes combined, 23.53%; female only, 20.66%), and the highest fixation indices (both sexes

combined, $\Phi_{ST} = 0.235$, $P < 0.001$; female only, $\Phi_{ST} = 0.207$, $P < 0.001$) (Table 3.32). Fixation indices were similar and did not always increase when the males were excluded from the analysis (both sexes combined vs. female only: 2005, 0.012 vs. 0.017; 2006 three colonies, 0.104 vs. 0.119; 2006 all, 0.235 vs. 0.207) (Table 3.32).

The genetic distance between colonies did not significantly increase with geographical distance. I analyzed the pattern of isolation-by-distance based on genetic markers for both sexes combined and adult female only with the four different combinations of either log-transformed applied to the linearized genetic distance or geographic distance, i.e., genetic distance vs. geographic distance, log (genetic distance) vs. geographic distance, genetic distance vs. log (geographic distance), log (genetic distance) vs. log (geographic distance) (Mitochondrial DNA: both sex combined, r = 0.319 – 0.358, NS; females only, r = 0.218 – 0.306, NS; Microsatellite DNA: both sex combined, r = 0.089 – 0.433, NS; female only, r = -0.431 – -0.168, NS) (Figure 3.14).

Parentage

The distribution of maternal and paternal relationship within and between colonies is listed in Table 3.33. One sex-unknown juvenile collected from Roosting Area 2 assigned to a mother from Roosting Area 4 was not on the list due to the unknown sex. In total, the number of identified parent-offspring dyads was low. Only 15.2% (23 out of 151) of the individuals roosted with a parent or offspring, and only 3.6% (4 out of 111 female) of females roosted with their mother or daughter.

Sex-biased dispersal and transients

The sex-dispersal test in the FSTAT could not reject the null hypothesis that both sexes dispersed equally (F_{ST}, female = -0.006, male = 0.056, $P = 0.917$). Similarly, the test detected no differences in F_{ST} between individuals observed only in the first year and those observed for both years (residents = -0.003, transients = -0.015, $P = 0.142$). The test also revealed no differences between individuals observed only in short term and those observed for long term (residents = 0.001, transients = -0.004, $P = 0.390$).

Chapter 4. Discussion

Part A. Causes of roost switching

The outcome of the predictions and hypothesis related to the causes of roost switching are presented in Table 4.1.

Roost types

My data on roost switching are similar to those presented for Rafinesque's big-eared bat, *Corynorhinus rafinesquii*, that showed a low day-to-day roost fidelity in the area with abundant tree roosts and a high roost fidelity to the man-made structures where tree roosts were less common (Trousdale et al. 2008). The most obvious differences between roost types were 1) availability 2) permanency and 3) space. The three factors of roosts are usually associated with each other and difficult to separate as comparison was based on different species (Lewis 1995; Kunz & Lumsden 2003). My data come from one species, and in my study area there is no indication that the roosts were limited in their availability to the house-roosting *Myotis formosus*. *Myotis sodalis* moved greater distances between roosts and foraging areas in areas where roost density was lower (Kurta et al. 1996; Kurta et al. 2002).

Furthermore, the difference of permanency between roost types does not explain roost switching behaviour. Although leaves are much less permanent than houses, except in a few cases, most roost switches at Roosting Area 1 did not reflect an obvious change in roost specifics, and bats often reused the same leaves as roosts. These data suggest that the ephemerality of roost-sites does not explain the frequency of roost switching. Frequent roost switching in the absence of destruction of roosts also occurred in several

tree roosting bat species. For example, the New Zealand long-tailed bat, *Chalinolobus tuberculatus*, switch roosts every 1.7 ± 2.0 days, even though the roost-tree has been occupied by bats for hundreds of years (O'Donnell & Sedgeley 1999). Big brown bats, *Eptesicus fuscus*, switch roosts every 1.7 ± 0.7 days and 73% of the tree roosts were reused in the subsequent year (Willis & Brigham 2004).

The difference of roost fidelity between roost types is consistent with the hypothesis that individuals roosting in a roost with limited space switch roosts more frequently than those using more spacious roosts. Large roost capacity allows larger groups and associated benefits, e.g. thermoregulation. In addition, O'Donnell (2000) suggested that *C. tuberculatus* use small tree cavities as roosts and increased social contact by frequent roost switching. Willis & Brigham (2004) further suggested that the roost fidelity to a physically large roost, i.e., buildings, tunnels, or caves, were essentially identical behaviour to the fidelity of bats to a small area of forest. Kunz & Lumsden (2003) observed that high roost fidelity was usually associated with large subgroup size, implying the importance of roost capacity.

Sex and reproductive status

In general in bats, higher roost fidelity by males has more often been reported in the tropics and has been considered to be associated territorial defense. Resident males defend their roosts which could be costly to construct (e.g., tent-roost - Storz et al. 2000) or less available (e.g., tree hole - Wilkinson 1985; Williams 1986). By defending a territory, males were thought to increase their probability of access to potential mates attracted by the roost resource (Kunz & McCracken 1996).

For bat species using more abundant roosts, especially temperate species, roost fidelity between sexes were usually similar (e.g., tree cavity or rock crevice – Brigham 1991; rock crevices – Lewis 1996; under exfoliating tree bark – Menzel et al. 2001). Roost availability has different impacts on males than on females. Chaverri et al. (2007) documented roosting behaviour of *Artibeus watsoni*, tent-making bat. When roost availability was low, male and females had higher and similar roost fidelity, but when roost availability increased, roost fidelity for both sexes dropped because the cost-benefit ratio associated with roost switching decreased. Roost fidelity of females dropped significantly more than that of males suggesting that the male might gain more advantage from territorial defence by staying in the same roost.

Foliage-roosting *M. formosus* are neither tent-making nor using a rare type of roost. Equally low roost fidelity for males and females implies that the cost-benefit ratio of roost switching was similar for both. The exception of higher roost fidelity shown by lactating females suggested that the cost-benefit ratio to switch roost is higher for them than for females in other reproductive states. High roost fidelity in lactating females was documented in *Barbastellus barbastellus* (Russo et al. 2005), and although lactating pallid bats, *Antrouzois pallidus*, did not show difference in roost fidelity from pregnant females, they reduced moving distance by about 50% compared to pregnant females (Lewis 1996). Lewis (1995) predicted that roost switching was costly and that the costs would be highest for lactating females which would switch roosts less often than other bats. Brigham & Fenton (1986) demonstrated that the reproductive success of *E. fuscus*, was decreased when they were excluded from their preferred roost suggesting site-specific benefits for breeding. Roost switching by lactating females was presumed to be

accompanied by moving non-volant young which was considered to be energetically expensive, and might increase the probability of predation (Kunz & Lumsden 2003).

M. formosus young of the year could not fly until two weeks of age and not skilfully until four weeks of age when they had reached about 70% of adult body mass (Shen & Lee 2000). Here the higher roost fidelity in lactating females suggests a higher ratio of costs to benefits for roost switching behaviour.

Disturbance

My results do not support the hypothesis that disturbance would result in a higher roost switching frequency in bats and indicated the capture in 2005 did not directly affect the roost switching frequency and the observation was reliable. Other researchers have documented change in roost switching behaviour immediately after disturbance. Fenton et al. (1993) documented this behaviour by radio-tagged *Noctilio albiventris*, and Barclay and Kurta (2007) classified it as emergency roost switching unlike recurrent roost switching. Other observers had documented emergency roost switching by *M. formosus* when individuals were flushed by the sudden and loud noise created by a running ambulance or the sudden coming thunder shower (Chang 2007). No significant increasing of roost switching frequency has also been documented for other bat species in response to disturbance associated with research (e.g., Lewis 1996; O'Donnell & Sedgeley 1999; Russo et al. 2005).

Predation

The short moving distance between consecutive roosts of foliage-roosting *M. formosus* and high roost-site fidelity of house-roosting individuals, which usually had a

larger subgroup size than the foliage-roosting ones, indicated that the attractiveness to the predator might not be an important issue of anti-predator strategies, but the increased subgroup size might be more important. This is consistent with the findings from their roost selection: female *M. formosus* roosted high up in trees and farther from the main trunk (compared to males), which were attributed to predator avoidance (Chang 2007). Like many studies attributing bat behaviour to predator avoidance, I had no direct evidence of predation on *M. formosus*, in both years of my study red-bellied squirrels, *Rubrisciurus rubriventer*, was active at Roosting Area 1 and were observed attempting to approach a group of roosting *M. formosus* (Chang 2007). All these observations imply that foliage-roosting *M. formosus* might spend more energy on vigilance or remaining mobile. A comparison of *E. fuscus* roosting in rock crevices versus buildings suggested a higher predation risk for crevice-roosting individuals and obliged bats roosting there to maintain higher body temperatures than those roosting in buildings (Lausen & Barclay 2006).

Ectoparasites

Ectoparasites can be common on tropical and temperate species of bats (Chilton et al. 2001; ter Hofstede et al. 2004; Patterson et al. 2007), and impose cost to their hosts (Lewis 1996; Giorgi et al. 2001). Roost switching by host bats could interrupt the reproductive cycle of ectoparasites (Reckardt & Kerth 2006; Bartonička & Gaisler 2007). Reckardt & Kerth (2007) demonstrated that *M. bechsteinii* use roost switching to avoid ectoparasites.

I observed no ectoparasites on any of the *M. formosus* I handled. The absence of ectoparasite load might be related to the exposed roosting habits of *M. formosus* because

the intensity of ectoparasites can correlate to the level of enclosure of the roosting environment (Patterson et al. 2007). Bats roosting in foliage and in other exposed situations may have lower rates, even no ectoparasites, of infestation by ectoparasites than those roosting in more sheltered situations (ter Hofstede & Fenton 2005; Patterson et al. 2007). While I expected low incidences of ectoparasites in foliage-roosting individuals, *M. formosus* roosting in the house would have been expected to harbour more ectoparasites. The zero infection on high fidelity, house-roosting *M. formosus* might also be due to its exposed roosting habits, which was in contrast with other house-roosting bats mostly roosting inside the house and the environment was enclosure.

Weather

Other researchers have reported that rain could prevent the bats from exiting to forage in the evening and thus reduce roost switching (Vonhof & Barclay 1996) and the same is true of crevices-roosting *A. pallidus* (Lewis 1996). *M. formosus* did not fail to emerge to forage during the rain (Yang 1996). There are at least two possible explanations of why foliage-roosting *M. formosus* switched roosts more frequently after rain while house-roosting individuals did not. Rain might change the suitability of the roosting leaves or obliterate chemical cues some bats use to identify roostmates (Kerth et al. 2002b; Safi & Kerth 2003). Sheltered sites like the building roost should be less subject to either influence of rain.

Commuting distance

In general, bats seem not to switch roosts to reduce commuting distance to foraging sites (Lewis 1995). My results did not support the hypothesis that bats switched

roost to reduce the commuting distance between foraging and roost-sites. I found that *M. formosus* moved short distances between roosts compared to the distances they travelled to forage. It seems common in forest-dwelling bats that switching roost frequently while having a fidelity to a small area but foraging over a larger range (Fenton 1983; Brigham & Fenton 1986; Lewis 1996; Vonhof & Barclay 1996; Kunz & Lumsden 2003; Russo et al. 2005).

Individuals associated

My results generally support the predictions that *M. formosus* lived in a fission-fusion society. The only discrepancy with the prediction was that the number of individuals associated decreased as the roost switching frequency increased, which was in contrast with the observation from *E. fuscus*, which increased the number of individuals associated by increasing the roost switching frequency (Willis & Brigham 2004). This is probably because *M. formosus* associated with more individuals by joining a larger subgroup rather than by switching roost-site or sometimes the whole group just switched together (personal observation).

Part B. Analysis of nonrandom association

Roost types

My data for foliage-roosting *M. formosus* support the work of others showing that a combination of the outcomes of the three indices was consistent with the description of the fission-fusion society for example in *M. bechsteinii* (Kerth and Konig 1999) or *Desmodus rotundus* (Wilkinson 1985), and other bat species, albeit based on different analyses (O'Donnell 2000; Willis & Brigham 2004; Rhodes 2007; Popa-Lisseanu et al.

2008). The fact that subgroup size remained the same while the colony size and number of subgroups declined in 2006 (Table 3.1) also is consistent with Kerth's and Konig's (1999) data for *M. bechsteinii* where colony size and number of subgroups decreased over three years of experiment, the subgroup size remained rather stable. Overall, my results support the hypothesis that the nonrandom association in subgroups reflects the importance of minimum subgroup size, and individuals formed fission-fusion society to increase the member to associate and overcome the limited subgroup size constrained by the physical structure. In addition, the significantly correlated association of dyads between years suggests a long term association between individuals for both types of roost.

Sex and reproductive status

My results showing the impact of reproductive status of females on roost switching behaviour mirrors those of other researchers. Kerth and Konig (1999) reported this for *M. bechsteinii* where reproductive females prefer to roost together while non-reproductive females associated less frequently with other individuals. At Roosting Area 2, the difference of the indices value between different reproductive status was not obvious and the only exception was pregnant females in 2005 that showed a lower value in SR-index and higher value in χ^2-index due to switching roosts more frequently (Figure 3.2). In addition, adult males seldom associated with other bats and most of time was solitary suggesting a difference of the requirement from the interaction of subgroup between sexes. Sexual segregation is common for most temperate bat species (McCracken & Wilkinson 2000; Zubaid et al. 2006), but it is not universal for bat species

to use a fission-fusion system (see Wilkinson 1985; O'Donnell 2000; Vonhof & Fenton 2004).

Genetic relatedness

My results for mean genetic relatedness, multiple matrilineal lines and no relation between association and genetic relatedness or matrilineal relationship are consistent with findings from the two other bat species using a fission-fusion social system, *E. fuscus* (Metheny et al. 2008) and *M. bechsteinii* (Kerth and Konig 1999). The data from bats contrast with those from other mammal species of fission-fusion society (e.g., African elephants, *Loxodonta Africana*, Archie et al. 2006). However, in *E. fuscus*, although the association was not related to genetic relatedness or matrilineal relationship, the individuals of the same matrilineal line were more likely to move together to establish a new colony (Metheny et al. 2008). Mother and daughter of *M. bechsteinii* and *Rhinolophus ferrumequinum* had more overlaps in the foraging sites than other individuals (Kerth et al. 2001; Rossiter et al. 2002). Together the evidence from bats suggests that they discriminate roost mates depending on the genetic relationship and the inclusive fitness is thus possible to be accrued by the kin recognition.

Why different association pattern between roost types

The different association pattern between the different roost types supports the hypothesis that when the physical space of roost is limited, individuals could use fission-fusion social system to associate with more individuals than one roost can house. The spacious house roost accommodated many more individuals in one localized area than foliage roosts. From 1994 to1996, the peak number of *M. formosus* in the house roost

each year was >200 (Yang 1996; Shen 1996) and most of these individuals roosted also at site B. Ten years later I found that most of the *M. formosus* in the house still roosted together at site B and formed a large subgroup, which sometimes consisting of more than 100 individuals. I never observed groups of this size roosting in foliage where the largest single group I saw was around 60 individuals. My data suggest that roost capacity limits subgroup size in *M. formosus*.

Many benefits associated with group living by bats could be positively correlated with subgroup size. Social thermoregulation could increase the capacity for thermal regulation within the roost and increase energy savings for individuals (Willis & Brigham 2007). Increasing vigilance and dilution could reduce the risk of predation (Speakman et al. 1999), while communal nursing could reduce the energy expenditure of females and increase the survival of young (McCracken 1984; Wilkinson 1992b). Exposed roosts used by *M. formosus* could provide less protection from the elements and from predators than roosts in hollows or cavities (Sedgeley 2001; Jacobs 2007), and impose higher costs of thermoregulation (Bartonička & Gaisler 2007; Willis & Brigham 2007). Saving energy by entering torpor during inclement weather might be costly for pregnant or lactating females because low ambient temperatures slow the growth of young (McNab 1982; Racey 1982; Tuttle & Stevenson 1982). Field studies of crevices-roosting *E. fuscus* showed that the pregnant or lactating female used torpor less frequently than post-lactating female, and the cost-benefit ratio of using torpor might be highest in the lactating female (Lausen & Barclay 2003). Therefore, behaviorally clustering in a large group, which could reduce heat loss (Roverud & Chappell 1991), might be more important for *M. formosus*. Most individuals in the house always roosted together also

implies a benefit associate with the subgroup size (Herreid 1967; Trune & Slobodchikoff 1976; Bonaccorso et al. 1992).

Association of individuals in the two roosting areas could have been a consequence of female philopatry as hypothesized for the fission-fusion colony of giant noctule bats, *Nyctalus lasiopterus* (Popa-Lisseanu et al. 2008). Individuals born in the maternity colony were already associated with other colony members and the increase of the repeated interaction would increase the chance of cooperation between individuals (Vehrencamp, 1979; Rothstein & Pierotti 1988). Familiarity with the roost and roost mates over long-term association might benefit philopatric individuals. There is evidence that foraging habitats are inherited in *M. bechsteinii* and *R. ferrumequinum* (Kerth et al. 2001; Rossiter et al. 2002). Long-term associations were important for the food sharing by vampire bats, *D. rotundus* (Wilkinson 1984; Wilkinson 1985). This interpretation is supported by high inter-annual fidelity of *M. formosus* to roosting areas, the virtual absence of exchange between roosting areas, association of dyads between years were all significantly and positively correlated.

Information transfer about foraging (Wilkinson 1992a) and/or roost-sites (Kerth & Reckardt 2003) have been reported in bats and suggested as a potential reason for formation of fission-fusion societies (Popa-Lisseanu et al. 2008). My data for the two roosting areas used by *M. formosus* do not fit this explanation. Although information transfer can be facilitated by the increase of the subgroup number, members do not necessarily roost together. Behaviours like "rallying behaviour", described as a period (15-45 mins) of rapid fight around the vicinity of potential roosts before dawn, by *A. pallidus* had been suggested for locating roosts and roost-mates (Vaughan & O'Shea

1976; Lewis 1996) have also been observed in *M. formosus*. Similar behaviour have also been reported for *B. barbastellus* (Russo et al. 2005) where it occurred before entering the roost-sites at dawn or before leaving for foraging in the night. *Myotis formosus* usually circled around the roost-tree for a few minutes and circling individuals seemed not all roost in the same tree. This behaviour suggested a potential communication between individuals.

