DYNAMICS OF AUTUMN SWARMING AND POPULATION STRUCTURE OF LITTLE BROWN AND NORTHERN MYOTIS BATS (*MYOTIS LUCIFUGUS* AND *M. SEPTENTRIONALIS*)

by

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To the memory of my father, Douglas Henderson.
The pursuit of knowledge can leave one happily restless.

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ABSTRACT

As a fundamental unit in evolutionary ecology and the base unit for management, the population is of immense interest in understanding a species' ecology. Individuals are the foundation of populations where their diversity in behaviours can scale up to variation in other traits that characterize population dynamics. Understanding the variation of individuals in their behaviours and traits within a population (i.e., their structure) is therefore of great importance in characterizing populations.

Bats are highly vagile and gregarious animals that show variation among sex and age classes in many stages of their annual cycle that are best understood during the summer and winter seasons. In this thesis I explored the dynamics in activity of two temperate bats, little brown Myotis (*M. lucifugus*) and northern Myotis (*M. septentrionalis*) during swarming in autumn to characterize aspects of their population structures. To examine intersexual differences in swarming activities bats were captured and tagged at multiple swarming sites to characterize intersexual differences in the frequency and extent of re-use of swarming sites. I explored the associations among sex and age classes of each species to test predictions of hypotheses on the functions of swarming of gene flow and information transfer from mother bats to offspring. Lastly, I characterized population genetic structure of *M. lucifugus* to study the reproductive cohesion of bats - gene flow- among swarming sites.

Male bats were found to have higher swarming activity compared to females which may reflect males spending more time devoted to swarming to maximize mating opportunities. Predictable age and sex class groups were found during swarming where young-of-the-year were found to have the highest associations with other bats and most preferentially with other young-of-the-year. Adult male and female bats were most often captured alone, but when males were captured they showed preference for grouping with other males, including male *M. lucifugus* having preferred male associates across nights. Genetic data for *M. lucifugus* were suggestive of high gene flow and thus a high degree of reproductive connectivity among swarming sites. Together these results provide information of how variation among individuals contributes to population structure.

LIST OF ABBREVIATIONS AND SYMBOLS USED

AIC	Akaike's information criterion
AMOVA	Analysis of molecular variance
BIC	Bayesian information criterion
BP	before present
bp	base pair
BSP	Bayesian skyline plot
CV	Coefficient of variation
DR	Deviation ratio
df	degrees of freedom
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
FAMD	Factor analysis of mixed data
g	gram
GG	general gregariousness
GPS	global positioning system
h	haplotype diversity
HWI	half-weight association index
HWE	Hardy Weinberg Equilibrium
HV II	hypervariable domain II
IBD	isolation-by-distance
Km	kilometre
LGM	last glacial maximum
m	metre
mm	millimetre

mM	millimolar
mtDNA	mitochondrial DNA
N_e	effective population size
ng	nanogram
π	nucleotide diversity
PAI	pairwise affinity index
PCA	principle components analysis
PCR	polymerase chain reaction
PE	parameter estimate
PIC	phylogenetically independent contrasts
PIT	passively integrated transponder
Φ_{ST}	Phi ST
S	social differentiation
SD	standard deviation
SE	standard error
UK	United Kingdom
µL	micro litres
WNS	white-nose syndrome
YOY	Young-of-the-year

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CHAPTER 1 INTRODUCTION

1.1 BACKGROUND

The population is a key unit in describing the biology of a species. It is essential from an evolutionary perspective because it represents an important level at which evolution occurs by way of evolutionary change among individuals (Freeman & Herron 2004). It is also important from a more practical conservation and management side because it is the fundamental unit of management (Lacy 1988; Lande 1988). At their foundation, populations are composed of interacting individuals where these interactions can be ecological (e.g., competition) and genetic (e.g. mating or cloning). Some species are highly social with individuals interacting regularly with preferred associates where these interactions can be particularly intense; thus social structure is an important component to the structure of many populations (Chesser 1991; Storz 1999). Within the vertebrates, well known examples that exhibit complex social structures include primates, cetaceans, equids and bats (Wilkinson 1985; Smolker *et al.* 1992; Pepper *et al.* 1999; van Schaik 1999; Baird & Whitehead 2000; Sundaresan *et al.* 2007; Kerth 2008). For other species, the main interactions of individuals may be less social in nature but still important in maintaining cohesion of a population through gene flow (Slatkin 1985). Within populations, how individuals interact with each other can have important demographic and genetic consequences, in influencing mating systems, birth and death rates, and immigration and emigration rates (Mills 2013). Thus characterizing the

dynamics of populations (e.g., demographic and genetic structure) remains an important undertaking for understanding populations.

There are two main approaches to describe population structure (Slatkin 1994; Waples & Gaggiotti 2006). The first emphasizes demographic cohesiveness where individuals are often directly characterized in terms of movements and interactions. From these observation-based studies, clusters of individuals are defined to delineate groups. The second approach emphasizes reproductive cohesiveness where often indirect means are used to assess patterns and boundaries of gene flow to define groupings of individuals. These approaches are not mutually exclusive as demographic interactions such as competition and behavioural interactions or responses, such as dispersal, will influence mating and gene flow (Clutton-Brock 1989; Storz 1999). Characterizing population structure from both approaches is necessary to provide the most comprehensive view (Lowe & Allendorf 2010).

An important concept in characterizing population structure regardless of the approach is dispersal. In this thesis, dispersal is defined in a broad, classical sense as an ecological concept: the movement of individuals away (one way) from their source in search of resources such as food, shelter or mates (Elton 1927; Clobert *et al.* 2001). Dispersal will usually affect an individual's fitness (Sinclair 1992) and therefore many proximate mechanisms may act singly, or together to influence dispersal. These include mate and resource competition, inbreeding avoidance and territory bequeathal (Lambin 1997; Perrin & Mazalov 1999; Berteaux & Boutin 2000; Ronce *et al.* 2001). Although migration (i.e., 2-way, seasonal movements) can also influence gene flow if mating is associated with the movements (Webster *et al.* 2002), I consider this as a separate

concept from dispersal. In several places in this thesis I use a specific context for dispersal where it is the one-way movement of individuals from their source breeding group to a new breeding group in a manner such that genetic exchange has occurred (Allendorf & Luikart 2007). As such, characterizing the movements of individuals, such as dispersal, can play a significant role in our understanding of populations by providing key information on how individuals are interconnected and thus structured relative to each other in a population.

As long lived, highly mobile and gregarious animals, bats are interesting animals to examine the dynamics of individuals in forming and maintaining populations. Population structure in temperate bats was first characterized using the demographic cohesiveness approach primarily based on banding studies (e.g., Griffin 1945; Beer 1955; Tuttle & Stevenson 1977). Although banding studies provided insight into movements and population structure, they fell out of wide use, particularly in North America, partly owing to the injury and death of animals (Hutterer *et al.* 2005; Ellison 2008). With advances in technology in the past 25 years, the characterization of the ecology of many species during the active summer period has shed light on many aspects of their populations, such as details of intra-specific resource use (e.g., Barclay 1991; Wilkinson & Barclay 1997; Broders & Forbes 2004), social structure (Kerth & König 1999; Willis & Brigham 2004; Senior *et al.* 2005; e.g., Garroway & Broders 2007) and reproductive phenology (Grindal *et al.* 1992; e.g., Feldhamer *et al.* 2001; Frick *et al.* 2010). Many species have now had population structure characterized from a reproductive cohesiveness approach using genetic techniques, although primarily as an assessment of structure among summer maternity colonies (e.g., Burland *et al.* 1999; Petit & Mayer

2000; Rossiter *et al.* 2000; Castella *et al.* 2001; Kerth & Morf 2004; Vonhof *et al.* 2008; Dixon 2011).

For many temperate bat species the autumn is a transition period where individuals migrate from summering to winter areas, mate and deposit fat stores for hibernation; how this season relates to their overall population structure remains poorly characterized. Several species that are sexually-segregated in the summer come together in early autumn in large mixed-sex aggregations to engage in swarming activity just prior to hibernation (Davis 1964; Humphrey & Cope 1976; Parsons *et al.* 2003; Glover & Altringham 2008). Swarming is the term used to describe the event of mass visitations by bats to underground sites where they engage in chasing and mating behaviours (Davis 1964). It is thought to be the primary mating period for many species (e.g., Fenton 1970; Veith *et al.* 2004; Rivers *et al.* 2005; Furmankiewicz & Altringham 2007). However, autumn swarming may also facilitate social interactions for information transfer regarding suitability of hibernation sites, knowledge of migration routes and may include the orientation of young-of-the-year to overwintering sites (Davis 1964; Fenton 1969; Parsons *et al.* 2003). If swarming is the primary mating period, then partially discrete breeding bat populations may be characterized around swarming sites (Rivers *et al.* 2005). Few studies have examined the role of swarming in structuring bat populations genetically and only in European species (Rivers *et al.* 2005; Furmankiewicz & Altringham 2007; Bogdanowicz *et al.* 2012).

Studies examining movements or social groups during swarming have been primarily limited to basic assessments of nightly capture rates of sexes and/or age groups to infer the types of interactions that may occur (e.g., Cope & Humphrey 1977; Rivers

2005; Glover & Altringham 2008; Piksa *et al.* 2011) although some detailed behavioural studies have been carried out (Barclay *et al.* 1979; Thomas *et al.* 1979). The details of how individual bats balance their activities and what resources they use during swarming are generally not as well characterized as summer or winter activities for most species. Some exceptions occur in documenting intra-specific variation in pre-hibernation fat deposition (Kunz *et al.* 1998; McGuire *et al.* 2009), characterization of roosting resources (Parsons & Jones 2003; Furmankiewicz 2008) and patterns of torpor use by males (Encarnação *et al.* 2004; Becker *et al.* 2013). However, these studies on their own represent only a small glimpse into the complex dynamics of the swarming season for bats.