Part C. Genetic subdivision among colonies

Population differentiation

Together my results suggest that gene flow between colonies is mainly male-mediated. Difference in pattern of inheritance between the two genetic markers I used influences effective population size. The effective population size of uniparentally transmitted mtDNA is four times smaller than biparental nuclear DNA which would result in a more rapid genetic drift for mtDNA than nuclear DNA (Birky et al 1989; Avise 2004). My results indicated that the overall level of colony structure based on mtDNA was 16 times greater than that inferred from nuclear DNA (2006 all individuals: mtDNA, $\Phi_{ST} = 0.235$; microsatellite DNA, $F_{ST} = 0.014$), and when only females included, the level of colony structure increased to >200 times (2006 females only: mtDNA, $\Phi_{ST} = 0.207$; microsatellite DNA, $F_{ST} = 0.001$). The data suggest that the contrasted pattern of colony structure obtained from the two different markers of different mode of inherence can be attributed to different gene flow patterns between males and females.

Such contrasted patterns of biparentally and maternally inherited genes have been documented in several bat species and were considered to be the results of female philopatry and male-mediated gene flow. It seems to be a common pattern in non-tropical bat species (e.g., *Plecotus auritus*, Veith et al. 2004; Furmankiewicz & Altringham 2007; *Rhinolophus monoceros*, Chen et al. 2008; *M. bechsteinii*, Kerth et al. 2000, Kerth et al. 2002a; *E. fuscus*, Vonhof et al. 2008), but not necessarily in migratory species (*Nyctalus noctule*, Petit & Mayer 1999). Overall, however, the F_{ST} for microsatellite markers was low but significantly different from zero (2006), indicating some colony structure and suggested that *M. formosus* does not form a panmictic population within the sampling range.

Behaviourally, male-mediated gene flow among bat colonies might occur in two ways. First by dispersal of males, it is a common pattern in mammals to avoid inbreeding or competition (Greenwood 1980). Greater horseshoe bats, *R. ferrumequinum*, disperse from their natal roosting area to peripheral areas and find a territory for mating, which could be reoccupied by the same male for more than ten years, and revisited by the same females. In this way male *R. ferrumequinum* mate with females from their natal or different colonies so there was no genetic subdivision between adjacent colonies (Rossiter et al. 2000; Rossiter et al. 2006). We do not know the pattern of male dispersal in *M. formosus*, but evidence from several lines suggests that not all males dispersed. First there was no significant difference between F_{ST} inferred from male and female (from the test of sex biased dispersal) although this could reflect small sample size for males or that the dispersal rate was beyond the sensitivity of the test (Goudet et al. 2002; Handley & Perrin 2007). Second, although not significant, the F_{ST} values from males

were slightly higher than those for females, and the overall F_{ST} for females only was lower than for both sexes (in 2006) combined. This suggests that the genetic structure of males might not be lower than that of females which does not meet with the prediction that the dispersed sex would have a lower F_{ST}. Third, from banding records, I found that adult males also show inter- and intra-annual fidelity to the roosting area. Moreover, although all juvenile males in this study did not appear in the second year compared to the small portion of females that did return, the difference could reflect the effect of mortality in first year bats (Zahn 1999), especially for males (Wilkinson 1992b). In another banding study conducted at Roosting Area 2 in 1994-1995, a small proportion of juveniles of both sexes returned to the same roost in the second year (female: 5/38; male: 2/44; Shen 1996). However, the male from Roosting Area 4 carrying the haplotype H1, was only found elsewhere at Roosting Area 1 where it was common, suggesting that the male could have originated from Roosting Area 1.

Gene flow between colonies could be facilitated by mating outside the roosting area, for example during swarming or hibernation. Normally, genetic differentiation of migratory bat species is low or almost absent in both maternally and biparentally inherited genetic markers (Burland & Worthington Wilmer 2001) because individuals fly over a long geographical range during migration, which increases the range of potential dispersal (Wilkinson & Fleming 1996). Although winter and autumn roosts remain unknown for *M. formosus*, the significant genetic differentiation among colonies over a small geographical range based on both nuclear and mitochondrial DNA suggested *M. formosus* might not migrate.

For many vespertilionidae species, during late summer to autumn, bats are active and chasing each other rather than roosting at the swarming site, which is usually male-biased, associates with several adjacent colonies, and covers a large catchment area (the areas from which bats are drawn) (Fenton 1969; Rivers et al. 2006; Furmankiewicz 2008). Swarming sites serve as a center for extra-colony copulation and they facilitate gene flow among adjacent colonies (e.g. *Myotis bechsteinii*, Kerth et al. 2003; Kerth & Morf 2004; *P. auritus*, Veith et al. 2004; Furmankiewicz & Altringham 2007; *Myotis Nattereri*, Rivers et al. 2005).

Summer colonies of *P. auritus* consist of both natal philopatric male and female, and thus the mtDNA showed high degree of segregation in different summer colonies due to the natal philopatry. However, a high genetic variety of nuclear genes and a mixture of mtDNA haplotypes were found in the swarming site, which was associated with several adjacent colonies, suggesting that mating occurred at the swarming sites and gene flow between summer colonies was via male-mediated (Burland et al. 1999; Entwistle et al. 2000; Burland et al. 2001; Veith et al. 2004; Furmankiewicz & Altringham 2007). Although swarming behaviour seems prevalent among temperate vespertilionids (e.g. Barbour and Davis 1969; Furmankiewicz & Górniak 2002; Parsons et al. 2003), the movement of *M. formosus* after summer is still unknown, and no swarming behaviour in this species has not been recorded to date.

Inter-colony transfer of females

Although female philopatry seems to be common in non-tropical bat species, few studies have addressed the degree of female philopatry (Vonhof et al. 2008). Absolute female philopatry has only been demonstrated in *M. bechsteinii*, where each colony

usually contains 1-2 matrilines and numbers around 20 individuals. Within a similar geographical range as my study, the mtDNA differentiation between colonies of *M. bechsteinii* was extraordinary high (F_{ST} = 0.96) and the nuclear DNA differentiation was weak but significant (F_{ST} = 0.015), indicating that almost all females were philopatric to their natal roosting areas and that there was extreme male-biased dispersion (Kerth et al. 2000; Kerth et al. 2002a). Theoretically, inbreeding avoidance was the only explanation for such extreme sex-biased dispersal (Perrin & Mazalov 1999). Confrontation tests indicated that female *M. bechsteinii* discriminated among conspecifics by colonies from which they had originated and responded aggressively to intruders from other colony. This behaviour was interpreted as a defense of limited resources (roosts) or a result of cooperation which might be costly, and could be further enhanced by female philopatry (Kerth et al. 2002b; Safi & Kerth 2003). I found much lower levels of genetic differentiation of mtDNA than reported from *M. bechsteinii*. Although the different mutation rate of different mtDNA loci could contribute to the outcome of the fixation index (e.g. Kerth et al. 2000; Kerth et al. 2002a), the more than 4-fold difference in the fixation index between the two studies is not likely explicable by different mutation rates among mtDNA markers used. Most of the mtDNA variance occurred within, not among colonies, and there was no pattern of haplotype segregation in contrast to the situation in *M. bechsteinii*. My data suggest that there is some inter-colony transfer of female *M. formosus* which is in contrast to the results of my roost censuses. The difference may be explained by the small number of animals sampled.

Comparable results from *E. fuscus* revealed low genetic structure among colonies using nuclear DNA markers (F_{ST} = -0.0001 – 0.012; 99.5% variation within colonies), a

low to medium genetic structure among colonies using mtDNA markers (F_{ST} = -0.007 – 0.491), high haplotype diversity (mean h = 0.83), a large number of matrilines (5 -15) per colony, and small number of individuals were found to roost with a mother or daughter in the same colony. Vonhof et al. (2008) concluded that female dispersal may occur among some colonies.

Why no isolation-by-distance for both markers?

The absence of isolation-by-distance for both biparentally and maternally genetic markers suggests that direct exchange of genes among colonies was not restricted by distance. Therefore gene flow among colonies might not follow a stepping stone model (Kimura 1953). This may be partly explained by the relatively short distances among most *M. formosus* colonies compared to their longer commuting distance to foraging sites. Except the most distant roosting areas (Roosting Area 7 and Roosting Area 9), other colonies were within 11 km of one another. Among bats an example from the opposite extreme is provided by *P. auritus*, which are slow, maneuverable fliers unlikely to travel long distances. Here there are reports of isolation by distance for groups of colonies in an area with less than 50 km in diameter, apparently reflecting restricted dispersal abilities (Burland et al. 1999). The absence of isolation by distance for *M. formosus* might further support the hypothesis that in this species mating occurs away from nursery colonies. Bat species that mate at swarming sites show little evidence of isolation by distance among colonies associated with one swarming site due to the mixed of individuals from several adjacent colonies would break down the colonies boundaries (Rivers et al. 2005; Furmankiewicz & Altringham 2007).

What is a colony?

The colonies of *M. formosus* I studied behaved as distinct groups as indicated by the following lines of evidence: 1) frequent roost switching, 2) nonrandom associations between dyads, 3) high inter- and intra- annual fidelity to the small roosting area, 4) the near absence of movement among colonies and 5) genetic differentiation within a micro-geographic range. Our concept of "colony" of bats has improved as our understanding of the nature of their sociality has increased in recent decades (Fenton 1997; Fenton 2003). Instead of typically referring to a colony as an aggregation of individuals in a roost, Burland & Worthington Wilmer (2001) suggested that colonies are aggregations that contain individuals that interact with one another to a distinctly greater degree than with other conspecifics. My results support this concept, which is consistent with data for several bat species (*D. rotundus*, Wilkinson 1985; *M. bechsteinii*, Kerth & Koenig 1999; *C. tuberculatus*, O'Donnell 2000; *Thyroptera tricolor*, Vonhof et al. 2004; *E. fuscus*, Willis & Brigham 2004; *N. lasiopterus*, Popa-Lisseanu et al. 2008). The common theme is individuals interacting frequently but not always roosted together, and in some species groups used overlapping areas and did not mix with one another. Recent studies further suggested that this network between individuals was not limited in roosting together during daytime, but could be connected in the night by individuals from several satellite roosts visiting one communal roost constituting a roost network (Rhodes et al. 2006; Rhodes 2007).

Kin selection does not account for the major causes of sociality in *M. formosus*. This is indicated by low mean relatedness, low numbers of parent-offspring pairs identified, and multiple matrilineal lines within the colony combined with the absence of

correlation between dyad association and genetic relatedness or matrilineal relationship. Rather, cooperation, which has been demonstrated in other bat species and explained sociality in the fission-fusion society (e.g. *D. rotundus*, Wilkinson 1984; *M. bechsteinii*, Kerth 2006), might be a root cause for the sociality of *M. formosus*. Among male temperate bats, information transfer is an important factor to promote sociality (Safi & Kerth 2007).

Both foliage and building roosts of *M. formosus* are highly associated with human activity making them more vulnerable to potential loss of roosts and/or foraging sites. The significant decrease of colony size at Roosting Area 1 and Roosting Area 2 and the long-term decrease at Roosting Area 2 (50% within 10 years), indicates population decline and the need for better protection. Determining the social boundary and social cohesiveness of a bat colony would be crucial for conservation management if they further influence the influx of individuals in a micro-geographical scale (Racey & Entwistle 2003). However, to date our understanding about the formation and the nature of a colony of bats is still lacking and limited to a few well-studied species. Further research to clarify the degree of social cohesiveness and boundary of colony, the interaction of individuals within and among colonies, and the connection, both in strength and in the geographical range, between maternity colonies and mating sites would benefit both our understanding of the bat colony and the practices of conservation management.

Chapter 5. Conclusions

1. In general, roost switching frequency of foliage-roosting *Myotis formosus* was high and associated with intrinsic and environmental factors.

2. *Myotis formosus* showed flexibility in roost fidelity depending on the type of roosts. *Myotis formosus* roosting in a house almost always returned to the same roost-site every day while those roosting in foliage switched roost-site about every two days.

3. Availability and permanency do not appear to explain differences in patterns of roost switching. Rather the space available in a roost appears to provide a better explanation for different patterns of roost switching because bats roosting in a roost with limited space switched roosts more often than those in spacious roosts.

4. In general, both sexes of *M. formosus* had similar roost fidelity.

5. Lactating females usually had higher roost fidelity than females of other reproductive status, suggesting that the cost-benefit ratio of roost switching might be higher during lactation.

6. The recurrent roost switching of *M. formosus* is not explained by responses to disturbance which had more immediate impact.

7. The frequency of roost switching by *M. formosus* could be partly explained by reduction of risk of predation by the increased vigilance and dilution because the large subgroup size would decrease the tendency for roost switching, but not explained by reducing the attractiveness to predators.

8. Roost switching by *M. formosus* had no relationship with ectoparasite load because I found no ectoparasites in either foliage-roosting or house-roosting *M. formosus*.

9. Rain increased the frequency of roost switching by foliage-roosting *M. formosus* but not house-roosting individuals. This is probably because the house provided better protection from weather.

10. *Myotis formosus* did not switch roosts to reduce the commuting distance between foraging and roost-sites. Compared to their foraging ranges, they showed fidelity to a small roosting area.

11. *Myotis formosus* in the large subgroup switched roosts less frequently than those living in smaller subgroups. Bats were faithful to specific roosting areas, and the nonrandom association between individuals indicated that they live in a fission-fusion society and switched roost to enhance the social interaction.

12. *Myotis formosus* had distinctly different association pattern in different types of roost suggesting that the roost environment shaped the social interactions among bats.

13. In the house roost, which was relatively stable and spacious, females usually roosted together at the same roost-site. In foliage roosts, females frequently split into subgroups and some individuals associated with each other more often than expected even after the effects of roost and subgroup were removed.

14. In general, reproductive status affected the association pattern of foliage-roosting *M. formosus*. Dyads of post-lactating female associated less often than other reproductive status.

15. Adult males seldom associated with other bats and most of time were solitary, suggesting a difference of the requirement from the interaction of subgroup between sexes.

16. Low mean genetic relatedness, multiple matrilineal lines and no relation between association and genetic relatedness or matrilineal relationship of *M. formosus* suggested that kin selection does not explain the fission-fusion social system.

17. Different association pattern between the different roost types supports the hypothesis that when the physical space of roost is limited, individuals could use fission-fusion social system to associate with more individuals than one roost can house.

18. Benefits associated with cooperation or clustering as well as female philopatry are possible reasons for the fission-fusion society of *M. formosus*.

19. Weak colony structure based on biparentally inherited nuclear DNA and the much stronger colony structure based on maternally inherited mtDNA suggest that gene flow between colonies is mainly male-mediated.

20. Behaviourally, male-mediated gene flow among bat colonies might occur by male dispersal and/or mating outside, including swarming site, hibernacula, or on the migration route.

21. Low numbers of mother-offspring pairs found within the colony, high mtDNA haplotypes diversity, several matrilines found within a colony and a relatively lower colony structure based on mtDNA indicated female dispersal occurs among colonies.

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Table 1.1. Summary of the hypothesis and prediction related to the roost switching of *Myotis formosus*.

Hypothesis	Prediction
To reduce the risks of predation	1. Individuals roosting in larger subgroups would switch roost less frequently than those in small subgroups
	2. After a roost switching, the size of the new subgroup would be similar or larger than the previous subgroup
	3. Most roost switching would be among roost-tree (or house) instead of within roost-tree (or house)
To lower ectoparasite loads	1. Individuals with higher level of ectoparasite loads would switch roosts more frequently
	2. The roost switching frequency would increase as the subgroup size increase
	3. Lactating females would switch roost more frequently than other females
To respond to disturbance	1. Newly caught individuals would switch roosts more frequently than those caught long time ago
To reduce commuting costs to foraging areas	1. Individuals would switch to the roost-site which is closer to the foraging site
	2. Moving distance between consecutive roosts would be in a comparable scale to the moving distance between roosting and foraging sites
To response to the change of microclimate in the roost	1. The roost switching frequency of foliage roosting bats would increase after a rain
	2. The roost switching frequency of house roosting bats would be affected less after a rain
To enhance the social interaction	1. Individuals live in the large subgroup would switch roost less frequently than those lives in small subgroup
	2. The number of bats associated with a given individual would increase as its roost switching frequency increased
	3. A nonrandom association between individuals
	4. Showing fidelity to the roosting area while switching roost frequently
	5. Individuals roosting in a foliage roost (limited space) would switch roost more frequently than those in the house (spacious roost).

Table 2.1. Summary of the sampling localities for *Myotis formosus* on the Southwest plain, Taiwan.

Roosting area	Abbreviation	Location	Coordinate	Types of roost
1	BS	Beigang sugar refinery, Beigang, Yunlin	23°34'8 N, 120°17'49 E	foliage
2	SH	A house in Shueilin, Shueilin, Yunlin	23°33'39 N, 120°15'7 E	house
3	BP	Beigang Sports Park, Beigang, Yunlin	23°34'35 N, 120°18'12 E	foliage
4	CY	Chao-Yang Elementary School, Beigang, Yunlin	23°33'25 N, 120°17'11 E	foliage
5	CG	Chen-Guang Elementary School , Beigang, Yunlin	23°35'15 N, 120°19'3 E	foliage
6	CJ	Chen-Jheng Elementary School, Shueilin, Yunlin	23°37'16 N, 120°15'43 E	foliage
7	WJ	Wun-Jheng Elementary School, Shueilin, Yunlin	23°32'23 N, 120°13'25 E	foliage
8	CT	Rao-Ping Elementary School, Cihtong, Yunlin	23°46'40 N, 120°31'21 E	foliage
9	YS	An-Nei Elementary School, Shueilin, Tainan	23°19'45 N, 120°15'38 E	foliage

Table 2.2. Number of censuses made at Roosting Area 1 and Roosting Area 2 in 2005 and 2006, respectively.