Two wide ranging North American species where little is known of their swarming activities are *Myotis lucifugus*, the little brown Myotis and *M. septentrionalis*, the northern Myotis (van Zyll de Jong 1985; Broders *et al.* 2003; Naughton 2012). These species are year-round residents in Atlantic Canada and conform to a typical temperate hibernating seasonal cycle that includes swarming and hibernation at known sites within Nova Scotia and New Brunswick. In the summer, *M. septentrionalis* is a forest specialist species where it forages within the forest and typically roosts in trees (Foster & Kurta 1999; Jung *et al.* 2004; Henderson & Broders 2008). Female *M. septentrionalis* form maternity colonies and exhibit a fission-fusion social system where associating individuals regularly move among multiple interconnected groups (Garroway & Broders 2007; Patriquin *et al.* 2010). Males tend to roost solitarily also in trees (Broders & Forbes 2004; Jung *et al.* 2004; Safi & Kerth 2007). *Myotis lucifugus* is a more generalist species, roosting in buildings and trees and foraging in or along more open areas such as

ponds, wetlands and forest gap/edge margins (Anthony & Kunz 1977; Fenton & Barclay 1980; Broders & Forbes 2004). Female *M. lucifugus* also form maternity colonies (Humphrey & Cope 1976; Fenton & Barclay 1980) and recent work suggests forest-dwelling female *M. lucifugus* also exhibit dynamic changes in roost use and group size (Olson & Barclay 2013). However, social structure has not been quantified in forest- or building-dwelling maternity colonies of *M. lucifugus*. Male *M. lucifugus* roost singly, in the same structure as females (buildings) or with other males (Davis & Hitchcock 1965; Humphrey & Cope 1976; Broders & Forbes 2004). Subtle differences between the species in morphology and echolocation call characteristics relate to their different foraging and potentially migratory dynamics (Fenton & Bogdanowicz 2002; Ratcliffe & Dawson 2003). Comparatively, more is known about *M. lucifugus* compared to *M. septentrionalis* likely owing to their more conspicuous nature in occupying human structures and large distributional range.

1.2 THESIS GOAL AND OBJECTIVES

The goal of this thesis was to explore the dynamics of temperate bats as they interact during the swarming period and, specifically, how the activities they engage in during swarming may influence population structure. Although both species share many general life-history characteristics related to reproductive and seasonal cycles such that the two species may be quite similar, the subtle differences between them in ecomorphology and behaviour may result in differences in swarming activities. Thus, this study looks for general concordance in patterns that are perhaps common for many

temperate swarming bats, and attempts to characterize the subtle nuances of each species in terms of their swarming dynamics.

I first studied the interactions among individuals using an approach aimed at understanding the demographic cohesiveness of the two species. Here bats were captured and tagged at different swarming sites that were subsequently monitored, to characterize the intraspecific patterns in the frequency and extent of re-use of autumn swarming sites by individual bats. I also explored the social dynamics of swarming bats, as the intraspecific associations at swarming sites, and tested predictions of hypotheses on the functions of swarming (gene flow and maternal information transfer). Second, I studied the reproductive cohesiveness of bats during swarming by characterizing population genetic structure of *M. lucifugus* sampled at different swarming sites in eastern Canada. Finally, I took a broad perspective in examining population genetic structures across order Chiroptera to examine what correlates of dispersal at the species level may predict the degree of genetic structuring observed across bats. The specific objectives addressed as chapters in this thesis were:

Chapter 2: I studied autumn swarming activity of *Myotis lucifugus* and *M. septentrionalis* to test predictions based on intersexual variation in behaviours to maximize fitness. Capture-mark-recapture surveys were conducted at swarming sites to characterize the nature and extent of intersexual variation in behaviour during swarming to determine if males spend more time swarming by visiting swarming sites more often than females.

Chapter 3: Given the social nature of bats during the summer, and a high degree of swarming site fidelity for some individuals, there is a high potential for social

interactions to occur during swarming beyond simple aggregation. I investigated the occurrence of social groups for different age and sex classes of *M. lucifugus* and *M. septentrionalis* during swarming. This included examining if preferred associations occur among males among nights. I also tested if adult female and young-of-the-year groups of *M. septentrionalis* are composed of highly related individuals. The later would suggest mother-offspring pairs are maintained during swarming consistent with the maternal guidance hypothesis.

Chapter 4: I characterized genetic variation and population genetic structuring in *M. lucifugus* sampled from 15 swarming sites in south-eastern Canada. Contemporary gene flow was examined using nuclear markers and historical population structure and demography was examined using mitochondrial DNA. I assessed differences in the degree of structuring between the sexes to evaluate asymmetry in gene flow between the sexes.

Chapter 5: I compared five dispersal extent predictors (morphological and ecological) with population genetic structure among 43 species of bats by conducting a comparative analysis based on data from the literature. Owing to the co-variance that many of these traits exhibit, I used a statistical framework to account for this co-variance that has not been previously examined.

Chapters 2, 3, 4, and 5 are written as independent manuscripts for publication. In all chapters I played the primary role in all aspects of research from literature review and development of ideas for the research design, through to the planning and carrying out of fieldwork, data analysis and writing up the publications and the thesis. My supervisor and members of my committee provided expertise in conducting various aspects of the

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CHAPTER 2 MAXIMIZING MATING OPPORTUNITIES: HIGHER AUTUMN SWARMING ACTIVITY IN MALE VERSUS FEMALE MYOTIS BATS

2.1 ABSTRACT

Many animal taxa exhibit intersexual differences in sociality and resource selection that can result in variation in energy allocation budgets. For example, asymmetry of reproductive energetics between the sexes can lead to variation in behaviour to maximize their lifetime reproductive success. Temperate bats are known to show marked intersexual differences during the summer when sexual segregation occurs. Differences in activities engaged in during the mating period of autumn swarming are likely but many questions remain. I studied autumn swarming activity of little brown and northern Myotis bats (*Myotis lucifugus* and *M. septentrionalis)* in Nova Scotia, Canada to test predictions based on intersexual variation in behaviours to maximize fitness. I conducted capture-mark-recapture surveys at swarming sites to characterize the nature and extent of intersexual variation in behaviour during swarming. Relative to females, males: A) occurred in disproportionally large numbers; B) had longer swarming seasons which wholly overlapped that of the female swarming season; and C) accounted for a disproportionately large number of the recaptures at the swarming sites suggesting they had returned more frequently. No movements among swarming sites were detected for either species. Activity at swarming sites was highest in the first four weeks for both species. For *M. lucifugus*, this period was dominated by a disproportionally large number of transient individuals than later in the season. As predicted, males engaged more frequently in swarming activities than females which likely reflect males maximizing

opportunities for mating. Although their activities overlap during this period, the differences suggest sex-specific activity budgets and it is likely that within each sex, individuals of each group reconcile energetic constraints differently to maximize fitness.

2.2 INTRODUCTION

For many animal taxa, marked intersexual differences occur in degree of sociality and resource selection strategies and these differences can lead to significant differences in activity budgets. For example, male and female Cepero's ground-hoppers (*Tetrix ceperoi*) use different substrates for perching and spend different amounts of time resting, foraging and on mating behaviours (Hochkirch *et al.* 2007). Forest birds show intersexual differences in foraging site and time allocations (Holmes 1986). Lastly, male and female grey seals (*Haliochoerus grypus*) have striking differences in home range locations before and after the breeding season (Breed *et al.* 2006). Intersexual differences are largely thought to reflect the asymmetry of reproductive energetics and parental investment (i.e., reproductive cost) that leads to different strategies to maximize their own expected lifetime reproductive success (Trivers 1972).

In mammals, sexual segregation in spatial organization or resource use in the non-breeding season is common for species where the sexes live in separate groups or as solitary individuals (Ruckstuhl & Neuhaus 2000). Several proximate level, social and ecological hypotheses have been proposed to explain sexual segregation (reviewed in Main 2008). For example, the activity budget hypothesis proposes that asynchrony in foraging patterns can lead to sexual segregation (Conradt 1998; Ruckstuhl & Neuhaus 2002). These hypotheses have been examined most thoroughly in ungulates where

sexual size dimorphism is intrinsic to the segregation mechanisms stemming from dimorphic energetic costs (Ruckstuhl & Neuhaus 2000; Bowyer 2004). Although much debate exists, an alternative hypothesis that is generally favoured is the reproductive strategy hypothesis because it is applicable across taxa and operates at an ultimate level where multiple environmental, behavioural and physiological factors explain intersexual differences (Main 2008). During the breeding season, the degree of sexual segregation decreases to varying extents across taxa although many of these same factors continue to act differentially on the sexes in promoting individual fitness which includes facilitating courtship and mating behaviours. Regardless of where individuals are in the seasonal and reproductive cycles, they must continue to strategically allocate time to specific activities, or the timing of these activities to optimize the balance between costs and benefits to achieve higher fitness.

Male mammals generally maximize fitness by securing many mating opportunities (Bateman 1948; Andersson 1994), potentially at the expense of other activities such as foraging (Miquelle 1990; Alberts *et al.* 1996). This strategy may be possible due to physiological mechanisms such as metabolic compensation that may also allow males to reduce foraging time during the mating season by reducing their metabolic resting rate and lowering their energetic costs (Becker *et al.* 2013). Female mammals are physiologically limited in the number of offspring they can produce, so they maximize fitness by investing more energy into fewer offspring and do not need to secure as many mating opportunities as males (Andersson 1994). Thus, during the breeding season, mating strategies and activities may differ for males and females, which has been shown

in a diversity of taxa including rodents (Michener 1998), ungulates (Tettamanti & Viblanc 2014) and pinnipeds (McCann 1983).

Male and female temperate bats show sexual segregation during the summer (Senior *et al.* 2005), where intersexual differences are well documented in foraging activity (Wilkinson & Barclay 1997; Kerth & Morf 2004; Dietz & Kalko 2007), roost selection (e.g., Broders & Forbes 2004; Barclay & Kurta 2007) and use of torpor; the latter two being tightly linked to microclimate preferences (Willis 2006; Boyles 2007). Females incur higher energetic costs during the reproductive period (Kurta & Kunz 1987; Kurta *et al.* 1990; Mclean & Speakman 2000) which occurs during the temperate spring and summer (Racey & Entwistle 2000). During this same period, males incur relatively lower energetic costs associated primarily with their own self-maintenance (Racey & Entwistle 2000). However, as the summer progresses spermatogenesis progresses to a peak in late summer in preparation for mating which may impose some energetic costs (Wimsatt 1969), albeit lower than that experienced by reproductive females. During the autumn, many hibernating temperate bats migrate from summering areas to winter areas, mate, and deposit fat stores for hibernation; several species form large mixed-sex aggregations of individuals within which they engage in swarming activities (Parsons & Jones 2003; Rivers *et al.* 2005; Furmankiewicz & Altringham 2007). During swarming, bats congregate at underground sites prior to hibernation and engage in chasing and mating behaviours. It is thought to be the primary mating event for many species occurring primarily in autumn (e.g., Kerth & Morf 2004; Veith *et al.* 2004; Rivers *et al.* 2005) but also in spring for some species (Furmankiewicz *et al.* 2013). Visits to swarming sites by individuals and species are highly variable and may occur on an hourly

or nightly basis but are not well characterized (Fenton 1969; Humphrey & Cope 1976; Rivers *et al.* 2006; Furmankiewicz 2008). Swarming bats may also gather or exchange information regarding suitability of hibernation sites, knowledge of migration routes or orient young-of-the-year to such sites (Davis 1964; Fenton 1969; Parsons *et al.* 2003b).

Compared to the summer, very little is known about the intersexual variation in behaviour and resource use of bats during the swarming season. Literature documenting resource use (e.g., roosts) is limited (although see Parsons & Jones 2003; Furmankiewicz 2008), likely owing to the difficulty in tracking highly vagile animals during the migratory period where they roost away from swarming sites. However, physiological studies quantifying the energetics of fat storage prior to hibernation characterize intersexual differences in the timing patterns of fat deposition, which suggests the possibility of similar differences in activities undertaken during the swarming period (Kunz *et al.* 1998; Ingersoll *et al.* 2010). Further, a large observed male bias during swarming (e.g., Cope & Humphrey 1977; Thomas *et al.* 1979; Rivers *et al.* 2006; Piksa 2008), may suggest differences between the sexes in the seasonal timing of use and time spent at swarming sites. Lastly, evidence of intersexual differences in dispersion on the landscape with respect to elevation has been documented which may reflect energetic demands and foraging efficiency trade-offs of females that precludes the extensive use of high elevation swarming sites by those females that are energetically stressed (Piksa *et al.* 2011). These studies collectively suggest energetic constraints may underlie the activities engaged in by each sex during swarming. Understanding these intersexual differences may therefore provide insight into optimal fitness strategies of each sex that may in turn lead to insights into population dynamics.

In this study, I conducted capture-mark-recapture surveys at swarming sites to establish if intersexual differences in visits to swarming sites, as a proxy measure of swarming activity, were present in two temperate insectivorous bats species. The little brown Myotis (*Myotis lucifugus*) and the northern Myotis (*M. septentrionalis*) are widely distributed temperate insectivorous species of North America. Both species make regional migrations from summering areas to winter hibernacula and are known to swarm during the autumn (Fenton & Barclay 1980; Caceres & Barclay 2000) and are the only two species with significant year-round populations in this area of Canada (Broders *et al.* 2003; Naughton 2012). In the summer, *M. septentrionalis* is a forest specialist species where it forages within the forest and typically roosts in trees (Jung *et al.* 2004; Henderson & Broders 2008). *Myotis lucifugus* is a more generalist species, roosting in buildings and trees and foraging in or along more open areas such as ponds, wetlands and forest gap/edge margins (Anthony & Kunz 1977; Fenton & Barclay 1980; Broders & Forbes 2004). Although more is known about *M. lucifugus* compared to *M. septentrionalis*, including swarming activities, the similarity of the general life-history characteristics related to reproductive and seasonal cycles support similar expected intersexual differences in both species.

I hypothesized that differences between the sexes in energy budgets would lead to different strategies in the frequency and timing of activities during swarming. Specifically, males having spent the summer mainly on self-maintenance, would allocate more energy to mating activities during swarming to secure as many mating opportunities as possible. In contrast, reproductive females having spent the summer rearing young, may not need as many copulations to maximize fitness and should allocate more

activities to rebuild their own energy stores in preparing for hibernation and less to mating activities. This hypothesis leads to four predictions that can be tested again my mark-recapture data: that for bats captured at swarming sites (1) there would be a male bias resulting from male bats spending more time at swarming sites and (2) the swarming season would be longer for males than females. In recaptures of bats I predicted (3a) a higher proportion of male recaptures than females and (3b) individual males to be recaptured, on average, more often than females because to maximize copulations males should stay longer at swarming sites or visit more frequently than females. Lastly, (4) a greater proportion of male recaptures would be at the site of initial capture compared to females as males should have a higher swarming site fidelity using fewer swarming sites to allow them to visit them more frequently.

2.3 METHODS

2.3.1 Capture and tagging

Bats were captured at 6 swarming sites in Nova Scotia, Canada (Figure 2.1, Table 2.1), during the autumn and spring seasons of 2008 to 2011 using harp traps (Austbat Research Equipment, Lower Plenty, Victoria, Australia) or mist nets (Avinet, Dryden, New York). Sites were separated by distances ranging from 27.9 to 98.9 km. Individuals were identified to species and sex with age (young-of-the-year YOY; or adult) determined by examining the degree of ossification and shape of the epiphyseal growth plates of the metacarpals (Anthony 1988). Depending on the nightly capture numbers, I tagged all or a subset of captures with permanent, passively integrated transponders (PIT

tags; Trovan ID 100, EIDAP Inc., Sherwood Park, Alberta) for individual identification. PIT-tags are microchips, encased in biocompatible glass, that are activated when they pass within range of a reader enabling each unique PIT-tag code to be recorded with a time/date stamp. Following injection of the tag, the injection site was sealed using surgical glue (Torbot, Cranston, Rhode Island) and bats were held for a period of 5-10 minutes in separate bags to ensure the injection site was sealed and that bats were active and ready for flight following release. All bats were released prior to sunrise with a mean total handling time from capture to release of 42 (10-180) minutes. Methods for the capture and handling of bats were approved by the Saint Mary's Animal Care Committee (Protocols 09-24, 10-11, 11-18) under yearly issued permits from the Nova Scotia Department of Natural Resources.

An emergent fungal pathogen, *Pseudogymnoascus desctructans,* which causes white-nose syndrome (WNS), has resulted in both study species suffering dramatic recent declines in their populations in eastern North America (Blehert *et al.* 2009; Turner *et al.* 2011; Minnis & Lindner 2013). Therefore, I used the most up-to-date precautionary WNS decontamination protocols provided by the US Fish and Wildlife Service to try and minimize potential spread of fungal spores via my capture and handling methods (available from http://whitenosesyndrome.org/topics/decontamination). In the late winter of 2010/2011, WNS was detected in Nova Scotia, although not yet at the study sites. However, to reduce the chance of transmission from my work, I reduced the number of active trapping and tagging sessions in 2011 with no tagging in the spring.

Recaptures of bats were assessed during the autumn swarming period (2009-2011) via three methods. First, in all three years I conducted active trapping sessions at

swarming sites (autumn only) to actively hand-scan all captured bats for PIT-tags. Second, in 2010 I set up harp traps with PIT-tag antenna fitted in holes I cut in the sides of the harp trap bags (PIT-harp trap). This facilitated bats being captured and passively scanned for a tag as they escaped out through the holes housing the antenna. These modified PIT-harp traps were left out over multiple nights (6-24) at secure sites to passively scan bats, and traps left out >1 week were checked minimally on a weekly basis. Prior to deploying the PIT-harp traps without personnel present, I conducted trials where I observed the behaviour of bats in the traps and confirmed that captured bats found the holes quickly and escaped. Third, in 2011 I installed PIT-tag antenna in temporary mesh gates constructed at 4 swarming site entrances to passively scan bats as they entered underground sites. The mesh allowed air flow into the sites to minimize any effect of the gates on hibernacula microclimates. Therefore, recaptures encompass PIT-tagged bats detected via one of the three methods. I discontinued using the passive PIT-harp traps in 2011, because they had the potential to be a vector for the spread of WNS. Active trapping and scanning was conducted at sites in autumn 2011 where gates could not be constructed with a few sessions still occurring at gated sites.

2.3.2 Analyses

Young-of-the-year were excluded from analyses because they may represent another intraspecific group with distinct activity patterns. Therefore, the data do not represent total captures at each site but rather a subset composed of in-hand sex identified adults. I used *G*-tests to examine differences in swarming activity measures between male and female bats because of their additive properties which allow for more elaborate

experimental designs, such as replications, to be tested (Sokal & Rohlf 1995; Macdonald 2009). For all tests, significance was considered at $\alpha = 0.05$.

To determine if there was male-bias in bats using swarming sites (prediction 1), I compared the proportions of adult male and adult females captured during autumn swarming, for each species, using *G*-tests at three levels. I first tested if the overall proportion of males was greater than that of females across all sites, for each year, using a replicated goodness of fit *G*-test. Second, I tested the proportions of each sex at the site level, across all years, using the same procedure as above. Lastly, I conducted an unplanned test of the homogeneity of replicates *G*-test using the simultaneous test procedure, to examine if the proportions of each sex captured at swarming sites changed during the swarming season. Here, I classified the swarming season into 8, week-long periods for the capture data that began on August 11 (the earliest survey date of all years). Sites were not sampled on the same night every year, or with equal frequency owing to variability in weather among years and to meet other concurrent study objectives. Therefore, I pooled sampling nights across sites and across years for each week-long period with the sum of captures of each sex during each week-long period used for analysis. The mean number of sampling nights included in each period was 7.8 (range: 4-13). Sample weeks were ordered from highest to lowest proportions of males observed. Varying sets of weeks, starting from the largest and from the smallest proportion of males observed, were examined in sequence for homogeneity in the magnitude of the observed proportions until heterogeneous sets were identified. These heterogeneous sets are indicative of significant changes in the proportions of each sex.