Roosting Area	Year	Census period			Total census	Census per week (mean ± SD)
1	2005	1-May	to	16-Aug	95	5.9 ± 1.3
	2006	7-May	to	26-Aug	91	5.7 ± 1.3
2	2005	30-Apr	to	17-Aug	95	5.9 ± 1.4
	2006	7-May	to	26-Aug	98	6.1 ± 1.1

Table 2.3. Description of the seven nuclear microsatellite loci used to estimate the genetic relatedness, variability and subdivision. PCR conditions for different loci and the source paper and the original species that each locus was designed for were listed.

Locus	Primer sequence	MgCl2 (mM)	Primer (µM)	Template (µl)	Source species	Source of the primers
H19	GGAATCCGAATCCCTGGC GACATCCCCTCACCCCAAC	1.5	0.25	2	*Myotis myotis*	Castella and Ruedi 2000
B15	TAAGGTATAAAGAGAAATACC AAAGGGTCTTGTTTAACTTT	3	0.25	1	*Myotis bechsteinii*	Kerth et al. 2002
F19	CCCAAATCTGTCTTTCAGGC GCTAGCCATGGAGAAGGAAG	2	0.2	1	*Myotis myotis*	Castella and Ruedi 2000
D15	GCTCTCTGAAGAGGCCCTG ATTCCAAGAGTGACAGCATCC	3	0.25	1	*Myotis myotis*	Castella and Ruedi 2000
EF5	AAACTCTCCCATCTGCTCT TCTCACTTCCTCATCAATCA	3	0.25	1	*Eptesicus fuscus*	Vonhof et al. 2002
H29	GCTTTATTTAGCATTGGAGAGC TCAGGTGAGGATTGAAAACAC	1.5	0.25	1	*Myotis myotis*	Castella and Ruedi 2000
EF6	ATCACATTTTTGAAGCAT ATCTGTTTTTCTCTCCTTAT	2	0.25	1	*Eptesicus fuscus*	Vonhof et al. 2002

Table 3.1. Mean colony size, number of subgroups and subgroup size found at each roosting area in *2005* and *2006*, respectively. The data were shown in mean ± SD and the data range shown in parentheses.

Roosting Area	Year	Colony size	Number of subgroups	Subgroup size
1	2005	84. ± 24.8 (23-135)	21.9 ± 10.4 (1-46)	3.8 ± 7.0 (1-57)
	2006	67.4 ± 21.8 (13-104)	17.8 ± 6.6 (3-32)	3.9 ± 6.2 (1-55)
2	2005	104.9 ± 24.4 (41-143)	7.9 ± 3.5 (1-21)	13.6 ± 29.2 (1-131)
	2006	82.2 ± 25.5 (10-118)	5.4 ± 2.7 (1-15)	15.5 ± 30.4 (1-108)

Table 3.2. Year and number of individuals of *Myotis formosus* of different age and sex caught in the three roosting areas.

Roosting Area	Year	Adult		Juvenile		Total
		Female	Male	Female	Male	
1	2005	47	8	5	11	71
2	2005	6	2	0	0	8
	2006	2	0	0	1	3
3	2005	17	0	2	2	21

Table 3.3. Year and number of *Myotis formosus* of different age and sex observed in the three roosting areas.

Roosting Area	Year	Adult		Juvenile		Total
		Female	Male	Female	Male	
1	2005	33	4	2	6	45
	2006	35	3	0	0	38
	Both year	25	2	1[a]	0	28
2	2005	9	0	0	0	9
	2006	7	0	0	0	7
	Both year	6	0	0	0	6
3	2005	15	0	0	0	15
	2006	9	0	0	0	9
	Both year	7	0	0	0	7

[a] The individual was caught as juvenile in 2005 and returned as adult in 2006.

Table 3.4. Number of times *Myotis formosus* observed and number of times the observed bat-day could be identified as switch or stay in the roost-site or roost-tree (or house) at BS (Roosting Area 1) and SH (Roosting Area 2), in 2005 and 2006.

Site-year	Total sighting	No. bats observed /day	No. times observed /bat	% of stay observed	No. identified as switch or stay	
					Roost-site	Roost-tree (or house)
BS-2005	1162	12.2 ± 4.9	25.8 ± 19.4	59.4 ± 22.3	1072 (92.2%)	792 (68.1%)
BS-2006	1616	16.9 ± 6.4	42.3 ± 22.8	64.6 ± 27.4	1482 (91.9%)	1220 (75.6%)
SH-2005	650	7.5 ± 1.4	72.2 ± 16.2	87.5 ± 7.0	600 (92.3%)	600 (92.3%)
SH-2006	493	5.4 ± 1.5	70.4 ± 19.9	94.1 ± 4.2	466 (94.5%)	466 (94.5%)
BP-2005	168	3.3 ± 1.6	9.9 ± 9.7	N/A	N/A	N/A
BP-2006	37	2.5 ± 1.6	2.2 ± 3.1	N/A	N/A	N/A

Table 3.5. Summary of the radio-tracking data from 13 adult female *Myotis formosus* gathered in 2005 in Yunlin County, Taiwan.

Bat ID	Roosting Area	Reproductive status	Date transmitter affixed	Date of last contact	Tracking period (days)	Days bat located	days bats in study area (%)	No. roost-site found	No. roosting tree or house found	No, roost-site switching	Fidelity to roost-site	Moving distance (M)
B	3	PF	1 May	7 May	6	3	3 (50%)	2	1	2	0.50	809
I	2	PF	2 May	10 May	8	5	5 (63%)	2	1	3	0.50	N/A
O95	1	PF	30 Apr	4 Apr	4	4	4 (100%)	1	1	0	1.00	N/A
G11	1	PF	20 May	28 May	8	6	6 (75%)	5	5	4	0.20	229
G14	1	PF	22 May	4 Jun	13	10	10 (77%)	2	1	2	0.80	2
G19	1	PF	26 May	30 May	4	3	3 (75%)	1	1	1	0.67	N/A
G11	1	LF	25 Jun	30 Jun	5	4	4 (80%)	2	2	3	0.25	83
G29	1	LF	28 Jun	7 Jul	9	9	7 (78%)	3	1	4	0.56	119
G32	1	LF	29 Jun	8 Jul	9	7	7 (78%)	2	1	1	0.86	N/A
O95	1	LF	29 Jun	3 Jul	4	3	3 (75%)	3	1	3	0.00	4.3
G35	1	LF	3 Jul	13 Jul	10	10	7 (70%)	3	2	5	0.50	82.5
G38	1	LF	3 Jul	10 Jul	7	3	2 (29%)	3	2	2	0.33	110.5
G48	1	PLF	26 Jul	6 Aug	11	9	5 (45%)	8	5	7	0.22	672
G49	1	PLF	26 Jul	1 Aug	6	3	2 (33%)	2	1	1	0.67	863.5
G5	1	PLF	2 Aug	8 Aug	6	3	3 *50%)	3	3	3	0.00	29.5
Mean (Roosting Area 1)					7.4	5.7	4.9 (66.5%)	2.9	2	2.80	0.47	219.5
Mean (All)					7.3	5.5	4.7 (65.2%)	2.8	1.9	2.70	0.47	273.1

Table 3.6. Sample size and mean fidelity to roost-site and roost-tree (or house) of adult female (AF), adult male (AM), and juvenile (J), and the results of the Mann-Whitney tests between fidelity to roost-site and roost-tree (or house) in the same roosting area of the same year.

			Roost-site fidelity			Roost-tree (or house) fidelity				
Roosting Area	Year	Sex-age	n	mean	SD	n	mean	SD	z	*p*
1	2005	AF	33	0.45	0.23	29	0.84	0.16		
		AM	4	0.11	0.14	2	0.71	0.4		
		J	8	0.39	0.19	7	0.95	0.07		
		All	45	0.41	0.23	38	0.85	0.16	-6.958	< 0.001
	2006	AF	35	0.45	0.15	34	0.87	0.14		
		AM	3	0.36	0.34	2	0.94	0.09		
		All	38	0.44	0.17	36	0.87	0.13	-6.991	< 0.001
2	2005	AF	9	0.84	0.08	9	0.94	0.06	-2.65	< 0.01
	2006	AF	7	0.94	0.02	7	0.95	0.01	0.520	NS

Table 3.7. Sample size and mean fidelity to roost-site and roost-tree (or house) of adult male (AM), pregnant female (PF), lactating female (LF), post-lactating female (PLF) and non-reproductive female (NRF) from Roosting Area 1 and Roosting Area 2 in 2005 and 2006, respectively

Roosting Area	Year	Reproductive status	Roost-site			Roost-tree (or house)		
			n	Mean fidelity	SD	n	Mean fidelity	SD
1	2005	PF	19	0.48	0.22	19	0.79	0.28
		LF	18	0.65	0.20	17	0.91	0.12
		PLF	15	0.39	0.17	15	0.84	0.18
		NRF	3	0.13	0.22	2	1.00	0.00
		AM	4	0.11	0.14	2	0.71	0.40
	2006	PF	27	0.42	0.17	26	0.81	0.19
		LF	26	0.64	0.13	25	0.96	0.07
		PLF	19	0.41	0.26	16	0.86	0.20
		NRF	2	0.35	0.08	2	0.85	0.12
		AM	3	0.36	0.34	2	0.94	0.09
2	2005	PF	6	0.69	0.11	6	0.94	0.13
		LF	5	0.98	0.06	5	0.98	0.06
		PLF	5	0.91	0.10	5	0.94	0.07
		NRF	3	0.79	0.09	3	0.90	0.06
	2006	PF	4	0.96	0.05	4	0.97	0.03
		LF	4	0.99	0.02	4	0.99	0.02
		PLF	4	0.91	0.03	4	0.92	0.01
		NRF	2	0.92	0.04	2	0.94	0.01

Table 3.8. Results of the Kruskal-Wallis tests comparing the fidelity to roost-site and roost-tree (or house) of *Myotis formosus* belonging to different sex and reproductive status within the same roosting area and year.

Roosting Area	Year	Roost-site		Roost-tree (or house)	
		H	*p*	*H*	*p*
1	2005	23.033	<0.001	1.976	NS
	2006	21.045	<0.001	10.482	<0.01
2	2005	11.553	<0.01	3.807	NS
	2006	5.948	0.051	7.406	<0.05

Table 3.9. Results of pairwise Mann-Whitney tests with the Bonferroni correction for each test comparing the fidelity to roost-site and roost-tree (or house) of adult male (AM), pregnant female (PF), lactating female (LF), post-lactating female (PLF) and non-reproductive female (NRF) within the same roosting area and year.

			Roost-site			Roost-tree (or house)		
Roosting Area	Year	Categories	*Z*	*p*	after Bonferroni correction	*z*	*p*	after Bonferroni correction
1	2005	PF, LF	-2.114	0.035	NS			
		PF, PLF	-1.406	0.160	NS			
		PF, NRF	-2.204	0.028	NS			
		PF, AM	-2.599	0.009	NS			
		LF, PLF	-3.329	0.001	Significant			
		LF, NRF	-2.514	0.012	NS			
		LF, AM	-2.981	0.003	Significant			
		PLF, NRF	-1.839	0.066	NS			
		PLF, AM	-2.503	0.012	NS			
		NRF, AM	0.000	1.000	NS			
1	2006	PF, LF	-4.667	< 0.001	Significant	-3.381	0.001	Significant
		PF, PLF	-0.246	0.806	NS	-1.186	0.236	NS
		PF, AM	-0.139	0.890	NS			
		LF, PLF	-2.898	0.004	Significant	-1.171	0.242	NS
		LF, AM	-1.613	0.107	NS			
		PLF, AM	-0.240	0.810	NS			
2	2005	PF, LF	-2.810	0.005	Significant			
		PF, PLF	-2.114	0.035	NS			
		PF, NRF	-1.296	0.195	NS			
		LF, PLF	-1.294	0.196	NS			
		LF, NRF	-2.065	0.039	NS			
		PLF, NRF	-1.650	0.099	NS			
2	2006	PF, LF				-0.833	0.405	NS
		PF, PLF				-2.045	0.041	NS
		LF, PLF				-2.381	0.017	NS

Table 3.10. Results of independent Mann-Whitney tests comparing the fidelity to roost-site and fidelity to roost-tree (or house) of pregnant female (PF), lactating female (LF), post-lactating female (PLF) and non-reproductive female (NRF) between different roost types (Roosting Area 1 and Roosting Area 2) in the same year.

Year	Reproductive Status	Roost-site		Roost-tree (or house)	
		z	p	z	p
2005	PL	-2.166	<0.05	-1.481	NS
	LF	-3.214	<0.001	-1.099	NS
	PLF	-3.277	<0.001	-0.733	NS
	NRF	-1.993	<0.05		
2006	PL	-3.189	<0.001	-1.856	0.063
	LF	-3.175	<0.001	-0.634	NS
	PLF	-3.088	<0.01	-0.694	NS

Table 3.11. Sample size, mean and SD of the fidelity to roost-site and roost-tree (or house) of *Myotis formosus* caught within ten days and one year later, and the Wilcoxon signed rank test comparing the fidelity of individuals at Roosting Area 1 and Roosting Area 2 in 2005 and 2006.

			Immediately after disturbance		One year later			
Roosting Arae	Categories of fidelity	*n*	Mean	SD	Mean	SD	*z*	*p*
1	site	17	0.42	0.27	0.44	0.27	-0.259	NS
	tree	12	0.81	0.29	0.75	0.27	-1.114	NS
2	site	4	0.61	0.08	0.96	0.08	-1.89	NS
	house	4	0.94	0.13	0.96	0.08	-1.00	NS

Table 3.12. Results of the independent Mann-Whitney test comparing the subgroup size of *Myotis formosus* which switched roost-site or roost-tree (or house) next day with individuals which did not switch at Roosting Area 1 and Roosting Area 2 in 2005 and 2006.

Roosting Area	Year	Roost-site		Roost-tree (or house)	
		z	*p*	*z*	*p*
1	2005	-8.667	<0.001	-5.507	<0.001
	2006	-6.483	<0.001	-5.099	<0.001
2	2005	-6.065	<0.001	-1.598	0.110
	2006	-3.337	<0.01	-3.036	<0.01

Table 3.13. Sample size, mean and SD of the size of subgroup(s) a bat joined in two consecutive days, categorized by whether the bat remained staying in the same or switched to another roost-site, and the independent Wilcoxon signed-rank test comparing the subgroup size before and after a roost-site switch or stay of *Myotis formosus* within Roosting Area 1 andRoosting Area 2 in 2005 and 2006.

Roosting Area	Year	Categories	*n*	Before Mean	Before SD	After Mean	After SD	*z*	*p*
1	2005	Switch	228	12.34	13.85	10.83	11.69	-0.403	NS
		Stay	556	19.79	15.66	20.25	16.02	-2.345	< 0.05
	2006	Switch	469	13.61	12.83	13.46	13.03	-0.29	NS
		Stay	702	18.1	15.49	17.9	15.04	-0.783	NS
2	2005	Switch	56	64.13	29.02	77.79	23.71	-1.877	NS
		Stay	490	90.66	23.83	92.42	24.73	-2.392	< 0.05
	2006	Switch	5	42.4	47.83	52	44.84	-0.135	NS
		Stay	435	88.84	16.34	88.63	16.39	-0.302	NS

Table 3.14. Results of the independent χ^2-test comparing the frequency of *Myotis formosus* which switched roost-site or roost-tree (or house) after a rain in previous day or night and individuals which did not switch at Roosting Area 1 and Roosting Area 2 in 2006.

Roosting Area	weather	Roost-site		Roost-tree (or house)	
		X^2	p	χ^2	p
1	rain	27.176	<0.001	1.184	NS
	rain in the night	22.122	<0.001	0.027	NS
2	rain	0.001	NS	0.041	NS
	rain in the night	1.524	NS	2.523	NS

Table 3.15. Year and number of *Myotis formosus* of different age and sex from Roosting Area 1 and Roosting Area 2 included in the analysis of three association indices and genetic relatedness. Number in the parenthesis indicated the number of individuals not genotyped for microsatellite DNA and mitochondrial DNA.

Roosting Area	Year	Adult		Juvenile		Total
		Female	Male	Female	Male	
1	2005	31	3	2	6	42
	2006	35 (2,3)	3	0	0	38 (2,3)
	Both year	23	2	1[a]	0	26
2	2005	9 (4,4)	0	0	0	9 (4,4)
	2006	7 (2,2)	0	0	0	7 (2,2)
	Both year	6 (2,2)	0	0	0	6 (2,2)

[a] The individual was caught as juvenile in 2005 and returned as adult in 2006.

Table 3.16. Sample size, mean and SD of the SR-index, X^2-index and SGS-index of dyads belonging to different sex-age combinations from Roosting Area 1 (BS) and Roosting Area 2 (SH) in 2005 and 2006. Range for SR-index, X^2-index and the number of positive and negative significance were showed in the parenthesis. AF: adult female, AM: adult male, J: juvenile.