Small sample sizes resulting from a limited number of swarming seasons and the necessary exclusion of the reduced 2011 capture season due to the appearance of WNS, precluded statistical assessment of season length between the sexes (prediction 2). However, I qualitatively present data trends as supplementary data to inform my research question. First I restricted the data to one site, Rawdon, which was sampled in every week of the 8 week long swarming period in 2009 and 2010. I calculated the minimum season length for each sex by considering the number of nights between the first and last identified capture of each sex at this site for each year. As a second measure of season length at the site, I used recapture data of tagged individuals from 2010 when I had a PIT-harp trap deployed for most of the season at the site from 14 August to 19 October and concurrently actively trapped bats on 9 nights during this period. In 2011 I had continuous nightly PIT-tag gate sampling of tagged individuals entering or exiting the site from 28 April through to 8 November 2011. I first excluded any individuals from the dataset that were detected using the site in June or July as I considered these individuals as 'local summer residents' of the surrounding swarming area. Spring work in 2009 and 2010 suggested bats emerge from winter hibernation in this region during late April through the month of May. I then calculated the minimum season length of recaptured, 'autumn transient' bats by considering the number of nights between the first and last recapture detection of each sex between the dates of 01 August and 31 October, 2011.

To test if the recapture rates for males were greater than that of females (predictions 3a and 3b); I compared the total number of individual males recaptured to that of females using only those individuals that were adults at the time of recapture. Recaptures were classified on a per night basis where individuals were detected at least

once. Since there were many instances of only 1 sex recaptured per site for a given season, I pooled the data over sites and years testing total male and female recaptures. I used a 2-sample equality of proportions test to determine if the proportion of tagged males that were recaptured was greater than the proportion of tagged females that were recaptured. I also examined recaptures for 2011 at Rawdon at the individual level where I summed the total number of recaptures detected per adult and compared the number recaptures per individual for males and females using a Mann Whitney *U*-test (Sokal & Rohlf 1995). For visualization of the data I classified them into 4 categories of recapture histories; 1,2,3 and >4 recaptures because there were few recapture histories that exceeded 4 recapture events for females of both species.

As a second assessment of weekly seasonal swarming activity patterns derived from capture data, I compared the changes in the proportion of male and female captures over the season from pooled capture data to that of recapture data collected in 2011 from Rawdon. I reduced the Rawdon 2011 recapture dataset to encompass 11 August to 05 October, for the same weekly intervals, and summed the total bats recaptured of each sex in each week. Finally, no recaptures of males or females tagged at the swarming sites were detected using different swarming sites precluding any analysis for prediction 4.

2.4 Results

From 2008-2011 (spring and autumn seasons), I tagged 865 *M. lucifugus* (220 females, 645 males; Table 2.2) and 482 *M. septentrionalis* (167 females, 315 males). No inter-swarming site movements were detected as all recaptures were at the site of capture. Over the three autumn swarming seasons, I captured and identified in-hand (but not

necessarily tagged) 725 adult *M. lucifugus* (262 females, 432 males) and 387 adult *M. septentrionalis* (142 females, 245 males) at the six swarming sites (Table 2.2). The overall proportions of adult males and adult females captured during autumn swarming had a large male-bias (prediction 1). In examining by each season (year), there was a male bias observed for both *M. lucifugus* ($G_T = 66.3$, $P < 0.001$) and *M. septentrionalis* ($G_T = 30.0$, $P < 0.001$; Table 2.3; Table 2S1 Supplementary Material). A similar male-bias was detected when data were examined by site (G_T MYLU= 99.9, $P < 0.001$ and G_T MYSE= 63.4, $P <0.001$; Table 2.3; Table 2S2 Supplementary Material). Despite the overall male bias, for *M. lucifugus*, the magnitude of male bias differed among years and among sites as shown by the heterogeneity *G*-test (G_H years = 16.5 $P < 0.001$ and G_H sites= 50.1, $P < 0.001$). In *M. septentrionalis*, a similar magnitude of male bias was detected at all sites and in each year as shown by the non-significant heterogeneity *G*-test (G_H years = 2.3 $P = 0.325$ and G_H sites= 7.9, $P = 0.159$).

Variability in the degree of male bias observed was found for different weeks in the swarming season in *M. lucifugus*. The simultaneous test procedure indicated that weeks 2, 7 and 8 had a higher proportion of females relative to week 1 which had a larger degree of male bias (Figure 2.2). From the recapture data collected in 2011, I detected more females in weeks 2,3 and 7 supporting general trends of the capture data (Figure 2.3). Notably, total recaptures of both male and female *M. lucifugus* were dramatically reduced during weeks 2 and 3 despite this being the period of high captures from capture surveys. For *M. septentrionalis* from capture data, weeks 2,3,4 and 6 had a higher proportion of females compared to the latest period of the swarming season in week 8. This reflects that over the three swarming seasons sampled, no adult female *M.*

septentrionalis were ever captured and identified during week 8. Recapture data from 2011 for *M. septentrionalis* showed a similar seasonal pattern to that characterized by capture data with more females detected during weeks 2 through 5 and then slowly declining through to a larger degree of male bias in weeks 7 and 8.

Trends from data collected at Rawdon suggest that male bats may have had longer swarming season lengths than females (prediction 2). From in-hand capture data, adult male *M. lucifugus* had estimated minimum season lengths of 53 and 51 days in 2009 and 2010, respectively. For adult females, minimum season length was estimated at 48 days in both years. Male *M. septentrionalis* had estimated minimum season lengths of 38 and 51 days in 2009 and 2010, respectively. Female *M. septentrionalis* had estimated minimum season lengths of 27 and 37 days in 2009 and 2010, respectively. From recapture data of PIT-tagged bats collected during 2010, minimum season lengths were estimated at 58 and 36 days for male and female *M. lucifugus*, respectively. Minimum season lengths for *M. septentrionalis* in 2010 were estimated at 66 and 26 days for males and females, respectively. For the 2011 season where passive monitoring occurred from 01 August to 31 October 2011, male and female *M. lucifugus* had minimum season lengths of 79 and 65 days respectively. Minimum season lengths for male and female *M. septentrionalis* in 2011 were estimated at 74 and 57 days, respectively.

For *M. lucifugus*, a significantly higher proportion of recaptured males were detected than females (Prediction 3a: 17% male versus 6.3% female recaptures; $\chi 2 = 14.1$, $df = 1$, $P < 0.001$). The large male-biased pattern was the same for *M. septentrionalis* (18% male versus 12% female recaptures; $\chi 2 = 3.3$, $df = 1$, $P = 0.034$). Individual male *M. lucifugus* recapture histories ranged from detections of 1 to 19 times

and females, 1 to 4 times. Individual male *M. septentrionalis* recapture histories ranged from 1 to 22 recaptures and females, 1 to 11 recaptures. Although the range of the number of recaptures for individual females was smaller compared to males, the difference was not statistically significant for *M. lucifugus* ($U = 702$, $P = 0.813$) or *M. septentrionalis* ($U = 403.5$, $P = 0.079$; Figure 2.4).

2.5 Discussion

In line with my predictions, I found that male bats had higher autumn swarming activity compared to female bats of both study species. A male bias was found in captures across all sites and in all swarming seasons. Previous studies of *M. lucifugus* detected this male bias during swarming (Fenton 1969; Humphrey & Cope 1976; Schowalter 1980) and male bias has been shown in many European swarming species (Kerth *et al.* 2003; Rivers *et al.* 2006; Furmankiewicz 2008; Glover & Altringham 2008; Piksa 2008). Taken together, these data support differences between male and female swarming behaviour where these differences are observed in multiple species that face similar seasonal constraints during swarming. They lend support to the assertion that a large male bias is suggestive of intersexual variation in strategies to maximize fitness during this specific season rather than reflecting population level sex-ratios, although general sex-ratios in bats are not well known at this level. Although there are differences in the timing of use of swarming sites among species as noted in this and other studies (e.g., Schowalter 1980; Parsons *et al.* 2003a; Glover & Altringham 2008), a general large

male bias is detected regardless of when the peak of swarming activity of each species occurs.

Despite the overall male bias, differences in the degree of male bias can be found at varying temporal scales for swarming bats. For example, Piksa (2008) documented subtle differences in the nightly timing of swarming *M. mystacinus* at a high elevation site where, later in the night, influxes of females were captured compared to early captures in the evening. I did not sample continuously throughout each night to be able to assess if this nightly variation occurred at my study sites since trapping ceased periodically to fully process already captured bats and minimize overall handling time. Within a swarming season, I found a peak of female activity occurred early to mid-swarming season for *M. septentrionalis* and for *M. lucifugus*. I suggest this reflects the different activities of each sex during this period. Females appear to visit less often, and concentrate their activities in a shorter time window, possibly for mating, compared to males that likely visit more often to maximize potential copulations. Females may spend the majority of their time away from swarming sites allocating more time to foraging to rebuild depleted energy stores having reared young in the summer. Mid-season peaks in female visits to swarming sites have been found in other swarming species in Europe (Glover & Altringham 2008; Piksa 2008). The overall reduced female swarming season that is timed in the middle of the active season, further support the assertion that females visit swarming sites less often and may spend less time engaging in swarming activities than males. Further, the seasonal distribution of female activity is contained within the male activity distribution suggesting male activity blankets all female activity to maximally overlap female swarming.

In *M. lucifugus*, a second smaller peak of female activity was detected at swarming sites late in the season. I propose that female *M. lucifugus* may initially show up in the first wave in late August at the site to mate as has been found in other areas (Thomas *et al.* 1979; McGuire *et al.* 2009), and possibly assess the site for suitability for hibernation and then leave. The second peak would then primarily represent females returning to a site for immergence into hibernation. As a forest specialist, female *M. septentrionalis* may use autumn forest roosting and foraging resources in the surrounding area of the swarming site such that there is less of a gap between mating and/or assessment of sites for hibernation and actual selection and immergence at the site for hibernation. Therefore, their activity at swarming sites appears more continuous. Detailed tracking studies would be required to assess if differences in the timing of migration to sites and the location of day roosts differs between females of each species.

If female bats maintain distinct pulses of high swarming activity year after year, then males can potentially cue in on these female activity peaks to maximize copulations when more females are available. In examining recapture records where I had continuous scanning coverage in 2011, I found that for *M. septentrionalis*, the levels of activity by tagged males and females closely matched the seasonal pattern of activity from the capture data. Although not an entirely independent data set, this congruence suggests female *M. septentrionalis* may concentrate their swarming activity at approximately the same time each year and that at least some males appear to track this. For *M. lucifugus*, the pattern is partially discordant between the two data sets where the second peak of females is present near the end of the season, but notably, a large peak in female and male recaptures corresponding to the female capture peak was not detected. I

hypothesize that this is a period of high transiency by *M. lucifugus* since captures at swarming sites remain high during this period which shows that bats are still engaging in swarming activities during this time. However, these captures appear to be dominated by transient individuals rather than more local bats to the site and thus this may reflect a period of migratory or dispersal movements among swarming sites. The recapture of a few males and females during this time suggests at least some individuals show a degree of swarming site fidelity. There are several observations of *M. lucifugus* roosting in atypical locations and structures following summer colony breakup (early to mid-swarming; Davis & Hitchcock 1965; Schowalter 1980; Riskin & Pybus 1998). Further, a recent analysis of banding records found that *M. lucifugus* captured during swarming had the highest movement rates of all individuals studied (summer, winter or swarming; Norquay *et al.* 2013). Together these studies support this period as being one of high movement and transitioning by individuals.

Given that the maximum known life spans are at least 18.5 (*M. septentrionalis*; Caceres & Barclay 2000) and 34 years (*M. lucifugus*; Davis & Hitchcock 1995), it may be that some males learn to exploit this temporal peak in female abundance. This could include older more experienced males or those that roost near females in the summer that can track their movements to swarming areas. I speculate that this could have potential consequences for male individual reproductive success. The mating system for *M. lucifugus* (Thomas *et al.* 1979) and possibly for *M. septentrionalis*, is characterized as promiscuous, and males could have higher reproductive success if they match their activity to when more females are available and are thus able to secure more copulations. Reproductive skew in the number of offspring sired by males or male lineages has been

shown for *M. lucifugus* where mating during hibernation (Watt & Fenton 1995) or cryptic female choice (Wilkinson & McCracken 2003) have been posited to potentially explain this pattern.

Recent work on swarming *M. daubentonii* demonstrated that patterns of paternity at maternity colonies reflected resource availability during the summer (Senior *et al.* 2005; Angell *et al.* 2013). At areas of high roost and foraging resource availability, few males roosted in maternity colonies and most paternity was assigned to swarming males. Conversely, at low resource areas, more males roost with females where they were thought to potentially contribute thermoregulatory benefits to the colony, and they showed higher probability of fathering offspring. This proximity suggests that they gained access to females at the colony for copulations in addition to swarming copulations which has been documented in *M. lucifugus* (Humphrey & Cope 1976). Further work would be required to assess if this occurs frequently in *M. lucifugus* or *M. septentrionalis* as it too could explain reproductive skew. More broadly however, the *M. daubentonii* and my own work collectively suggest the possibility of different temporal, spatial or social aggregating mechanisms acting on female bats. These in turn may permit different male mating strategies to occur as individual males adapt in response to females in maximizing their own individual fitness.

The second component of my study using recapture records of tagged bats further supports the prediction that males have higher swarming activity compared to females. Although the inherent male bias in captures resulted in me tagging more males, after correcting for this, the total recaptures of females were still lower than that of males. This finding mirrors that of swarming *M. nattereri* in the UK where fewer females were

recaptured compared to males (Rivers *et al.* 2006). At the individual level I did not find differences in the recapture histories of males and females. However, I believe this is a result of limitations in tracking individuals continuously with an equal sample effort throughout the study and from the generally low recapture rate of tagged individuals. As is the case with many swarming tagging studies (Humphrey & Cope 1976; Rivers *et al.* 2006; Norquay *et al.* 2013) my overall recapture success was low where even among those that were recaptured, many were only recaptured once during the entire study. I believe this shows the high degree of mobility of these species during this time. Although I did not detect any inter-swarming site movements, I was not able to monitor all 6 sites continuously for the three seasons and there are many other swarming sites in Nova Scotia that I did not monitor (Moseley 2007; Randall & Broders 2014). A rich mining history in Nova Scotia combined with many natural geological formations that could contain caves in the province (i.e., gypsum; Davis & Browne 1996; NSDNR 2009), means there is high potential for many other underground swarming and hibernation sites to exist that I was not aware of. Since the study was restricted to 3 years of monitoring relative to the long lifespan of individual bats, age-related factors determining the tendency or frequency of movements of individuals among sites over a lifetime may also play an important role in the swarming dynamics of these species.

Capture and tagging data have inherent biases due to higher likelihood of capturing the most mobile, or easily trapped individuals (Biro & Dingemanse 2008), which may have influenced my study. Also, my recapture survey effort differed by the method used (capture vs. passive detection) and varied throughout the study which may also have impacted detection of recaptures. However, I believe the general concordance

of the various metrics via both methods, despite the inherent limitations and biases, support a clear signal of males exhibiting higher swarming activities compared to females. Since other studies of swarming species show similar trends (e.g., Fenton 1969; Rivers *et al.* 2006; Glover & Altringham 2008; Piksa 2008) I believe these results collectively demonstrate that male and female bats do show intersexual differences in swarming activities.

In conclusion, I have shown that intersexual differences in activities occur for two temperate swarming species of bats in the timing and frequency of swarming site visitations. During autumn swarming, males are more abundant at sites, and spend more time individually and collectively over the season visiting swarming sites compared to females. Although their activities overlap during this period, male activity may be strongly determined by female activity. This work shows important intersexual differences that may provide insight into optimal fitness strategies of each sex that are important to understand in characterizing higher level population dynamics. The differences in activities may further suggest that sexual segregation may occur in day roosting or foraging areas used despite the eventual meeting at swarming sites for mating and other activities. Since little is known of the resources bats use (foraging and roosting) and the movements they make (e.g., routes used and frequency of) during this time period, future work characterizing these aspects may provide additional insights into the intersexual differences among swarming bats.

Table 2-1 Number of in-hand identified, adult *M. lucifugus* and *M. septentrionalis* bats captured at 6 swarming sites in Nova Scotia, Canada, (2009-2011), by location.

	Map	Sample	*M. lucifugus*			*M. septentrionalis*		
Site	Code	Nights	Males	Females	Total	Males	Females	Total
Cave of the Bats	CVB	6	25	6	31	41	21	62
Cheverie Cave	CHC	6	38	25	63	14	17	31
Hayes Cave	HAY	11	105	112	217	26	19	45
Lake Charlotte Mine	LKCH	5	37	26	63	24	7	31
Lear Mine	LEAR	9	65	46	111	31	17	48
Rawdon Mine	RAW	26	187	53	240	109	61	170
Total		*63*	*457*	*268*	*725*	*245*	*142*	*387*

Table 2-2 Number of individual *Myotis lucifugus* and *M. septentrionalis* tagged and later recaptured, by sex (M = males, F = females) at 6 swarming sites in Nova Scotia (2008-2011).

	M. lucifugus				*M. septentrionalis*			
	Tagged		Recaptured		Tagged		Recaptured	
	M	F	M	F	M	F	M	F
Cave of the Bats	49	9	0	0	27	18	0	0
Cheverie Cave	33	21	1	0	16	15	1	1
Hayes Cave	135	98	2	0	34	24	1	0
Lake Charlotte Mine	53	22	15	2	29	6	6	0
Lear Mine	54	23	7	4	42	25	3	1
Rawdon	321	47	114	13	167	79	71	26
Total	*645*	*220*	*139*	*19*	*315*	*167*	*82*	*28*

Table 2-3 *G* test statistics and significance for heterogeneity G (G_H), pooled G (G_P) and total G (G_T) to test for a male bias in adult captures at swarming sites in Nova Scotia for *M. lucifugus* (MYLU) and *M. septentrionalis* (MYSE), 2009-2011. Calculations were performed by pooling the data and testing for a) site differences and b) yearly seasonal differences.

	By site						**By Year**					
	MYLU	*df*	*P* value	MYSE	*df*	*P* value	MYLU	*df*	*P* value	MYSE	*df*	*P* value
G_H	50.1	5	<0.001	7.9	5	0.159	16.5	2	<0.001	2.3	2	0.325
G_P	49.8	1	<0.001	55.5	1	<0.001	49.8	1	<0.001	27.7	1	<0.001
G_T	99.9	6	<0.001	63.4	6	<0.001	66.3	3	<0.001	30.0	3	<0.001

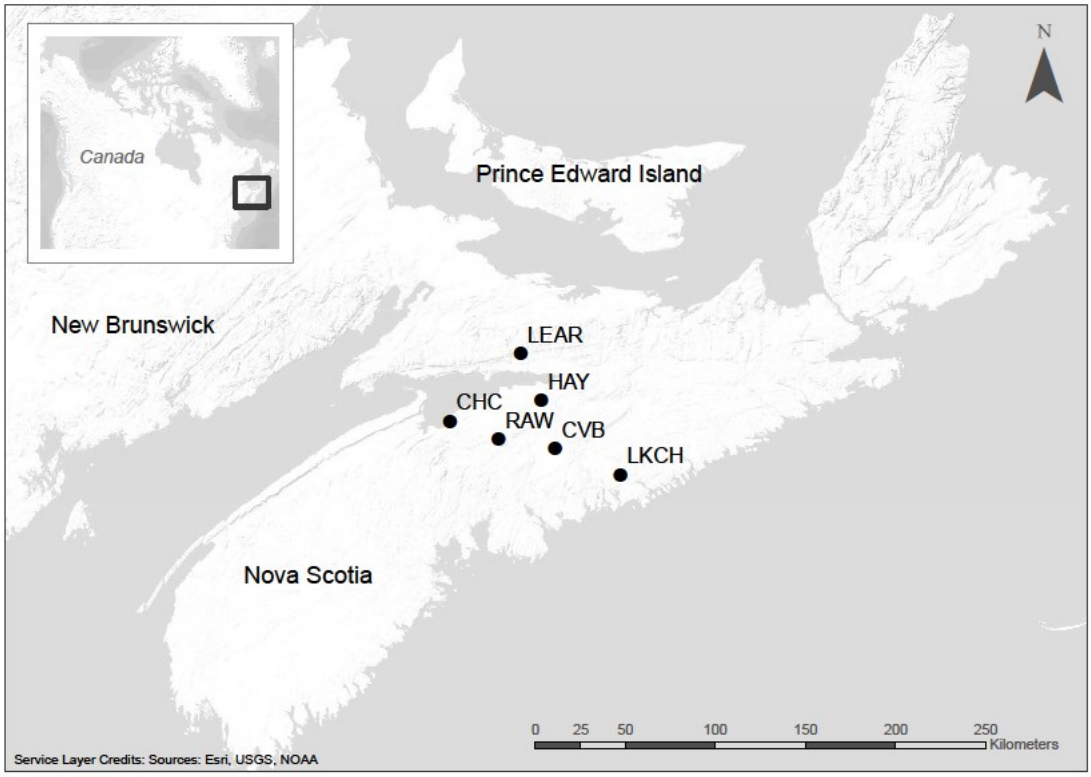

Figure 2.1 Locations of swarming sites (caves and abandoned mines) surveyed to assess intersexual differences in swarming activity of bats, Nova Scotia, Canada (2008-2011). Site codes are listed in Table 2.1.