	SR-index		χ2-index		SGS-index	
Sex-age class	No. dyads	mean ± SD (range)	No. dyads	mean ± SD (range)	No. dyads	mean ± SD (positive, negative)
BS in 2005	-	-	-	-	-	-
AF-AF	387	0.07 ± 0.14 (0 - 0.94)	155	12.24 ± 20.32 (0.01 - 189.26)	382	5.01 ± 14.69 (61,37)
AM-AM	3	0			3	0.12 ± 0.12 (0,0)
AF-AM	87	0.0002 ± 0.0017 (0 - 0.02)	3	9.20 ± 15.88 (0.02 - 27.53)	75	0.74 ± 0.70 (0,0)
AM-J	24	0.0036 ± 0.0175 (0 - 0.09)	1	20.53	17	0.68 ± 1.84 (1,0)
J-J	21	0.06 ± 0.15 (0 - 0.50)	3	0.40 ± 0.05 (0.35 - 0.44)	19	5.83 ± 12.49 (3,0)
AF-J	171	0.05 ± 0.12 (0 - 0.67)	36	4.80 ± 8.63 (0 - 40.05)	167	5.31 ± 15.61 (20,0)
Combined	693	0.05 ± 0.13 (0-0.94)	198	10.70 ± 18.68 (0-189.26)	663	4.49 ± 13.86 (85,37)
BS in 2006						
AF-AF	588	0.12 ± 0.14 (0 - 0.67)	433	26.65 ± 35.24 (0 - 218.94)	582	4.87 ± 7.49 (130,86)
AM-AM	3	0			2	0.24 ± 0.24 (0,0)
AF-AM	100	0.0020 ± 0.0165 (0 - 0.16)	9	21.47 ± 32.88 (0.01 - 80.03)	91	1.91 ± 3.74 (1,14)
Combined	691	0.10 ± 0.14 (0-0.67)	442	26.55 ± 35.16 (0-218.94)	675	4.46 ± 7.16 (131,100)
SH in 2005						
AF-AF	36	0.69 ± 0.11 (0.4-0.84)	36	1.55 ± 2.07 (0-7.79)	36	2.72 ± 2.86 (10,0)
SH in 2006						
AF-AF	21	0.89 ± 0.06 (0.75-0.97)	21	0.05 ± 0.05 (0-0.19)	21	3.33 ± 2.31 (6,0)

Table 3.17. Results of pairwise Mann-Whitney tests with the Bonferroni correction comparing the three association indices between the dyads of different sex and age combinations within and between the two roosting areas. AF: adult female, AM: adult male, J: juvenile. BS: Roosting Area 1. SH: Roosting Area 2.

Categories	SR-index		χ2-index	
	z	*p*	*z*	*p*
BS in 2005				
AF-AF, AM-AM	-2.531	NS*	N/A	
AF-AF, AM-AF	-13.141	<0.001	-2.08	NS
AF-AF, AM-J	-4.764	<0.001	-0.652	NS
AF-AF, J-J	-3.116	0.002	-1.901	NS
AF-AF, AF-J	-8.248	<0.001	-4.214	<0.001
AM-AM, AM-AF	-0.361	NS	N/A	
AM-AM, AM-J	-0.5	NS	N/A	
AM-AM, J-J	-0.962	NS	N/A	
AM-AM, AF-J	-1.181	NS	N/A	
AM-AF, AM-J	-0.627	NS	N/A	NS
AM-AF, J-J	-2.992	0.003	-0.866	NS
AM-AF, AF-J	-5.412	<0.001	-1.024	NS
AM-J, J-J	-1.244	NS	N/A	NS
AM-J, AF-J	-1.858	NS	N/A	NS
J-J, AF-J	-0.364	NS	-0.738	NS
BS in 2006				
AF-AF, AM-AM	-2.051	NS	N/A	
AF-AF, AM-AF	-10.89	<0.001	-1.934	NS
AM-AM, AM-AF	-0.303	NS	N/A	
BS vs. SH in 2005				
AF-AF	-10.97	<0.001	-5.48	<0.001
BS vs. SH in 2006				
AF-AF	-7.92	<0.001	-7.01	<0.001

* indicated the comparison was significant before Bonferroni correction.

Table 3.18. The mean number of significant positive and negative associations (SGS-index) and the fraction (shown in %) of the significant values to all other banded *Myotis formosus* calculated for the respective year, sex or age class, and the two roosting areas in 2005 and 2006. AF: adult female, AM: adult male, J: juvenile.

Roosting Area	Year	Sex-age class	No. bats	Number of associations per bat: mean ± SD (%)	
				Positive	Negative
1	2005	AF	31	4.61 ± 4.59 (11%)	2.39 ± 2.96 (6%)
		AM	3	0.33 ± 0.58 (1%)	0
		J	8	4.5 ± 4.34 (11%)	0
	2006	AF	35	7.46 ± 5.59 (20%)	5.31 ± 5.35 (14%)
		AM	3	0.33 ± 0.58 (1%)	4.67 ± 8.08 (13%)
2	2005	AF	9	2.22 ± 1.48 (28%)	0
	2006	AF	7	1.71 ± 1.38 (29%)	0

Table 3.19. Observed and expected number of significant positive and negative associations in SGS-index as a function of different reproductive status of two adult females. P: pregnant female; L: lactating female: PL: post-lactating female.

			Number of significant associations (SGS-index)							
			positive				negative			
Roosting Aeea	Year		P-P	L-L	PL-PL	*p*-value	P-P	L-L	PL-PL	*p*-value
1	2005	obs.	26	23	5	NS	0	1	0	NS
		exp.	21.757	20.504	11.739		0.4029	0.3797	0.2174	
	2006	obs.	61	46	23	NS	39	1	0	<0.001
		exp.	56.864	50.215	22.921		17.497	15.451	7.0525	
2	2005	obs.	2	0	3	NS	0	0	0	N/A
		exp.	2.1429	1.4286	1.4286					
	2006	obs.	0	0	1	NS	0	0	0	N/A
		exp.	0.2941	0.3529	0.3529					

Table 3.20 Genetic variation calculated from 151 *M. formosus* at seven microsatellite loci. Genetic variation is described as the allele size range in base pairs (bp), number of alleles per locus, observed heterozygosity (H_{obs}) and expected heterozygosity (H_{exp}). The null allele frequency and the results of test for deviation from Hardy-Weinberg equilibrium (HW) are also given.

Locus	Allele size range (bp)	Number of alleles	H_{obs}	H_{exp}	Null allele frequency	HW
H19	86-108	12	0.861	0.881	0.0098	NS
B15	142-182	20	0.861	0.914	0.0283	NS
F19	190-232	21	0.473	0.862	0.289	***
D15	83-115	8	0.57	0.537	-0.0344	NS
EF5	114-142	15	0.8	0.844	0.0244	NS
H29	161-199	20	0.88	0.932	0.0273	NS
EF6	184-212	15	0.742	0.881	0.0822	NS

Table 3.21. The variable sites within 263 base pair regions of HVII from Roosting Area 1 and Roosting Area 2. From the 57 sequences sampled, 9 haplotypes (H1 – H21) were described based on 11 polymorphic sites.

nucleotide position	45	77	114	145	167	173	177	206	213	221	261
H1	T	A	A	C	A	G	G	T	G	A	A
H2										G	
H3		G								G	
H4		G									
H7		G	G	T	G		A	G			
H8	C	G	G	T	G		A	G			
H9	C	G	G	T	G		A	G			T
H10	C	G	G	T	G		A	G	A		
H21		G	G	T	G	A	A	G			

Table 3.22. The distribution of haplotypes within Roosting Area 1 and Roosting Area 2. The haplotypes are given in the first row. The number of *Myotis formosus* presented in each roosting area and year is given. The number of adult males is also given in the parenthesis.

		H1	H2	H3	H4	H 7	H 8	H 9	H 10	H 21	Total
1	2005	11(1)	1	1	1	11(1)	14(1)	1	2		42
	2006	10	1	1	1	8(1)	11(2)	1	2		35
2	2005					2	2			1	5
	2006					2	3				5

Table 3.23. Observed and expected number of significant positive and negative associations in SGS-index as a function of maternal relatedness among (a) two individuals or (b) two adult females.

(a) *All individuals included*

Roosting Area	Year		Number of significant associations (SGS-index)							
			positive				negative			
			Same	Different	χ2-value	*p*	Same	Different	χ2-value	*p*
1	2005	obs.	18	67	0.247	NS	7	30	0.425	NS
		exp.	19.94	65.06			8.68	28.32		
	2006	obs.	20	79	0.169	NS	23	63	1.176	NS
		exp.	21.69	77.31			18.84	67.16		

(b) *Only adult females included*

Roosting Area	Year		Number of significant associations (SGS-index)							
			positive				negative			
			Same	Different	χ2-value	*p*	Same	Different	χ2-value	*P*
1	2005	obs.	8	53	2.132	NS	6	30	0.356	NS
		exp.	12.62	48.38			7.45	28.55		
	2006	obs.	20	79	0.008	NS	16	57	0.081	NS
		exp.	20.37	78.63			15.02	57.98		
2	2005	obs.	2	4	0.667	NS	0	0		
		exp.	1.2	4.8						
	2006	obs.	1	3	0.375	NS	0	0		
		exp.	1.6	2.4						

Table 3.24. Number of different age-sex classes of *Myotis formosus* from different roosting areas during 205-2006 genotyped from seven microsatellite loci and one mitochondrial locus.

Roosting Area	Year	AF	AM	JF	JM	Sex unknown	Total
1	2005	47a	8	5	11a		71b
	2006	33a	5	0	0		38a
2	2005	6	2	0	0		8
	2006	7	0	0	1	5	13
3	2005	17	0	2	2		21
	2006	9	0	0	0		9
4	2006	1	2	2	0		5
5	2006	3	0	0	0		3
6	2006	2	0	0	0		2
7	2006	4	3	0	4a		11a
8	2006	7	0	0	3		10
9	2006	5	2	2a	2		11a

a. the number of individuals sequenced from mitochondrial DNA loci were one less than the number.

b. the number of individuals sequenced from mitochondrial DNA loci were two less than the number.

Table 3.25. The variable sites within 263 base pair region of HVII from 9 roosting areas. From the 147 sequences sampled, 22 haplotypes (H1 – H62) were described based on 21 polymorphic sites.

nucleotide position	8	45	77	83	114	143	145	164	166	167	173	177	203	205	206	211	213	217	221	259	261
H1	A	T	A	A	A	G	C	G	G	A	G	G	T	A	T	T	G	C	A	G	A
H2																			G		
H3			G																G		
H4			G																		
H5	G		G								A										
H6			G		G		T	A		G		A			G						
H7			G		G		T			G		A			G						
H8		C	G		G		T			G		A			G						
H9		C	G		G		T			G		A			G						T
H10		C	G		G		T			G		A			G		A				
H11	G		G																		
H12			G												G						
H13			G									A									
H14			G											G		C					
H15			G		G		T			G		A			G			T			
H16		C	G		G		T			G		A	C		G						
H21			G		G		T			G	A	A			G						
H22			G	G	G		T			G		A			G						
H23			G		G		T			G		A			G					A	
H31		C	G		G		T		A	G		A			G						
H61			G				T		A						G						
H62	G		G			A															

Table 3.26. The distribution of haplotypes within 9 roosting areas. The haplotypes are given in the first row. The total number of *Myotis formosus* sampled from each roosting area is given. The number of adult males is also given in the parenthesis.

Roosting Area		H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H21	H22	H23	H31	H61	H62
1		21(3)	1	2	2	1	2(1)	14(2)	21(2)	1	4												
2					2			4	6				1(1)					1	2	1(1)			
3					3			8	2			2	1	1	1	1	2						
4		1(1)								1(1)	1										2		
5								3															
6								1													1		
7					2(1)		5(1)	3(1)															
8					3										2				2			2	1
9					5(1)	2							2									1(1)	
Total		22	1	2	17	4	8	33	29	2	5	2	4	1	3	1	2	1	4	1	3	3	1
1	2005	21	1	2	2	1	2	14	21	1	4												
	2006	10	1	1	1		1	8	12	1	2												
2	2005				1			2	2				1					1		1			
	2006				1			4	6										2				
3	2005				3			8	2			2	1	1	1	1	2						
	2006				1			3	1			2		1	1								

Table 3.27. Genetic variability of the 151 *Myotis formosus* based on mitochondrial DNA and microsatellite DNA sampled from nine different roosting areas and two years. *N*: number of haplotypes; *H*: haplotypes diversity; *Π(%)*: nucleotide diversity shown in percentage; *A*: mean number of alleles per locus; H_{obs}: observed heterozygosity.

		Mitochondrial variability			Microsatellite variability	
Roosting Area	Sample size	*N*	*h*	π (%)	*A*	H_{obs}
1 (2005)	71	10	0.798	1.42%	13.8	0.812
1 (2006)	38	9	0.790	1.36%	12.3	0.820
1 (total)	71	10	0.778	1.40%	13.8	0.812
2 (2005)	8	6	0.867	0.96%	7.5	0.813
2 (2006)	13	4	0.718	0.60%	8.7	0.808
2 (total)	17	7	0.831	0.91%	9.3	0.784
3 (2005)	21	9	0.807	1.23%	10.0	0.762
3 (2006)	9	6	0.889	1.46%	7.7	0.759
3 (total)	21	9	0.838	1.29%	10.0	0.762
4	5	4	0.900	1.60%	5.0	0.800
5	3	1	0.000	0.00%	4.2	0.733
6	2	2	1.000	0.76%	3.7	0.833
7	10	3	0.689	0.89%	8.0	0.742
8	10	5	0.867	1.50%	8.2	0.717
9	10	4	0.733	0.60%	8.8	0.797

a. the number of individuals sequenced from mitochondrial DNA loci were one less than the number.

b. the number of individuals sequenced from mitochondrial DNA loci were two less than the number.

Table 3.28. Comparison of pairwise Fst (lower diagonal) for microsatellite loci and pairwise Φ_{ST} (upper diagonal) for mitochondrial HVII region of all *Myotis formosus* among colonies in 2005.

Roosting Area	1	2	3
1	-	0.014	0.016
2	0.002	-	-0.038
3	-0.014	-0.020	-

Table 3.29. Comparison of pairwise Fst (lower diagonal) for microsatellite loci and pairwise Φ_{ST} (upper diagonal) for mitochondrial HVII region of all *Myotis formosus* among colonies in 2006. The star sign indicates a significant difference.

Roosting Area	1	2	3	4	7	8	9
1	-	0.099*	0.048	0.022	0.104	0.204*	0.365*
2	0.008	-	0.294*	0.076	0.171*	0.463*	0.706*
3	0.004	-0.001	-	0.164	0.161	-0.007	0.198*
4	0.035*	0.041	0.014	-	0.202*	0.301*	0.575*
7	0.024*	0.047	0.040*	0.052	-	0.325*	0.589*
8	0.003	-0.003	-0.018	0.001	0.017	-	0.046
9	-0.002	0.000	-0.002	0.028	0.041*	-0.012	-

Table 3.30. Comparison of pairwise Fst (lower diagonal) for microsatellite loci and pairwise Φ_{ST} (upper diagonal) for mitochondrial HVII region of all female *Myotis formosus* among colonies in 2005.

Roosting Area	1	2	3
1	-	0.013	0.019
2	-0.008	-	0.012
3	0.002	-0.015	-

Table 3.31. Comparison of pairwise Fst (lower diagonal) for microsatellite loci and pairwise Φ_{ST} (upper diagonal) for mitochondrial HVII region of all female *Myotis formosus* among colonies in 2006. The star sign indicates a significant difference.

Roosting Area	1	2	3	8	9
1	-	0.153	0.036	0.122	0.348*
2	0.001	-	0.356*	0.422*	0.802*
3	0.005	-0.013	-	-0.043	0.182
8	0.009	-0.025	-0.018	-	0.131
9	0.001	-0.030	-0.002	-0.016	-

Table 3.32. AMOVA for the *Myotis formosus* for samples grouped by different years, roosting areas and sex.

				Microsatellite			mtDNA control region		
Sample	Year	colony included	Source of Variation	Variation (%)	F_{ST}	P-value	Variation (%)	Φ_{ST}	P-value
All	2005	3	Among colonies	-0.25	-0.003	NS	1.22	0.012	NS
			Within colonies	100.25			98.78		
	2006	3	Among colonies	0.63	0.006	NS	10.38	0.104	< 0.001
			Within colonies	99.37			89.62		
	2006	7	Among colonies	1.38	0.014	< 0.01	23.53	0.235	< 0.001
			Within colonies	98.62			76.47		
Female	2005	3	Among colonies	-0.01	0	NS	1.69	0.017	NS
			Within colonies	100.01			98.31		
	2006	3	Among colonies	0.27	0.003	NS	11.91	0.119	< 0.001
			Within colonies	99.73			88.09		
	2006	5	Among colonies	0.13	0.001	NS	20.66	0.207	< 0.001
			Within colonies	99.87			79.34		

Table 3.33. Distribution of maternal and paternal relationship within and between colonies.

Relationship	No. within colony		No. between colony	
	Same haplotype	Different haplotype	Same haplotype	Different haplotype
Mother - Daughter	2	N/A	0	N/A
Mother - Son	6	N/A	0	N/A
Father - Daughter	2	3	0	3
Father - Son	0	0	0	5

Table 4.1. Summary of the outcomes of each hypothesis and predictions related to the roost switching of *Myotis formosus*. Bold font and underline indicates the result is consistent with the prediction.

Hypothesis	Prediction
To reduce the risks of predation	**1. Individuals roosting in larger subgroups would switch roost less frequently than those in small subgroups**
	2. After a roost switching, the size of the new subgroup would be similar or larger than the previous subgroup
	3. Most roost switching would be among roost-tree (or house) instead of within roost-tree (or house)
To lower ectoparasite loads	1. Individuals with higher level of ectoparasite loads would switch roosts more frequently
	2. The roost switching frequency would increase as the subgroup size increase
	3. Lactating females would switch roost more frequently than other females
To respond to disturbance	1. Newly caught individuals would switch roosts more frequently than those caught long time ago
To reduce commuting costs to foraging areas	1. Individuals would switch to the roost-site which is closer to the foraging site
	2. Moving distance between consecutive roosts would be in a comparable scale to the moving distance between roosting and foraging sites
To response to the change of microclimate in the roost	**1. The roost switching frequency of foliage roosting bats would increase after a rain**
	2. The roost switching frequency of house roosting bats would be affected less after a rain
To enhance the social interaction	**1. Individuals live in the large subgroup would switch roost less frequently than those lives in small subgroup**
	2. The number of bats associated with a given individual would increase as its roost switching frequency increased
	3. A nonrandom association between individuals
	4. Showing fidelity to the roosting area while switching roost frequently
	5. Individuals roosting in a foliage roost (limited space) would switch roost more frequently than those in the house (spacious roost).