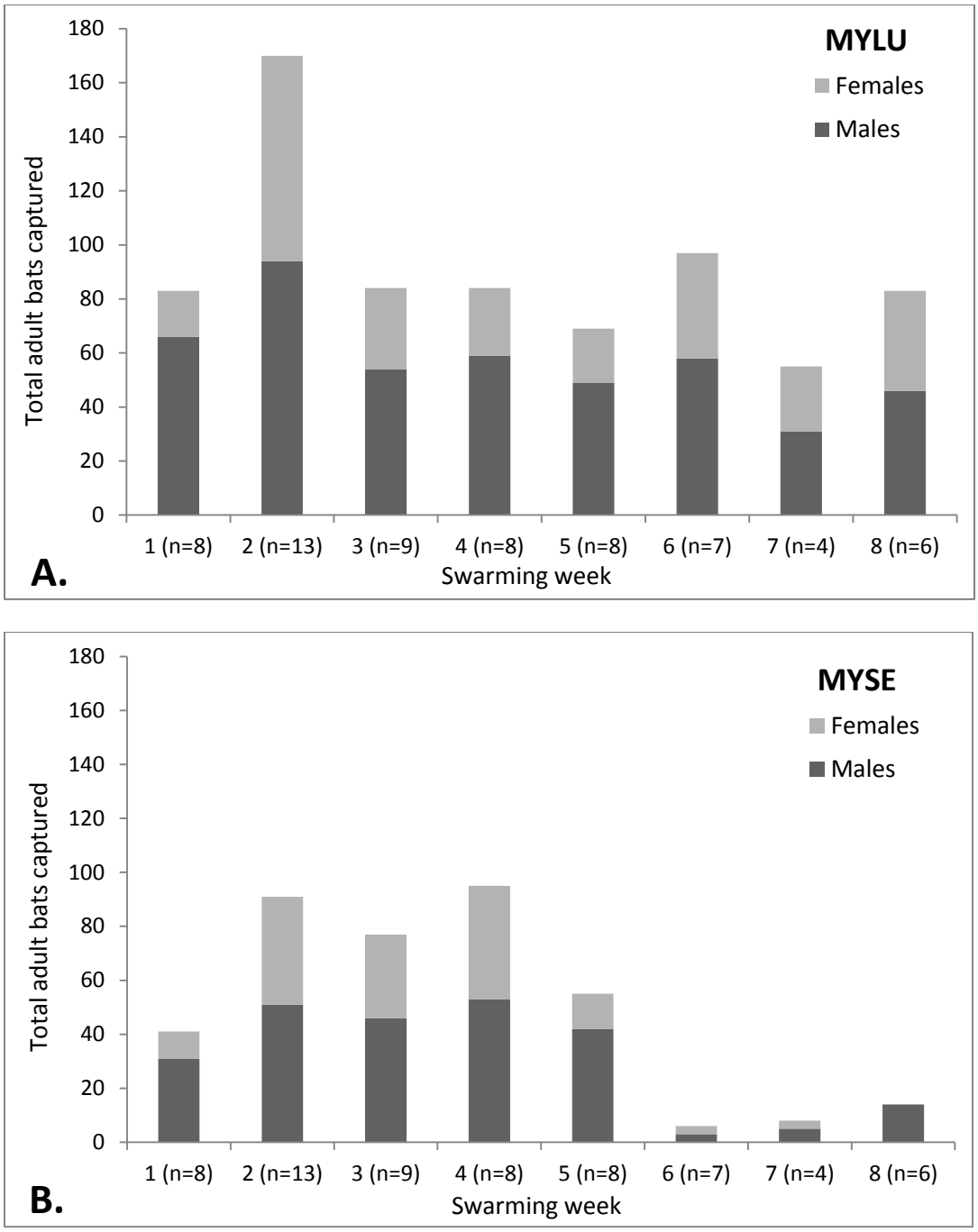

Figure 2.2 The proportion of total adult males and females identified in-hand during each week long period of the autumn swarming season at 6 sites in Nova Scotia (2009-2011) for A) *M. lucifugus* and B) *M. septentrionalis*. The number of sampling nights per week period is indicated in brackets where each week encompassed 7 days starting on 11-Aug of each year.

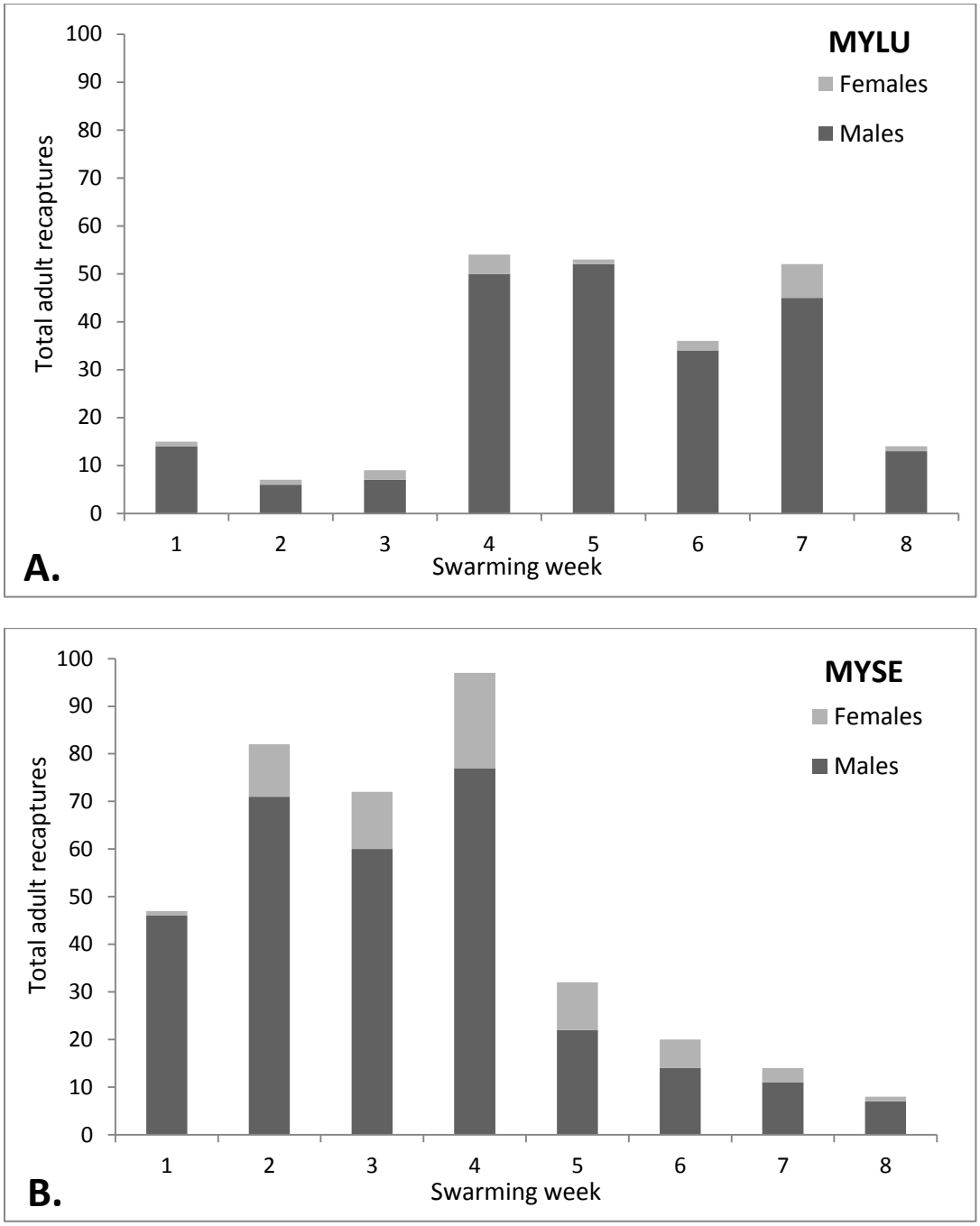

Figure 2.3 The proportion of total adult males and females recaptured during each week long period of the autumn swarming season at Rawdon, Nova Scotia (2011) for A) *M. lucifugus* and B) *M. septentrionalis*. Each week encompassed 7 days starting on 11-August through to 05-October.

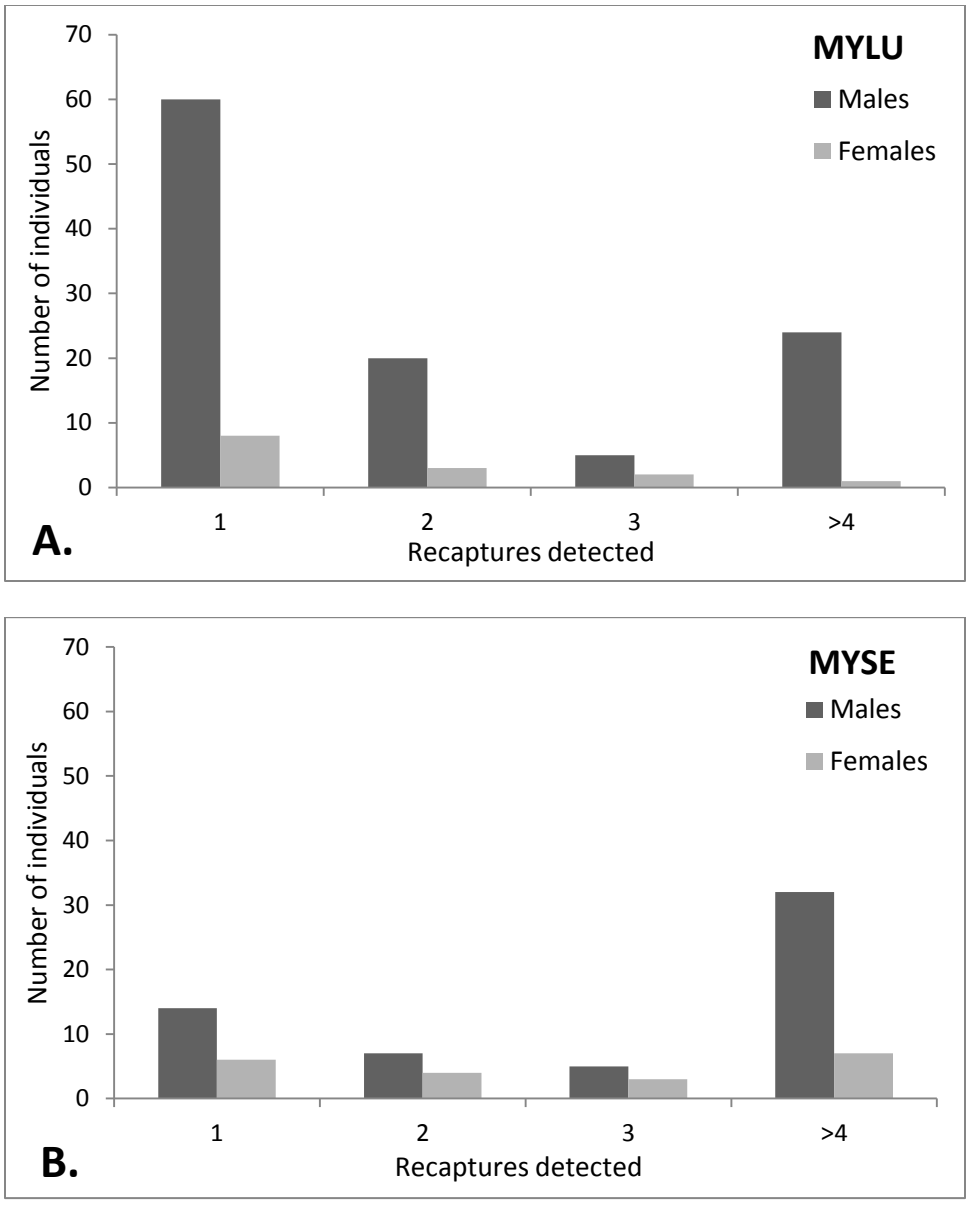

Figure 2.4 Number of individual male and female bats in each recapture history category of the total number of recapture detections observed for A) *M. lucifugus* and B) *M. septentrionalis*.

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2.7 SUPPLEMENTARY MATERIAL

Table 2S 1 Number of captured and identified in-hand adult bats, by year and sex, of little brown Myotis (*M. lucifugus*) and northern Myotis (*M. septentrionalis*) at six swarming sites in Nova Scotia.

	M. lucifugus			*M. septentrionalis*		
Year	Males	Females	*Total*	Males	Females	*Total*
2009	244	164	408	131	87	218
2010	108	74	182	90	43	133
2011	105	30	135	24	12	36
Total	457	268	725	245	142	387

Table 2S 2 Number of captured and identified in-hand adult bats, by capture site and sex, of little brown Myotis (*M. lucifugus*) and northern Myotis (*M. septentrionalis*) at six swarming sites in Nova Scotia (2009-2011).

	M. lucifugus			*M. septentrionalis*		
Site	Males	Females	*Total*	Males	Females	*Total*
Cave of the Bats	25	6	31	41	21	62
Cheverie	38	25	63	14	17	31
Hayes	105	112	217	26	19	45
Lake Charlotte	37	26	63	24	7	31
Lear	65	46	111	31	17	48
Rawdon	187	53	240	109	61	170
Total	457	268	725	245	142	387

CHAPTER 3 WHO SWARMS WITH WHOM? GROUP DYNAMICS OF MYOTIS BATS DURING AUTUMN SWARMING

3.1 ABSTRACT

For many animal taxa, group-living is a strategy where the togetherness provided by groups confers fitness benefits to individuals. Bats are highly gregarious with many species living in groups that show complex social structures. During the summer, many temperate species are sexually segregated among roosts and females have been found to exhibit dynamic social structures whereas males remain understudied. I studied the group dynamics of little brown and northern Myotis bats (*Myotis lucifugus* and *M. septentrionalis)* during autumn swarming, a period for which social interactions are largely unknown. Using capture-mark-recapture surveys, I characterized the occurrence and frequency of age and sex groups occurring at swarms. Within a night, young-of-the-year (YOY) associated more often with other bats than did adult males and females. Further, they associated more often with other YOY than adults. I found no evidence to support the maternal guidance hypothesis as a dispersal mechanism which predicts that there would be associations between mother-offspring pairs. Adult male and female bats associated less frequently with each other instead of together and tended to be most often captured alone. When males were captured in groups, these groups were more likely to be composed of multiple males and in *M. lucifugus*, males had preferred male associates they grouped with over multiple nights. Groups formed during the transitional autumn swarming season may reflect dynamic choices of individuals to maximize fitness.

3.2 INTRODUCTION

Group-living is a common strategy of many animal taxa where individuals gain fitness benefits via interactions with others provided by groups (Alexander 1974; Robinson *et al.* 2005). Group-living species can be categorized based on the impetus for group formation. This can range from passive aggregations around a common resource to highly social species where individuals seek specific group-mates. Passive grouping involves clustering of individuals without regard to the identity of those individuals, although fitness benefits are still obtained (Wilkinson 1985). For example, emperor penguins (*Aptenodytes forsteri*) aggregate to obtain thermal benefits during egg incubation (Ancel *et al.* 1997) and large-scaled girdled lizards (*Cordylus macropholis*) aggregate on plants for shelter when these are a limiting resource (Mouton 2011). Social species, on the other hand, actively seek specific individuals to group with such that fitness benefits depend on the interactions occurring among individuals (Whitehead 2008a). A variety of social structures of group-living animals have been revealed by characterizing the associations among individuals, including marine mammals, equids, primates, and birds (Myers 1983; Baird & Whitehead 2000; Connor *et al.* 2000; Flack *et al.* 2006; Sundaresan *et al.* 2007; Rutz *et al.* 2012).

Parallel to work describing social systems via association metrics, characterization of social behaviour in mating systems, particularly in mammals (Clutton-Brock 1989), has also provided important insights into the sociality of many taxa (Whitehead 2008a). Features of a mating system may have important implications for social interactions. For example, male bottlenose dolphins (*Tursiops aduncus*) form cooperative alliances during the mating season to gain or maintain access to receptive

females (Connor *et al.* 1992; Connor *et al.* 1999). This occurs in an environment where females are difficult for males to defend singly. Dolphins live in highly dynamic societies where individuals may move among higher level groups such that group sizes and composition are variable although individuals do have preferred associates (e.g., fission-fusion; Smolker *et al.* 1992; Connor *et al.* 2000). Males associate as pairs or trios having long-term associations where multiple pairs/trios can join to form alliances. Further, multiple alliances can join together to form higher level superalliances to compete against other alliances (Connor *et al.* 1999). These dynamics fit within the mating system category of 'multi-male groups with spatial defense by males (Clutton-Brock 1989; Randic *et al.* 2012). Similar cooperative and yet flexible alliances of males during the mating period have been shown in some primate species known to have fission-fusion social dynamics (Packer 1977; Watts 1998), but the extent to which this is common in the mating systems of other taxa is not well known. However, the flexibility of a fission-fusion social system may confer individuals with plasticity in their social interactions where during the mating season they can readily form groups that confer some reproductive advantage.

Bats are a diverse order of mammals with approximately 1,200 species (Wilson & Reeder 2005) where many live in groups that vary tremendously in group composition and stability within and among species (McCracken & Wilkinson 2000; Kunz & Lumsden 2003; Kerth 2008). Temperate zone bats experience strong seasonal cycles that impacts all aspects of their life-histories, and likely their social structures. Early work described a three phase annual cycle where two phases consist of mixed-sex associations (mating and winter) and sexually segregated groups during the female maternal care

period in the summer (Bradbury 1977). Exceptions to complete segregation in the latter are now known with some males associating to varying degrees with females and with other males (Altringham & Senior 2005; Safi & Kerth 2007). The majority of work examining social structure for temperate bats has focused on maternity colonies during summer likely owing to their designation as a critical demographic group for conserving their populations (Safi & Kerth 2007; Kerth 2008). Non-random associations among females, where group sizes and composition fluctuate frequently within larger cohesive groups (e.g., fission-fusion dynamics), are well documented in many temperate species (Kerth & König 1999; O`Donnell 2000; Willis & Brigham 2004; Popa-Lisseanu *et al.* 2008; Patriquin *et al.* 2010). With few studies on male associations in bats, the social dynamics of males during the summer are poorly known (Safi & Kerth 2007; Safi 2008). Further, inference of social structure has been primarily made from characterizing associations occurring during day roosting, for both sexes. Association patterns that occur during the night when bats could potentially be interacting during foraging or moving among day sites remain largely unknown.

In contrast to social dynamics during the summer, mixed-sex groups in temperate bats during the fall mating period are explained as being influenced primarily by mating strategies (Bradbury 1977; McCracken & Wilkinson 2000). Despite their importance in representing another dynamic level to the social organization for these animals, associations during the mating period are essentially unknown (McCracken *et al.* 2006). For some long-distance migratory species, such as *Nyctalus noctula* and *Pipistrellus nathusii*, males set up seasonal territories that are visited by females during the mating period after they have left summer maternity colonies (McCracken & Wilkinson 2000;

Petit *et al.* 2001; Hutterer *et al.* 2005). Other species that make smaller regional migrations, in the range of tens to hundreds of kilometres, gather at underground sites for swarming activity during the mating period prior to or following the hibernation period (Thomas *et al.* 1979; Rivers *et al.* 2005; Furmankiewicz 2008).