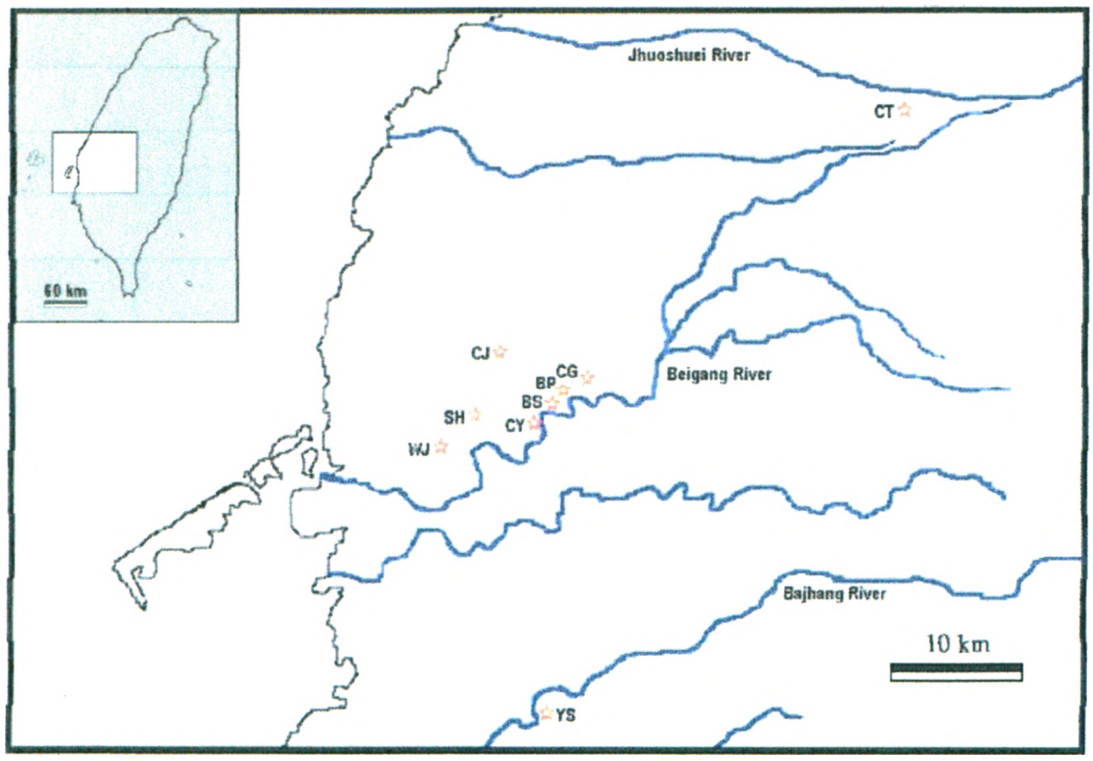
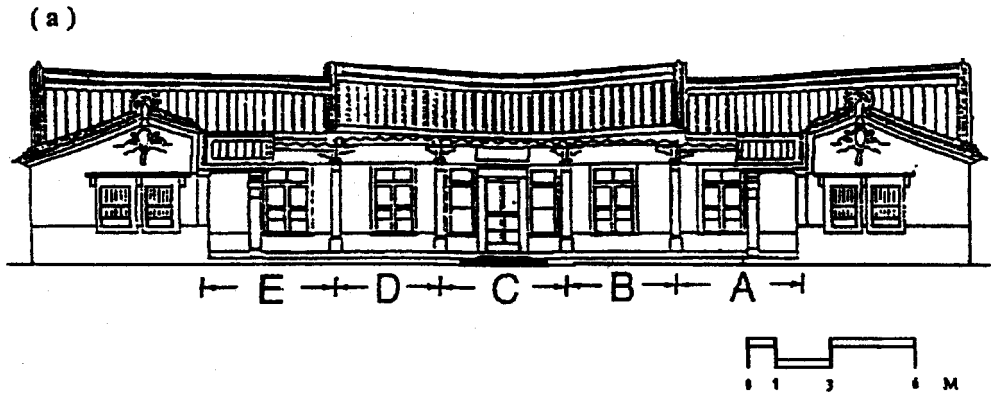


Figure 2.1. Map of sampling localities for *Myotis formosus* on the Southwest plain, Taiwan. Red stars indicated the localities of the colonies sampled, including 1) a park affiliated with the Beigang sugar refinery (BS, Roosting Area 1), 2) a old house (SH, Roosting Area 2), 3) Beigang Sports Park (BP, Roosting Area 3), 4) around Chao-Yang Elementary School (CY, Roosting Area 4), 5) the vicinity of Chen-Guang Elementary School (CG, Roosting Area 5), 6) the vicinity of Chen-Jheng Elementary School (CJ, Roosting Area 6), 7), a area around Rao-Ping Elementary School in Cihtong township CT, Roosting Area 7), 8) the vicinity of Wun-Jheng Elementary School (WJ, Roosting Area 8), 9) a area around An-Nei Elementary School in Yanshuei township (YS, Roosting Area 9).



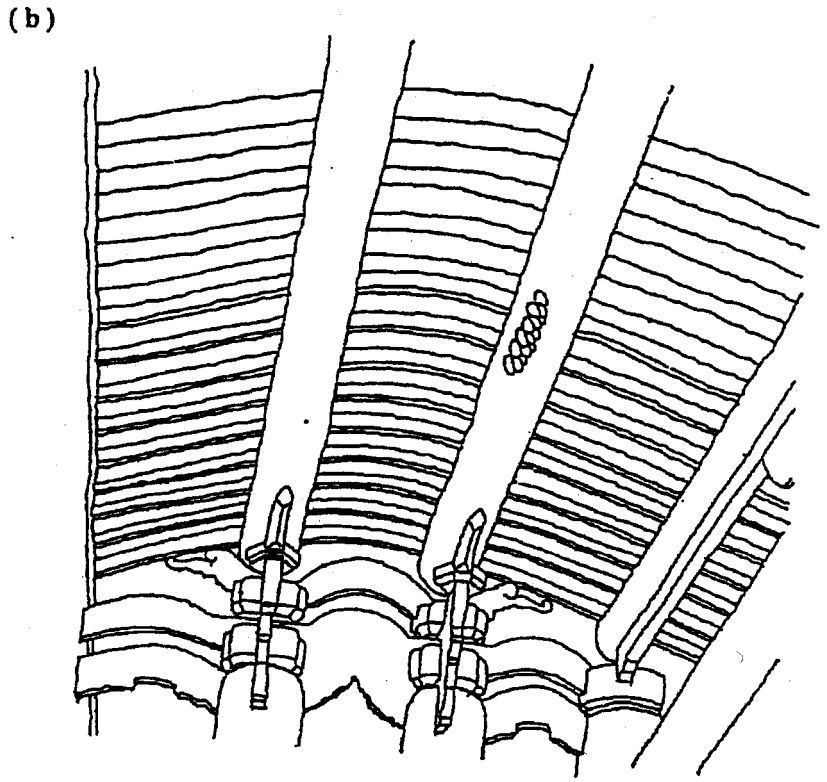


Figure 2.2. (a) The front-view of the traditional house, Roosting Area 2, and the position of the five roost-sites (A, B, C, D, and E) used by *Myotis formosus.* Also shown (b) are *M. formosus* hanging under a beam of the house. (adopted and modified from Yang 1996)

Figure 2.3. An aerial photograph of the Beigang sugar refinery (BS). Red circles indicate the areas of trees that were regularly censused. Yellow circles identify the areas of trees censused less frequently and non-regularly. (Adopted from aerial photo from Forestry Aerial Survey Bureau, Taiwan)

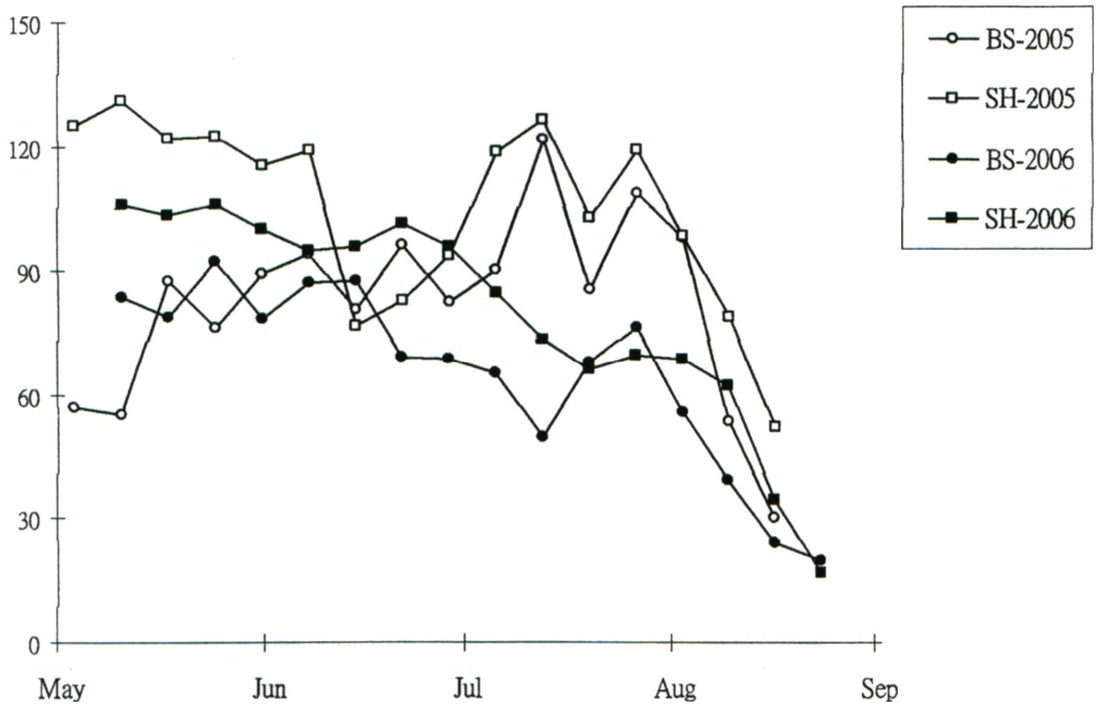


Figure 3.1. The daily number of *Myotis formosus* at , Roosting Area 1 and , Roosting Area 2 from May to August in 2005 and 2006, shown in weekly average.

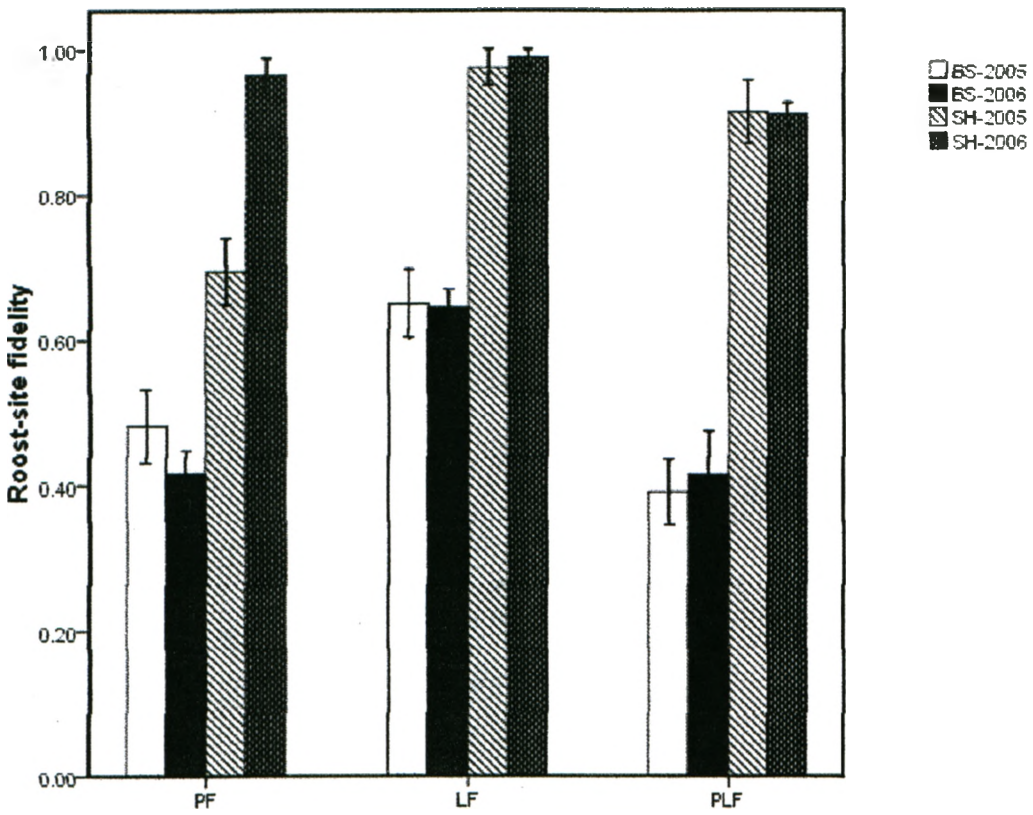


Figure 3.2. A comparison of roost-site fidelity between females of different reproductive status from Roosting Area 1 and Roosting Area 2 in 2005 and 2006, respectively. Data are presented in mean ±SE. PF: pregnant female; LF: lactating female; PLF: post-lactating female.

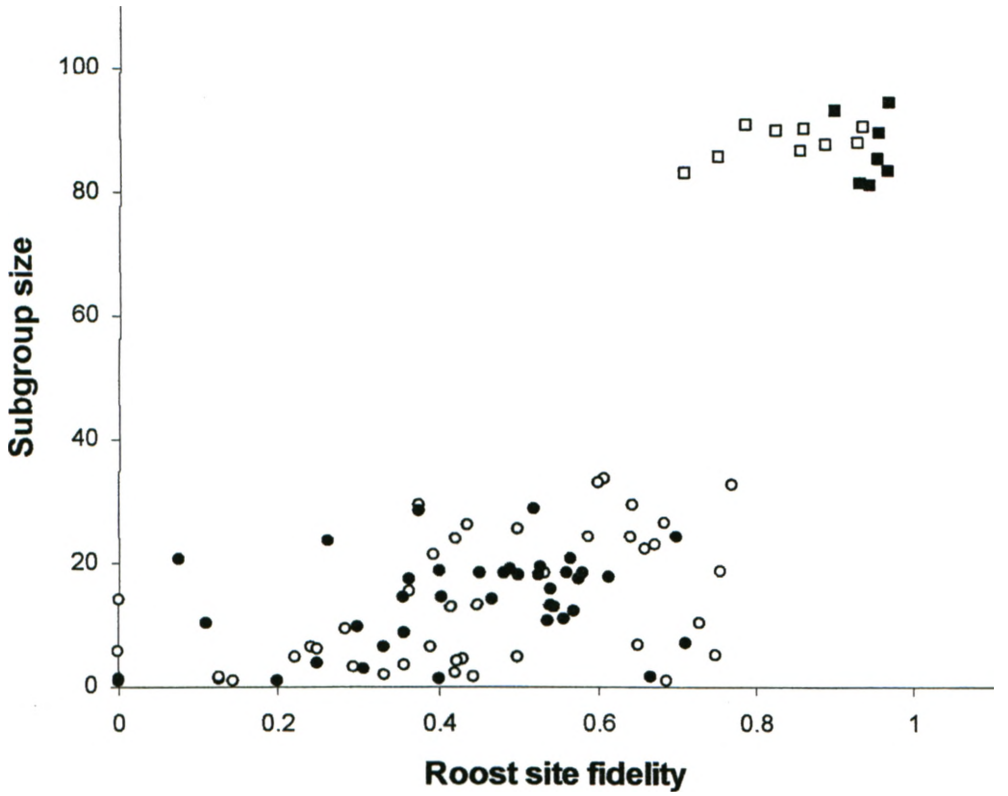


Figure 3.3. Scatter plots demonstrating the relationship between roost-site fidelity and the mean subgroup size of *Myotis formosus* at Roosting Area 1 and Roosting Area 2, in 2005 and 2006. Open circles indicate Roosting Area 1 in 2005, filled circles Roosting Area 1 in 2006; open squares indicate Roosting Area 2 in 2005 and filled squares Roosting Area 2 in 2006.

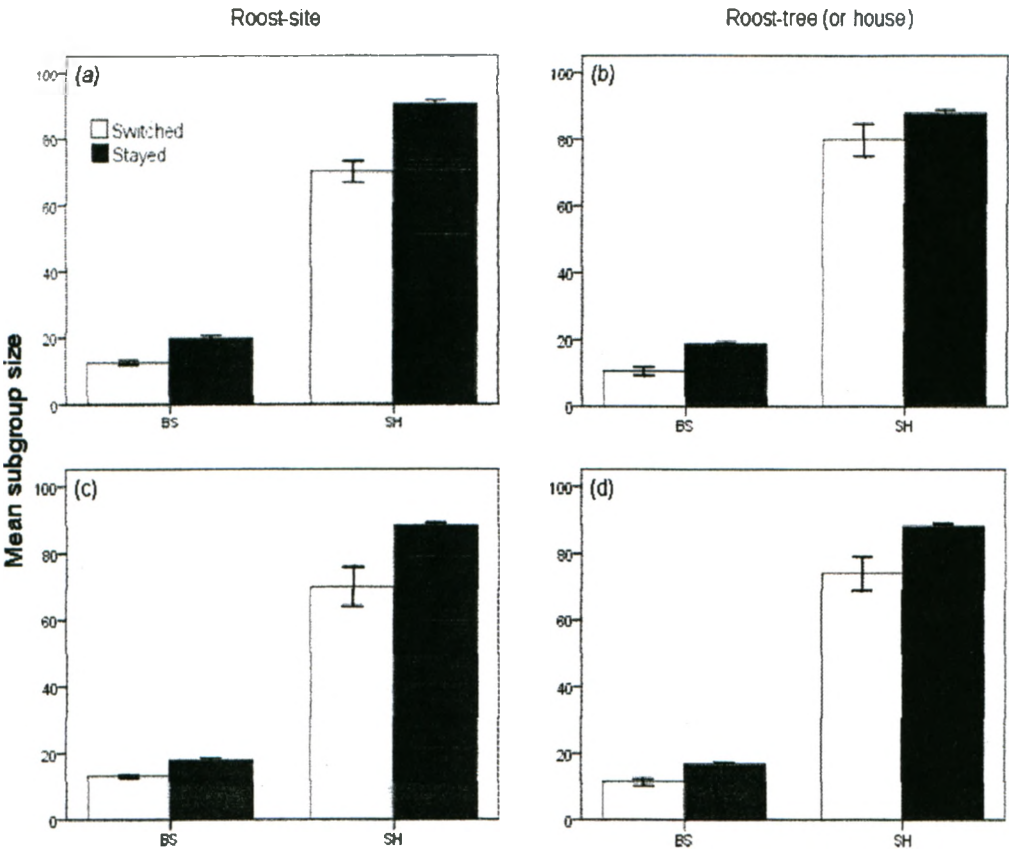


Figure 3.4. Mean subgroup size of *Myotis formosus* which switched roost-sites or roost-tree (or house) and returned the same roost-site or roost-tree (or house) the next day. Data for 2005 are shown in (a) and (b), those for 2006 in (c) and (d). BS: Roosting Area 1; SH: Roosting Area 2. Open bars indicate mean subgroup sizes of bats that switched roost-site or roost-tree (or house) the next day, and filled bars indicate mean subgroup size of bats that returned to the same roost-site or roost-tree (or house) next day. Error bars indicate mean ± 1.0 standard error.

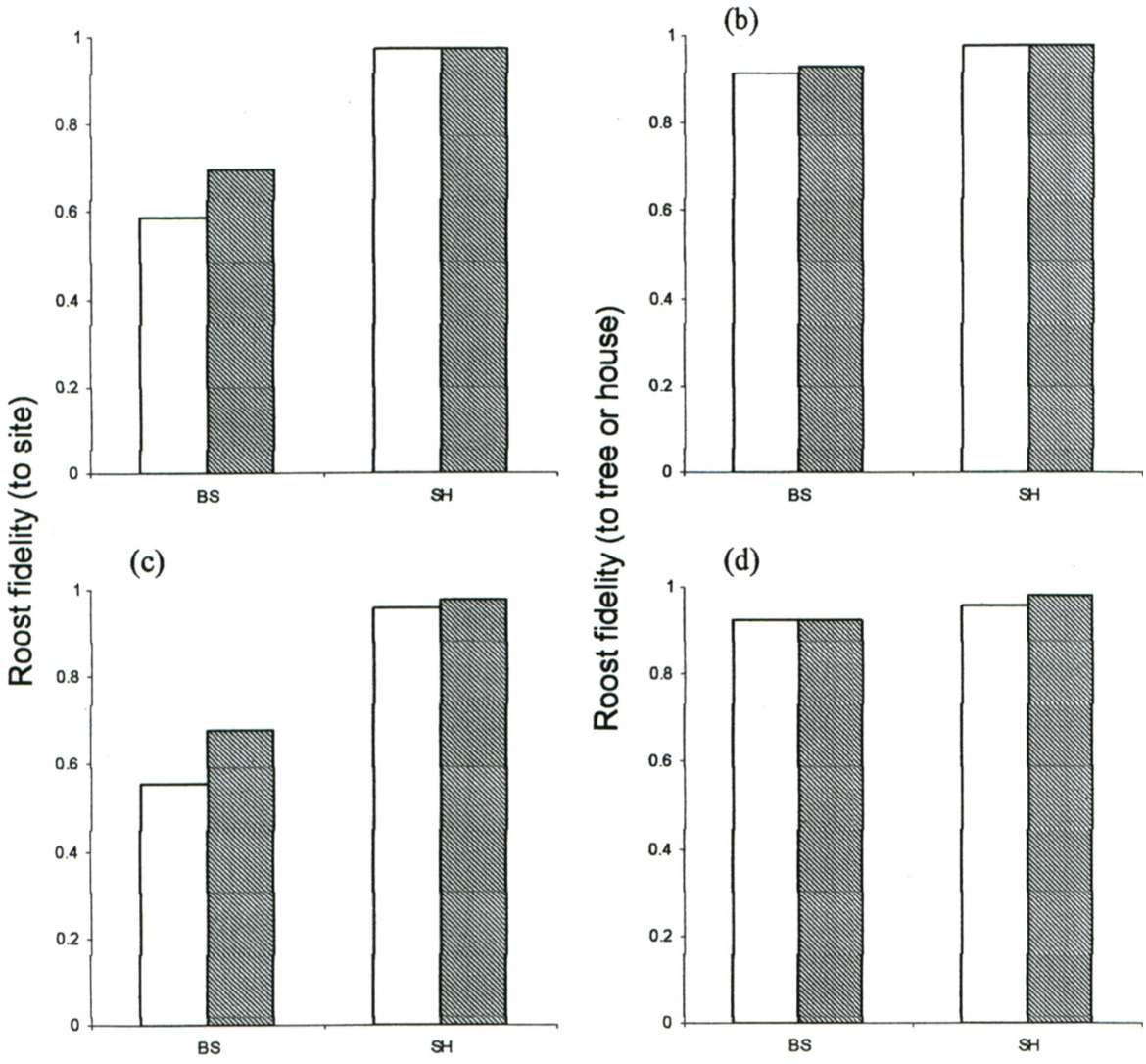


Figure 3.5. Fidelity to roost-site and roost-tree (or house) by *Myotis formosus* at Roosting Area 1 and Roosting Area 2 after a rainy day or rainy night. The effect of rain on the previous day (a) and (b) is compared to rain on the previous night (c) and (d).

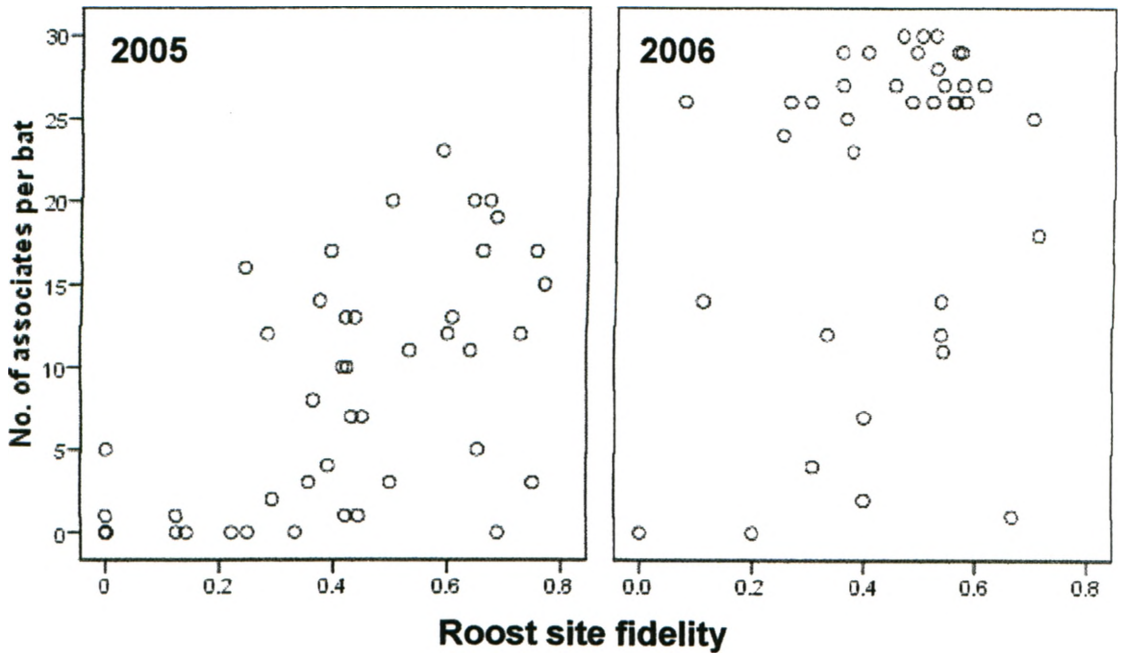


Figure 3.6. Scatter plots demonstrating the relationship between numbers of associations per bat and roost site fidelity at Roosting Area 1 in 2005 and 2006.

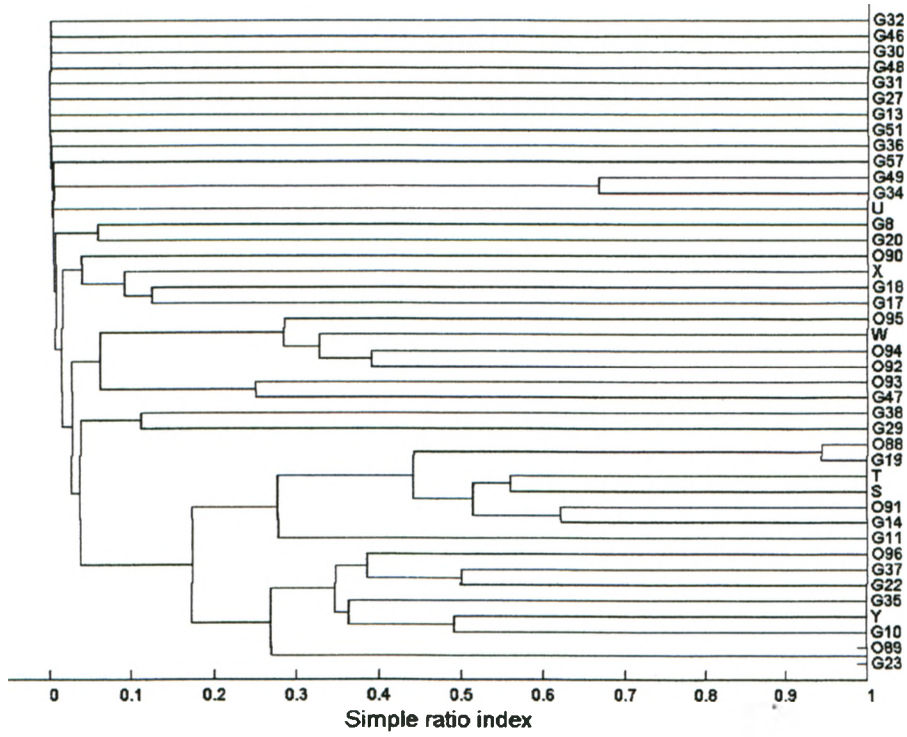


Figure. 3.7a. Average linkage cluster analysis diagram of simple ratio index (SR-index) values of *Myotis formosus* at Roosting Area 1, in 2005. The gray line shows a value of 0.1 indicating separation of clusters of individuals into distinct subgroups.

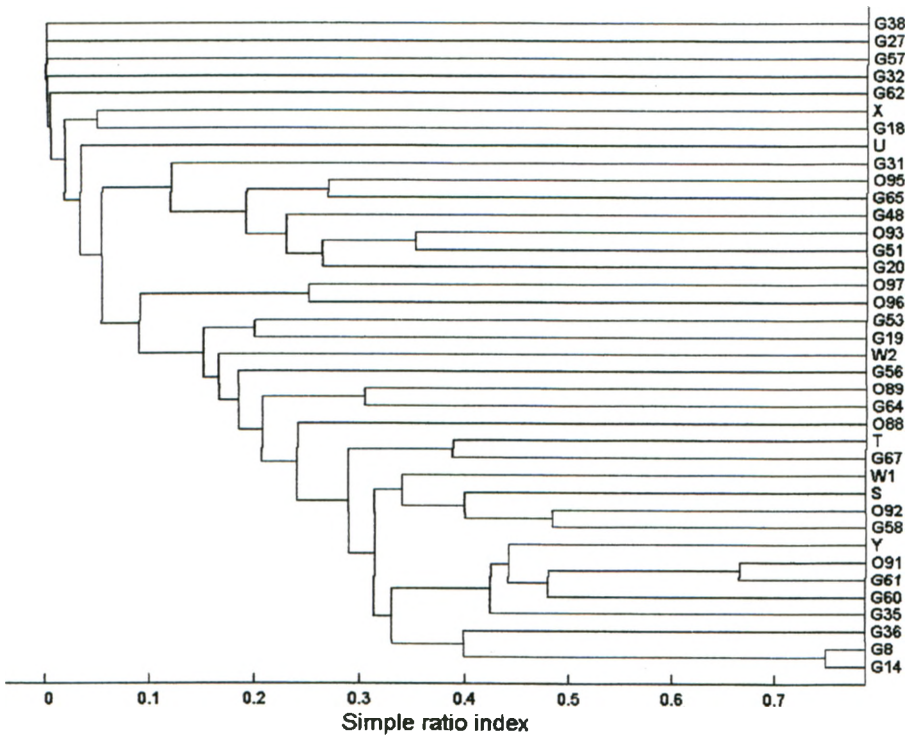


Figure. 3.7b. Average linkage cluster analysis diagram of simple ratio index (SR-index) values of *Myotis formosus* at Roosting Area 1, in 2006. The gray line of value 0.1 indicates the separation of the clusters of individuals into distinct subgroups.

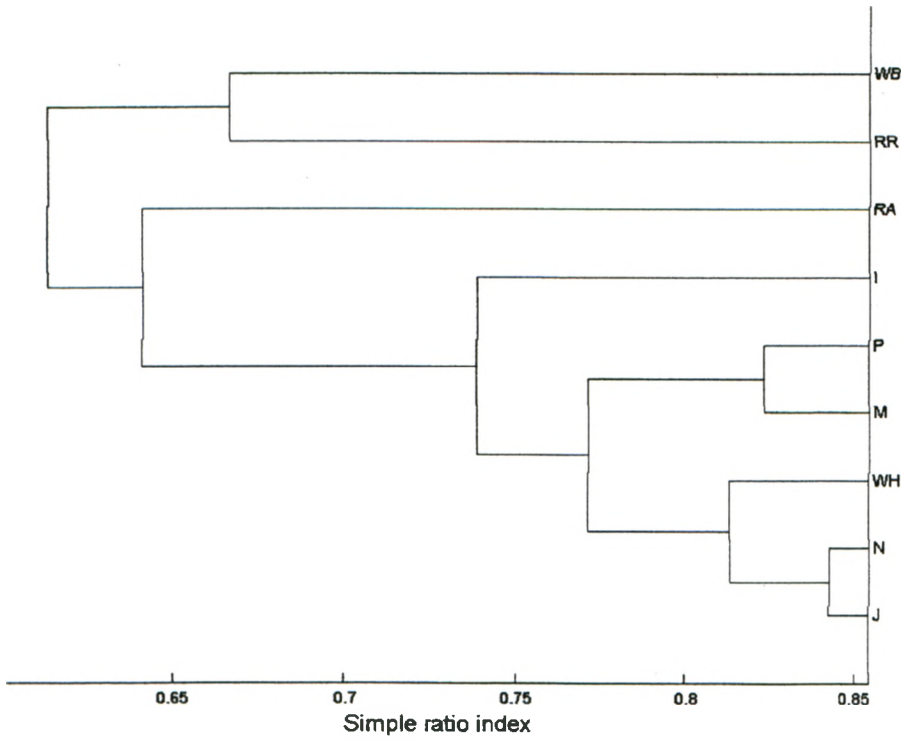


Figure 3.7c. Average linkage cluster analysis diagram of simple ratio index (SR-index) values of *Myotis formosus* at Roosting Area 2 in 2005.

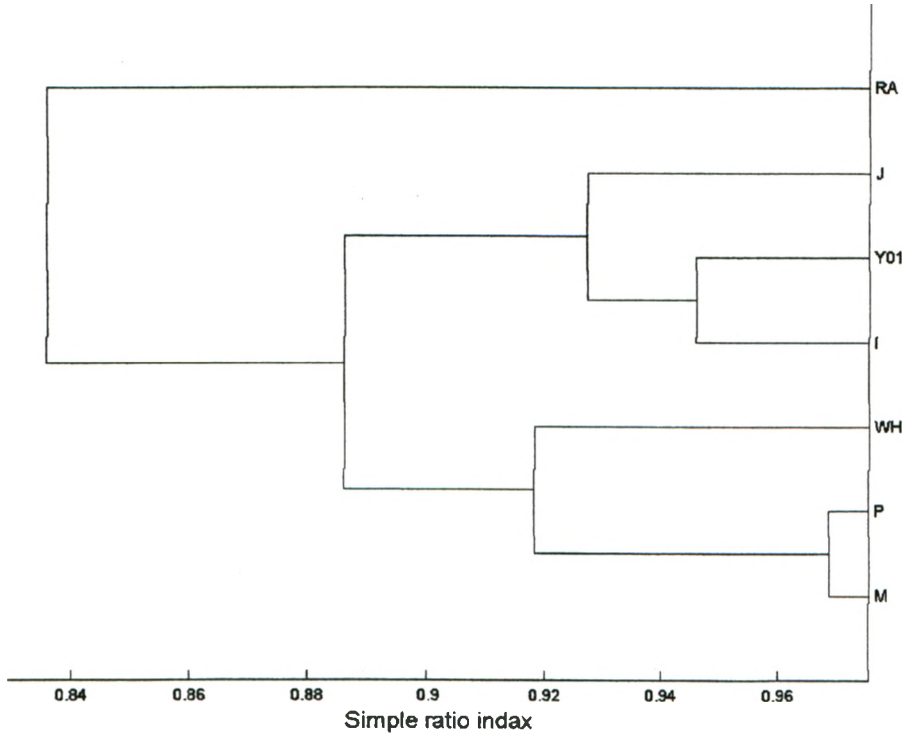


Figure 3.7d. Average linkage cluster analysis diagram of simple ratio index (SR-index) values of *Myotis formosus* at Roosting Area 2 in 2006.

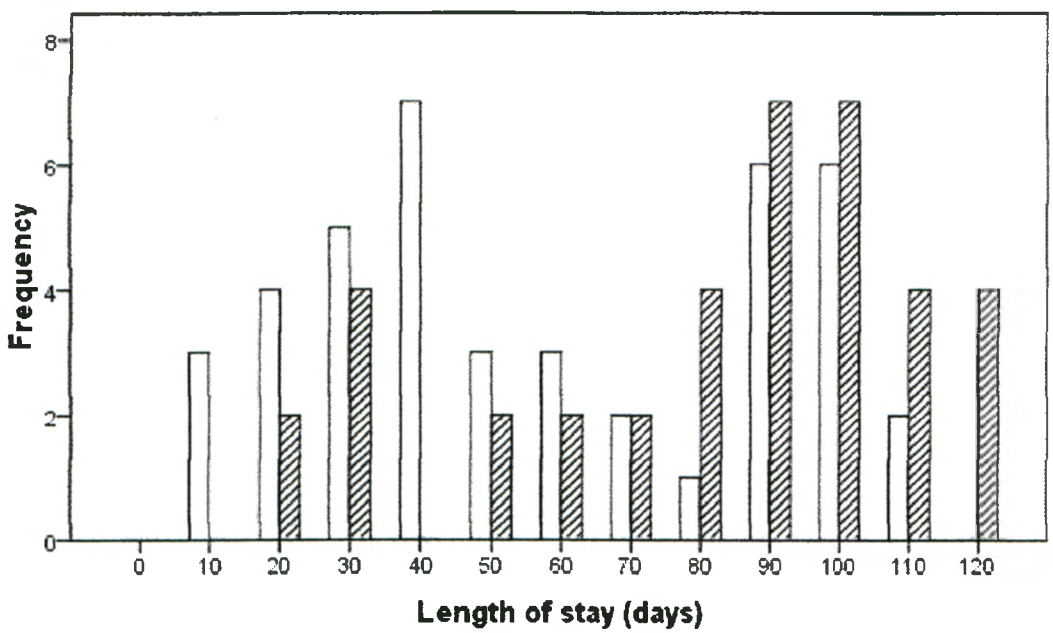


Figure 3.8. Histogram of amount of time each banded *Myotis formosus* was observed at Roosting Area 1 in 2005 (open bar) and 2006 (solid bar).

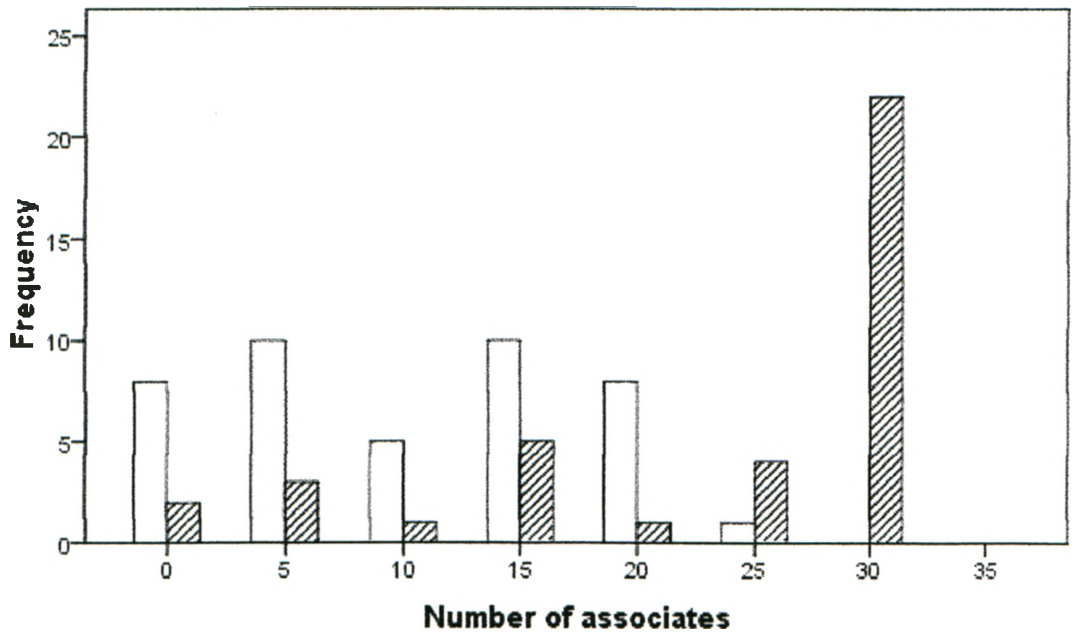


Figure 3.9. Histogram of the number of associations each banded *Myotis formosus* had during the census period at Roosting Area 1 in 2005 (open bar) and 2006 (solid bar).

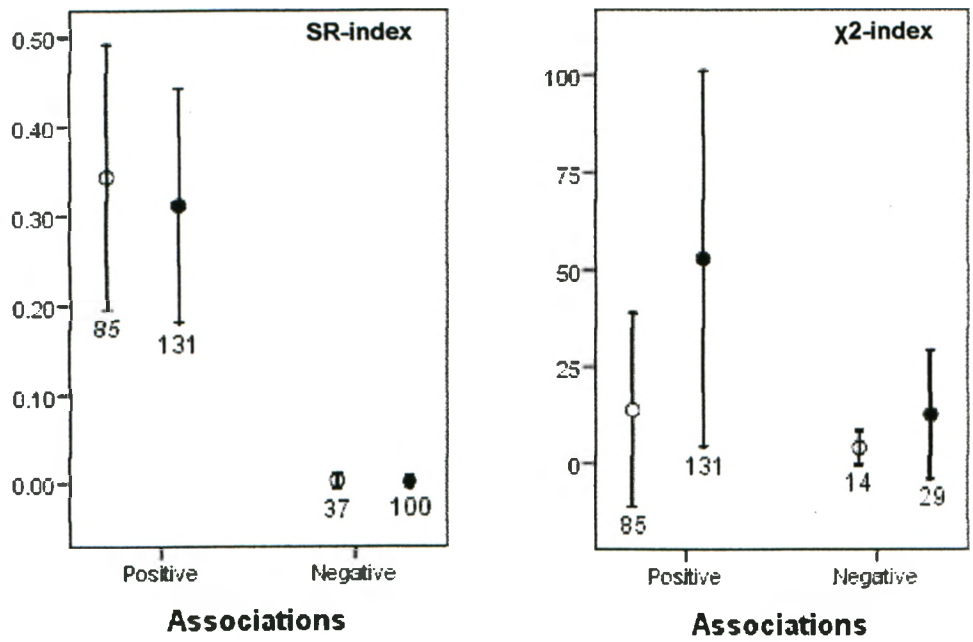


Figure 3.10. Comparison of the three indices used to analyze the associations among adult female *Myotis formosus* at Roosting Area 1 in 2005 and 2006. Mean ± SD of the SR-index and χ^2-index were given for female dyads with significant positive versus negative associations according to the SGS-index. Numbers below data points indicated the sample size (number of dyads). Open circles indicate bats in 2005 and solid circles bats in 2006.

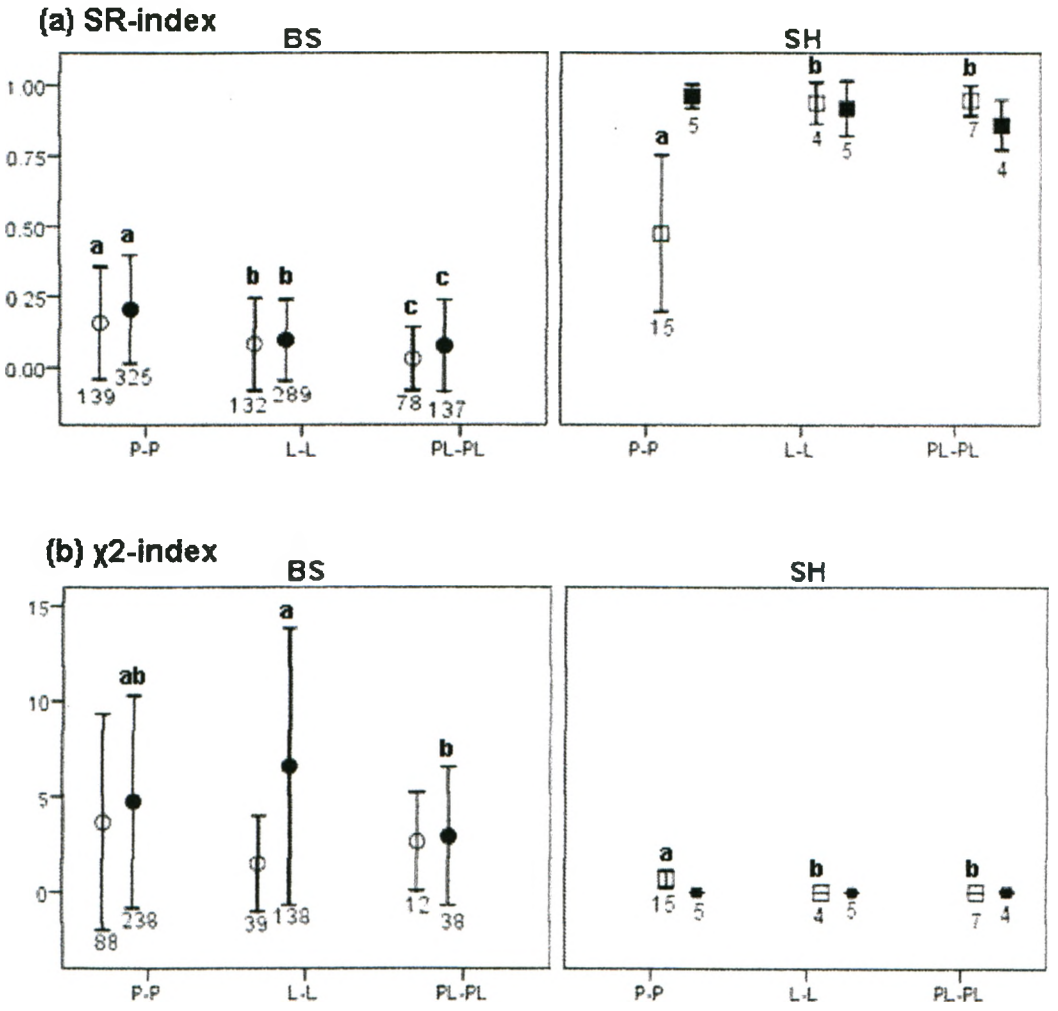


Figure 3.11. Comparison of the three indices used to analyze the associations among adult female *Myotis formosus* at Roosting Area 1 in 2005 and 2006. Mean ± SD of the SR-index and χ^2-index were given for female dyads with significant positive versus negative associations according to the SGS-index. Numbers below data points indicated the sample size (number of dyads). Open symbols indicate bats in 2005 and solid symbols bats in 2006.

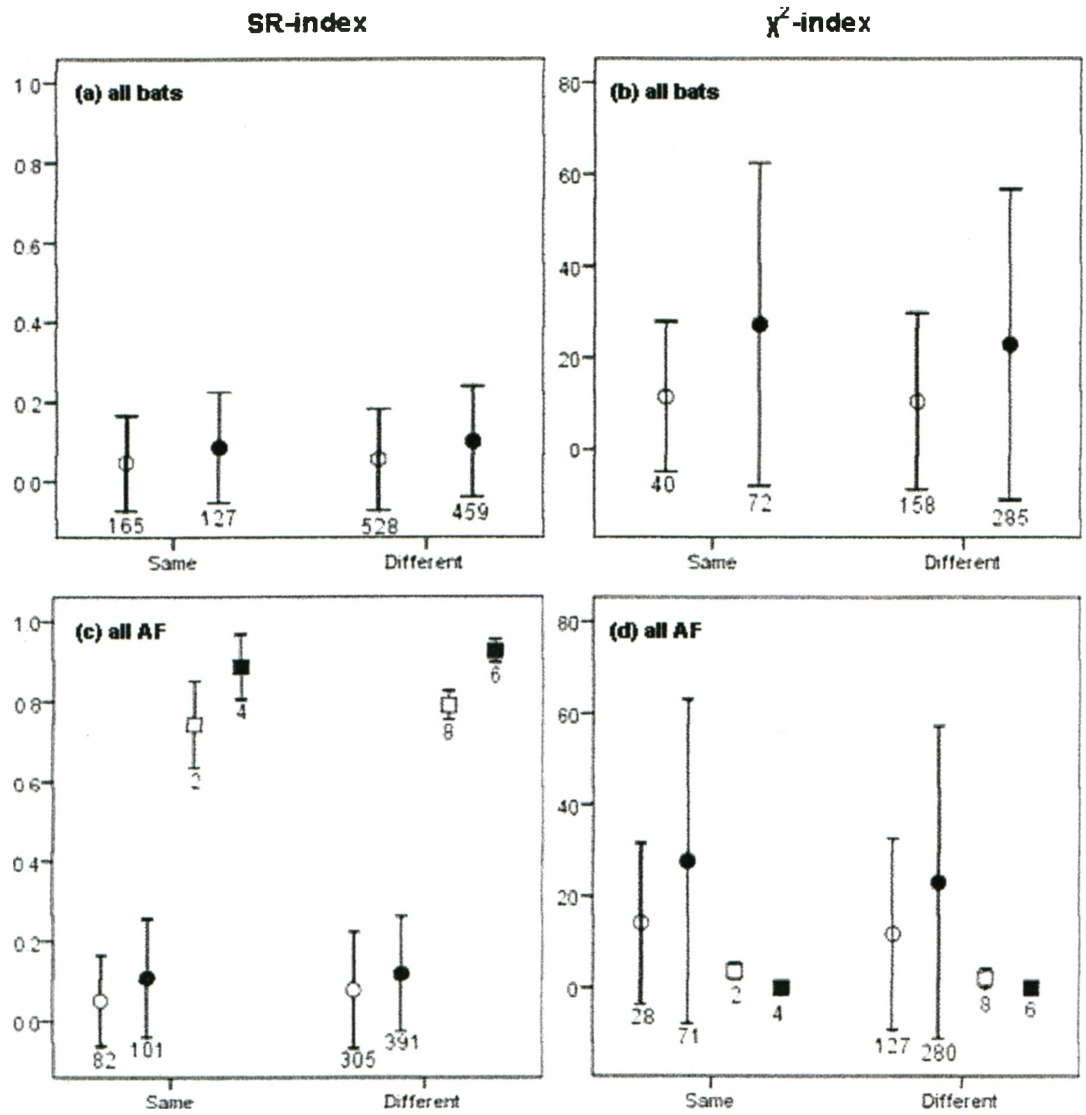


Figure 3.12. Comparison of the SR-index and χ^2-index between dyads of *Myotis formosus* with the same versus different mitochondrial DNA HVII sequence for the sample of all individuals and adult female only at Roosting Area 1 and Roosting Area 2 in 2005 and 2006. Mean ± SD of the SR-index and χ^2-index were given for female dyads with significant positive versus negative associations according to the SGS-index. Numbers below data points indicated the sample size (number of dyads). Open symbols indicated the bats in 2005 and solid symbols bats in 2006. Circles indicated the bats at Roosting Area 1 and squares indicated bats at Roosting Area 2.

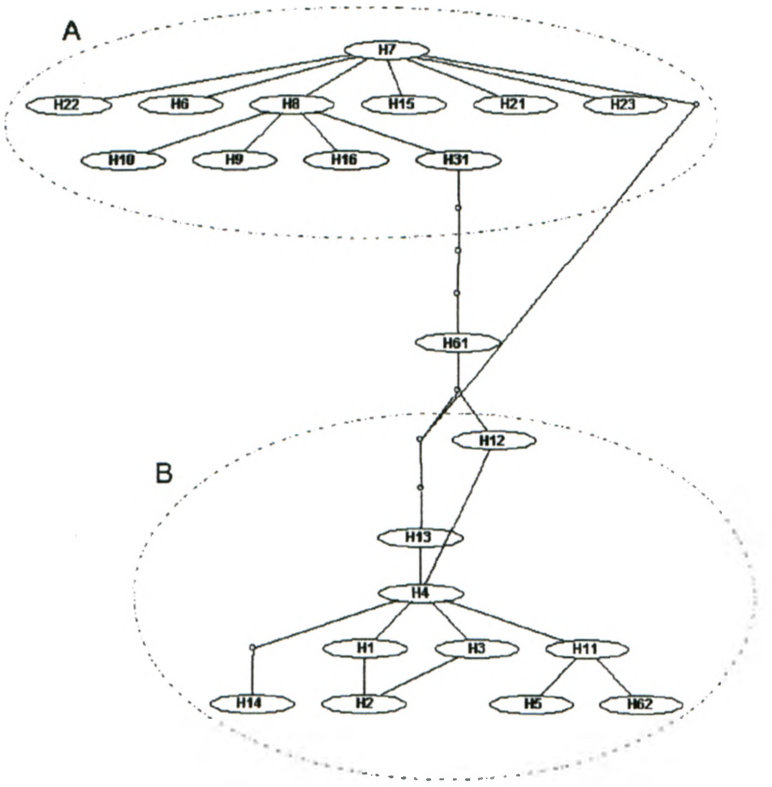


Figure 3.13. Maximum parsimony tree using 263 base pair region of HVII of mitochondrial DNA from 147 individuals. Large circles with haplotype name inside represent observed haplotypes. Small round circles indicate missing or un-sampled haplotypes expected to be intermediates between observed haplotypes. Dashed lines group the haplotypes into different clades. Each line between large or small circles indicates one step of mutation.

Appendix A. MATLAB COMMANDS FOR CALCULATING THREE ASSOCIATION INDICES

```
clear all; close all; format compact;
warning('off','MATLAB:xlswrite:AddSheet');

% Parameters:
%dataFile = 'Bats association 2005 & 2006.xls'; % sheets: 2005, 2006
%dataFile = 'Myotis association all 0501.xls'; % sheets: 2005 BS, 2006
BS, version 4
dataFile = 'Myotis association all 0508.xls'; % sheets: 2005 BS, 2006
BS (banded bats); 2005 BS group, 2006 BS group (population)
% File format: Row 1 is header, Row 2..N+1 is N observations
% Column 1: Date(MM/DD/YY) Time(HH:MM:SS PM), no leading zeros for MM
or DD
% Column 2: Location(floating point number)
% Column 3: banded bats: Individual ID: character string (1 or 3
characters)
% Column 3: population: Group size

for pp=1:2,
   % Read Excel data file (population):
   [junk1,junk2,xTemp] = xlsread(dataFile,[num2str(pp+2004),' BS
group']); % sheets 2005 BS group, 2006 BS group (population)
   % xRaw: Row 1 is header, Row 2...end-1 is data, last rows may be NaN
   xRaw = cell(0,3);
   for p=1:size(xTemp,1),
      if ~isnan(xTemp{p,1}),
         xRaw{end+1,1} = strtok(xTemp{p,1}); % discard time token from
string, keep date string
         xRaw{end,2} = xTemp{p,2};
         xRaw{end,3} = xTemp{p,3};
      end;
   end;

   % Extract lists of all possible day, location, and individual ID
from xRaw:
   listDay = {xRaw{2,1}}; % grow dynamically
   listLoc = xRaw{2,2};
   for p=3:size(xRaw,1),
      if isempty(strmatch(xRaw{p,1},listDay,'exact')),
         listDay{end+1} = xRaw{p,1}; % cell array of strings
      end;
      if ~any(xRaw{p,2}==listLoc),
         listLoc(end+1) = xRaw{p,2}; % vector of doubles
      end;
   end;

   % Convert listDay into numeric values:
   numDay = datenum(listDay);

   % Sort listDay, earliest day to most recent day:
   [junk,sortIndex] = sort(numDay);
   listDay = {listDay{sortIndex}};
   listDayPop = listDay; % make copy to compare w/ listDay from banded
bats
```

```
   % Count # times each individuals were observed at each roost on each
day:
   Lkt = zeros(length(listLoc),length(listDay)); % # bats
(banded+unbanded) in roost k on day t
   for p = 2:size(xRaw,1),
      t = strmatch(xRaw{p,1},listDay,'exact'); % day t
      k = find(xRaw{p,2}==listLoc); % location k
      Lkt(k,t) = xRaw{p,3};
   end;

   % Calculate probability of finding a pair of bats in same roost for
each day using K&K's method and Skowronski's method:
   NtPop = sum(Lkt,1); % Sum of all bats of population in all roost on
day t
   pLkt = Lkt.*(Lkt-1)./repmat(NtPop,size(Lkt,1),1)./repmat(NtPop-
1,size(Lkt,1),1);
   pLtKK = sum(pLkt,1); % probability of finding any 2 bats in same
roost on same day, Kerth&Konig method
   pLkt = Lkt./repmat(sum(Lkt,1),size(Lkt,1),1); % probability of
finding a bat in roost k on day t
   pLtSkow = sum(pLkt.^2,1); % probability of finding any 2 bats in
same roost on same day, Skowronski method

   % Read Excel data file (banded bats):
   [junk1,junk2,xTemp] = xlsread(dataFile,[num2str(pp+2004),' BS']); %
sheets 2005 BS, 2006 BS (banded bats)
   % xRaw: Row 1 is header, Row 2...end-1 is data, last rows may be NaN
   xRaw = cell(0,3);
   for p=1:size(xTemp,1),
      if ~isnan(xTemp{p,1}),
         xRaw{end+1,1} = strtok(xTemp{p,1}); % discard time token from
string, keep date string
         xRaw{end,2} = xTemp{p,2};
         xRaw{end,3} = xTemp{p,3};
      end;
   end;

   % Extract lists of all possible day, location, and individual ID
from xRaw:
   listDay = {xRaw{2,1}}; % grow dynamically
   listLoc = xRaw{2,2};
   listID = {xRaw{2,3}};
   for p=3:size(xRaw,1),
      if isempty(strmatch(xRaw{p,1},listDay,'exact')),
         listDay{end+1} = xRaw{p,1}; % cell array of strings
      end;
      if ~any(xRaw{p,2}==listLoc),
         listLoc(end+1) = xRaw{p,2}; % vector of doubles
      end;
      if isempty(strmatch(xRaw{p,3},listID,'exact')),
         listID{end+1} = xRaw{p,3}; % cell array of strings
      end;
   end;

   % Convert listDay into numeric values:
   numDay = datenum(listDay);
```

```
   % Sort listDay, earliest day to most recent day:
   [junk,sortIndex] = sort(numDay);
   listDay = {listDay{sortIndex}};

   % Calculate # locations:
   K = length(listLoc);

   % Count # times each individuals were observed at each roost on each
day:
   Nikt = zeros(length(listID),K,length(listDay)); % 1==individual i
observed at location k at day t;0==not observed
   for p=2:size(xRaw,1),
      t = strmatch(xRaw{p,1},listDay,'exact'); % day t
      k = find(xRaw{p,2}==listLoc); % location k
      i = strmatch(xRaw{p,3},listID,'exact'); % individual i
      Nikt(i,k,t) = Nikt(i,k,t)+1; % update counter (should be maximum
1)
   end;

   % Calculate terms:
   Ni = squeeze(sum(sum(Nikt,3),2)); % # days individual i observed at
any location, COLUMN vector
   Nk = squeeze(sum(sum(Nikt,3),1)); % # individuals in roost k over
all time, COLUMN vector
   Nt = squeeze(sum(sum(Nikt,1),2)); % # individuals in any roost on
day t, COLUMN vector
   Nik = sum(Nikt,3); % # days individual i at location k
   Nit = squeeze(sum(Nikt,2)); % 1==ith bat observed that day; 0==ith
bat not observed that day
   Nkt = squeeze(sum(Nikt,1)); % # individuals in roost k on day t

   % Find first/last day of observing each individual:
   firstDay = zeros(1,size(Nikt,1));
   lastDay = zeros(1,size(Nikt,1));
   for p=1:size(Nikt,1),
      tIndex = find(Nit(p,:));
      firstDay(p) = tIndex(1);
      lastDay(p) = tIndex(end);
   end;

   % Sort individuals by first day observed:
   [junk,orderInd] = sort(firstDay);
   [junk,orderIndInv] = sort(orderInd); % orderIndInv(i) =
find(orderInd==i)

   % Sort roosts by # bat-days:
   [junk,orderRoost] = sort(Nk);

   % Calculate coincident matrix:
   Nij = zeros(length(listID)); % # times individual i and individual j
observed in same roost on same day
   for t=1:size(Nikt,3) % for each day
      for i = 1:length(listID), % for each i
```

```
         ki = find(Nikt(i,:,t)==1); % could be empty if individual i
not observed on day t
         if ~isempty(ki),
            for j=1:length(listID), % for each j
               kj = find(Nikt(j,:,t)==1); % could be empty if
individual j not observed on day t
               if ~isempty(kj) && i~=j,
                  if ki==kj % same roost on day t
                     Nij(i,j) = Nij(i,j)+1; % update counter
                  end;
               end;
            end;
         end;
      end;
   end;

   % Calculate total number of observations of i and/or j in
overlapping history range:
   Tij = zeros(length(listID)); % # times individual i and/or
individual j observed on the same day in any roost in overlapping
history region
   BetaHatijWilk = zeros(length(listID)); % expected # days i and j
observed in same roost, considered over overlapping history region,
Wilkinson's method
   BetaHatijKK = zeros(length(listID)); % expected # days i and j
observed in same roost, considered over overlapping history region,
Kerth&Konig's method
   BetaHatijSkow = zeros(length(listID)); % expected # days i and j
observed in same roost, considered over overlapping history region,
Skowronski method
   OHRij = zeros(length(listID)); % overlapping history region size,
ignores days that neither bat was observed
   for i=1:length(listID),
      for j=1:length(listID),
         tRange =
[max(firstDay(i),firstDay(j)):min(lastDay(i),lastDay(j))]; % range of
days in overlapping history region
         wRange = Nit(i,tRange) | Nit(j,tRange); % index into tRange,
1==i or j observed, 0==neither observed that day
         bRange = Nit(i,tRange) & Nit(j,tRange); % index into tRange,
1==i and j observed, 0==one or neither observed that day
         Tij(i,j) = sum(bRange); % #days in tRange i and j observed on
same day in some roost
         OHRij(i,j) = length(find(wRange)); % #days in tRange that at
least 1 of the two bats was observed
         BetaHatijKK(i,j) = sum(pLtKK(tRange(bRange)));
         BetaHatijSkow(i,j) = sum(pLtSkow(tRange(bRange)));

         % Calculate beta using Wilkinson's method:
         Nitemp = squeeze(sum(sum(Nikt(:,:,tRange),3),2)); % # days
individual i observed at any location, COLUMN vector
         Niktemp = sum(Nikt(:,:,tRange),3); % # days individual i at
location k
         Pik = Niktemp./repmat(Nitemp+eps,1,K); % add eps in case
Nitemp=0 because OHRij=0
```

```
        T =
min(length(find(Nit(i,tRange))),length(find(Nit(j,tRange))));
        B = T*sum(Pik(i,:).*Pik(j,:)); % legacy variable name, from
version 1
        BetaHatijWilk(i,j) = B;
      end;
   end;

   % Calculate chi-squared (2-tailed):
   chi2Wilk = (Nij-BetaHatijWilk).^2./(BetaHatijWilk+eps); % add eps in
case denominator=0
   chi2Wilk = chi2Wilk.*sign(Nij-BetaHatijWilk);
   chi2KK = (Nij-BetaHatijKK).^2./(BetaHatijKK+eps) + ((Tij-Nij)-(Tij-
BetaHatijKK)).^2./((Tij-BetaHatijKK)+eps); % add eps in case
denominator=0
   chi2KK = chi2KK.*sign(Nij-BetaHatijKK);
   chi2Skow = (Nij-BetaHatijSkow).^2./(BetaHatijSkow+eps) + ((Tij-Nij)-
(Tij-BetaHatijSkow)).^2./((Tij-BetaHatijSkow)+eps); % add eps in case
denominator=0
   chi2Skow = chi2Skow.*sign(Nij-BetaHatijSkow);



   % Calculate simple index:
   SI = Nij./(Tij+eps); % add eps in case Tij=0
   SIhistory = Nij./(OHRij+eps); % add eps in case OHRij=0

   % Write chi2 and listID to Excel file:
   xOut = cell(length(listID)+1);
   for p=1:length(listID),
      xOut{p+1,1} = listID{p};
      xOut{1,p+1} = listID{p};
   end;
   xOutchi2Wilk = xOut;
   xOutchi2KK = xOut;
   xOutchi2Skow = xOut;
   xOutNij = xOut;
   xOutTij = xOut;
   xOutOHRij = xOut;
   xOutBijWilk = xOut;
   xOutBijKK = xOut;
   xOutBijSkow = xOut;

   xOutSI = xOut;
   xOutSIhistory = xOut;
   for i=1:length(listID),
      for j=1:length(listID),
         xOutchi2Wilk{i+1,j+1} = chi2Wilk(i,j);
         xOutchi2KK{i+1,j+1} = chi2KK(i,j);
         xOutchi2Skow{i+1,j+1} = chi2Skow(i,j);
         xOutNij{i+1,j+1} = Nij(i,j);
         xOutTij{i+1,j+1} = Tij(i,j);
         xOutOHRij{i+1,j+1} = OHRij(i,j);
         xOutBijWilk{i+1,j+1} = BetaHatijWilk(i,j);
         xOutBijKK{i+1,j+1} = BetaHatijKK(i,j);
         xOutBijSkow{i+1,j+1} = BetaHatijSkow(i,j);
```

```
            xOutSI(i+1,j+1) = SI(i,j);
            xOutSIhistory(i+1,j+1) = SIhistory(i,j);
        end;
    end;
    xlswrite('chi2Wilk_08.xls',xOutchi2Wilk,num2str(pp+2004));
    xlswrite('chi2KK_08.xls',xOutchi2KK,num2str(pp+2004));
    xlswrite('chi2Skow_08.xls',xOutchi2Skow,num2str(pp+2004));
    xlswrite('Nij_08.xls',xOutNij,num2str(pp+2004));
    xlswrite('Tij_08.xls',xOutTij,num2str(pp+2004));

xlswrite('OverlappingHistoryRegionij_08.xls',xOutOHRij,num2str(pp+2004)
);
    xlswrite('BetaHatijWilk_08.xls',xOutBijWilk,num2str(pp+2004));
    xlswrite('BetaHatijKK_08.xls',xOutBijKK,num2str(pp+2004));
    xlswrite('BetaHatijSkow_08.xls',xOutBijSkow,num2str(pp+2004));

    xlswrite('simpleIndex_08.xls',xOutSI,num2str(pp+2004));

xlswrite('simpleIndexHistory_08.xls',xOutSIhistory,num2str(pp+2004));
end;

% Bye!
```

Appendix B. MATLAB COMMANDS FOR AVERAGE CLUSTER ANALYSIS

```
clear all; close all; format compact;

% Random seed rand and randn:
rand('state',sum(100*clock));
randn('state',sum(100*clock));

% Parameters:
dataFile = 'BS SR-index (no missing data).xls'; % 2005, 2006

% Process each year:
for pp = 1:2,
   % Read Excel data file (lower triangle, no diagonal values):
   [A,batID] = xlsread(dataFile,num2str(pp+2004)); % numeric matrix,
NaN for non-numeric entries
   A = A+rand(size(A))*1e-8;

   % Convert lower triangle in A to vector (same format as output of
pdist.m):
   Aindex = find(tril(ones(size(A)),-1));
   Avector = A(Aindex); % Nx1 vector, N = size(A,1)*(size(A,1)-1)/2

   % Convert simple index to distance:
   Adist = 1-Avector(:)'; % ROW vector, compatible w/ pdist.m output

   % Form tree:
   Z = linkage(Adist,'average');

   % Plot:
   figure(pp);
   clf;
   dendrogram(Z,0,'orientation','left'); % add ,0 to display entire
tree (Matlab collapses larger trees otherwise)

   % Fix axes:
   xLabels = get(gca,'xticklabel'); % char matrix
   xLabels = num2str(1-str2num(xLabels));
   set(gca,'xticklabel',xLabels);
   yIndex = str2num(get(gca,'yticklabel'));
   set(gca,'yticklabel',{batID{1,yIndex+1}});
   c=get(gca,'children');
   set(c,'color',[0 0 0]); % make all lines black

set([gca,get(gca,'xlabel'),get(gca,'ylabel')],'fontname','arial','fonts
ize',12);
end;

% Cut everything below this line (after modifying figures) and paste at
Matlab prompt:
figure(1);
print -depsc2 'trees01 2005.eps';
print -dpng 'trees01 2005.png';
save 'trees01 2005.fig';
figure(2);
```

```
print -depsc2 'trees01 2006.eps';
print -dpng 'trees01 2006.png';
save 'trees01 2006.fig';


% Bye!
```

Appendix c. MATLAB COMMANDS FOR MANTEL TEST

```
clear all; close all; format compact;

% Random seed rand and randn:

rand('state',sum(100*clock));
randn('state',sum(100*clock));

% Parameters:

dataFile = '2006 genetics.xls'; % sheets: 2005 BS gene, 2005 BS SI,
2006 BS gene, 2006 BS SI
numTrials = 10e3; % Number of random permutations of matrix labels

% Process each year:
phiTrue = zeros(1,2); % true correlation coeff for each year
phiHat = zeros(numTrials,2); % correlation from random labels
randStat = zeros(1,2); % percentile of phiTrue compared to phiHat for
each year

for pp = 1:2,

   % Read Excel data file (lower triangle, no diagonal values):
   Atemp = xlsread(dataFile,[num2str(pp+2004),' BS gene']); % numeric
matrix, NaN for non-numeric entries
   Btemp = xlsread(dataFile,[num2str(pp+2004),' BS SI']); % numeric
matrix, NaN for non-numeric entries
   N = size(Atemp,1)+1; % include length from diagonal term
   % Construct transpose symmetric version of A and B:

   A = zeros(N);
   A(find(tril(ones(N),-1))) = Atemp(find(tril(ones(N-1),0)));
   A = A+A';
   B = zeros(N);
   B(find(tril(ones(N),-1))) = Btemp(find(tril(ones(N-1),0)));
   B = B+B';

   % Find true correlation between A and B:

   matrixIndex = find(triu(ones(N),1) & ~isnan(A) & ~isnan(B)); %
entries in upper triangle of A and B that are not NaN

   R = corrcoef(A(matrixIndex),B(matrixIndex));
   phiTrue(pp) = R(1,2);

   % Find correlations between Ahat and B for random permutations of A:

   for p=1:numTrials, % for each trial

      % Create random permutation of A:
      [junk,randIndex] = sort(rand(1,N));
      Ahat = A(randIndex,randIndex);
```

```
      matrixIndexAhat = find(triu(ones(N),1) & ~isnan(Ahat) &
~isnan(B)); % entries in upper triangle of Ahat and B that are not NaN

      % Find correlation:
      R = corrcoef(Ahat(matrixIndexAhat),B(matrixIndexAhat));
      phiHat(p,pp) = R(1,2);
   end;

   % Tabulate significance:
   randStat(pp) = sum(phiTrue(pp)<=phiHat(:,pp))/numTrials; %
percentage of all phiHat larger than phiTrue
end;

figure(1);
hist(phiHat(:,1),1000);
hold on;
plot(phiTrue(1)*[1 1],[0 50],'r-');
legend('Random matrix','Original matrix');
title('Histogram of corr. coeff., randomized matrices, 2005');
figure(2);
hist(phiHat(:,2),1000);
hold on;
plot(phiTrue(2)*[1 1],[0 50],'r-');
legend('Random matrix','Original matrix');
title('Histogram of corr. coeff., randomized matrices, 2006');
figure(3);
plot(phiHat(:,1));
xlabel('Trial number');
ylabel('Correlation coefficient');
hold on
plot([1 size(phiHat,1)],phiTrue(1)*[1 1],'r-');
legend('Random matrix','Original matrix');
title(['Corr. coeff., ',num2str(numTrials),' randomized matrices,
2005']);
figure(4);
plot(phiHat(:,2));
xlabel('Trial number');
ylabel('Correlation coefficient');
hold on
plot([1 size(phiHat,1)],phiTrue(2)*[1 1],'r-');
legend('Random matrix','Original matrix');
title(['Corr. coeff., ',num2str(numTrials),' randomized matrices,
2006']);

disp(['Corr. coeff., original matrix, 2005: ',num2str(phiTrue(1))]);
disp(['Corr. coeff., original matrix, 2006: ',num2str(phiTrue(2))]);
disp(['Percent of all corr. coeffs. from random matrices larger than
corr. coeff. from original matrix, 2005:
',num2str(randStat(1)*100),'%']);
disp(['Percent of all corr. coeffs. from random matrices larger than
corr. coeff. from original matrix, 2006:
',num2str(randStat(2)*100),'%']);


% Bye!
```