Autumn swarming is thought to be the primary mating period for many species (Kerth & Morf 2004; Rivers *et al.* 2005; Bogdanowicz *et al.* 2012). However, since it is a transition between two seasons, many other activities also take place during this time period that lasts for approximately 4-6 weeks. For example, the autumn swarming period also coincides with the period of fat deposition prior to hibernation (Kunz *et al.* 1998; McGuire *et al.* 2009; Becker *et al.* 2013), such that bats spend a portion of their autumn activity out on the landscape foraging. Bats may also assess sites for suitability for hibernation while swarming and possibly exchange information regarding hibernation suitability (Davis & Hitchcock 1965; Fenton 1969; Veith *et al.* 2004). Young born in the summer may learn of these sites from conspecifics that guide them to sites during swarming (Fenton 1969). The temporal overlap of adult females and young-of-the-year (YOY) while swarming has led some to suggest that this knowledge transfer occurs from mothers showing offspring these sites, termed the maternal guidance hypothesis (Sachteleben 1991). However, evidence in favour of this hypothesis is mixed (Sendor 2002; Kerth *et al.* 2003; Piksa 2008). Thus, swarming may facilitate many functions for bats (e.g., mating, various forms of information transfer) that are not mutually exclusive, although the relative importance of each activity may vary depending on the status of individuals (i.e., sex, body condition, or reproductive status) and their age.

Given the social nature of bats during summer, combined with a high degree of swarming site fidelity for some individuals (Rivers *et al.* 2006; Furmankiewicz 2008; Norquay *et al.* 2013), and their long lifespans (Austad & Fischer 1991; Holmes & Austad 1994) there is potential for social interactions to occur for bats during swarming beyond simple aggregation for mating. Previous work has anecdotally noted distinct groups of bats arriving or interacting at sites (Hall & Brenner 1968; Fenton 1969; Schowalter 1980; Rivers 2005) although the composition of these groups (e.g., sex and ages of bats) was not always determined or reported. Rivers (2005) documented stable coalitions of male *Myotis nattereri* during swarming suggesting male social groups may exist during swarming. In *M. mystacina*, the capture of female-YOY pairs together during swarming may suggest that some mother-offspring groups may also be present during swarming (Piksa 2008). It is not known if male coalitions or mother-offspring groups are common in other swarming species. Understanding the sociality of bats during swarming may provide important links in understanding the larger temporal nature of highly dynamic social structures that vary and yet persist over time despite seasonal constraints that may be impacting behaviours.

In this study I characterized group composition during swarming to make inferences on potential social interactions in two temperate species; the northern Myotis (*M. septentrionalis*) and little brown Myotis (*M. lucifugus*). Both species are widely distributed in North America and make regional migrations from summering areas to winter hibernacula and are known to swarm during the autumn (Fenton & Barclay 1980; Caceres & Barclay 2000). In the summer, female *M. septentrionalis* form maternity colonies, roosting primarily in trees (Foster & Kurta 1999; Henderson & Broders 2008)

and exhibit a fission-fusion social system (Garroway & Broders 2007; Patriquin *et al.* 2010). Males tend to roost solitarily also in trees (Broders & Forbes 2004; Jung *et al.* 2004) and the species is considered a forest specialist. Female *M. lucifugus* form maternity colonies roosting typically in buildings and in trees (Humphrey & Cope 1976; Fenton & Barclay 1980). Recent work suggests forest-dwelling female *M. lucifugus* also exhibit dynamic changes in roost use and group size (Olson & Barclay 2013), which may suggest a fission-fusion social system. However, social structure has not been quantified in forest-dwelling or building dwelling maternity colonies of *M. lucifugus*. Male *M. lucifugus* roost singly, in the same structure as females (buildings) or with other males (Davis & Hitchcock 1965; Humphrey & Cope 1976; Broders & Forbes 2004) where the dynamics of these mixed roosting patterns in relation to social structure have not been characterized. *Myotis lucifugus* is thought to exhibit a promiscuous mating system (Thomas *et al.* 1979); reproductive success can be skewed where some males sire more offspring, which may be due to mating during hibernation (Watt & Fenton 1995) or cryptic female choice (Wilkinson & McCracken 2003). Recent work on other temperate bat species (Jahelkova & Horáček 2011; Angell *et al.* 2013) demonstrates alternative mating strategies in the autumn occur for males, such as when and where they secure mating opportunities, which could also potentially explain this skew. Compared to *M. lucifugus*, less is known about the mating and swarming activities of *M. septentrionalis*, although the similarity of the general life-history characteristics related to reproductive and seasonal cycles suggest *a priori* that the two species may be quite similar.

I hypothesized that because swarming may serve multiple functions for temperate bats, including mating and information transfer, there would be distinct bat groups of

individuals actively associating with other individuals, composed of specific sex and age classes associated with individuals grouping to meet specific needs. If swarming congregations are comprised, at least in part, of individuals seeking mating opportunities, I predicted that within a night, bats captured at a swarming site would include: (1) adult male and female groups that are presumably mating groups; and (2) male coalitions where males form groups to gain or maintain access to females. Across nights I predicted there would be male coalitions that persist if these coalitions facilitate more mating opportunities for at least some of the males. In addition to mating, bats may also congregate at swarms to exchange information. This may occur specifically for YOY where they are shown swarming/hibernation sites by their mothers (the maternal guidance hypothesis). If this occurs, I predicted there would be adult female and YOY pairs occurring within a night that show a high degree of relatedness.

3.3 Materials and Methods

3.3.1 Study site

This study was conducted at the entrance of an abandoned gold mine in the Rawdon Hills (45°2'N, 63°49'W) of central Nova Scotia, Canada. The mine is a horizontal structure (adit) located in a hilly forested region where agricultural and forestry activities have created a landscape mosaic of disturbed and forested patches (Davis & Browne 1996). The site is a known swarming site and hibernaculum for three species of bats (*M. lucifugus*, *M. septentrionalis* and *Perimyotis subflavus*; Burns and Broders unpublished data; Moseley 2007).

3.3.2 Capture and tagging

Bats were captured using harp traps (Austbat Research Equipment, Lower Plenty, Victoria, Australia) set at the entrance of the mine at dusk during the autumn and spring from 2008-2011. Captures during the spring seasons facilitated me increasing the number of tagged individuals for the study. Individuals were identified to species and sex, with age (YOY or adult) determined by examining the degree of ossification and shape of the epiphyseal growth plates of the metacarpals (Anthony 1988). Two small tissue samples (≈ 9mm^2 each) were collected from each of the wings of individuals. I initially collected these from the uropatagium since this area was shown to heal faster and yield higher DNA quantities (Faure *et al.* 2009). However, after recaptured individuals were found with tears from these sample holes (in various stages of healing) and our lab received reports of bats found snagged on car antenna during a concurrent telemetry study, I discontinued this practice and returned to sampling from the plagiopatagium (Worthington Wilmer & Barratt 1996; Broders *et al.* 2013). Tissue samples were placed in either Allprotect Tissue Reagent (Qiagen N.V., Venlo, Netherlands) or 20% salt saturated DMSO solution (Seutin *et al.* 1991) and stored frozen at -20°C.

To track bats in their associations within and across nights via recaptures, I tagged all or a subset of captures with permanent, passively integrated transponders (PIT tags; Trovan ID 100, EIDAP Inc., Sherwood Park, Alberta) for individual identification. On a given night I tagged as many individuals as I could process until capture rates increased during the night outpacing my ability to efficiently and safely process bats. In 2010 and 2011 I stopped tagging bats after 05 September in concern for animals being able to recover effectively to maintain activities (e.g., forage and deposit fat stores) prior to the

hibernation. PIT tags are microtags (0.1 g) that are activated when they pass within range of a reader enabling each unique PIT tag code to be recorded with a time and date stamp. PIT tags have been used to study a variety of bat species with minimal impacts on behaviour, health or reproductive success (Kerth & König 1999; Patriquin *et al.* 2010; Rigby *et al.* 2012). Following injection of the tag, the injection site was sealed using surgical glue (Torbot Inc., Cranston, Rhode Island) and bats were held for a period of 5-10 minutes in separate bags to ensure the injection site was sealed and that bats were active and ready for flight following release. All bats were released prior to sunrise.

An emergent fungal pathogen, *Pseudogymnoascus desctructans,* which causes white-nose syndrome (WNS), has resulted in both study species suffering dramatic recent declines in their populations in eastern North America (Blehert *et al.* 2009; Turner *et al.* 2011; Minnis & Lindner 2013). Therefore, I used the most up-to-date precautionary WNS decontamination protocols provided by the US Fish and Wildlife Service to minimize potential spread of fungal spores via capture and handling methods (available from http://whitenosesyndrome.org/topics/decontamination). In the late winter of 2010/2011, WNS was detected in Nova Scotia and therefore to reduce the chance of transmission from my work, I reduced the number of active trapping and tagging sessions in 2011 with no spring tagging session occurring.

3.3.3 Assessment of swarming groups

Little is known regarding the variation in use of a site by individuals within and between nights or over multiple swarming seasons as swarming activity appears to be highly dynamic (Fenton 1969; Humphrey & Cope 1976; Rivers *et al.* 2006). Large variation can be found in the number and duration of visits made to swarming sites and

how far individuals travel to swarming sites. This made the selection of an appropriate temporal scale to characterize social associations problematic since a low recapture rate means following individuals is difficult. Therefore, two complementary approaches were used to examine group dynamics among swarming bats at different temporal scales.

First, since recapture rates were predicted to be low, I used a class-based approach to examine the tendency to form groups by age and sex classes rather than as individuals. Groups of bats flying at swarming sites (possibly arriving together or arriving separate and associating together) have been observed in many species (Hall & Brenner 1968; Fenton 1969; Schowalter 1980). Behavioural interactions observed in groups of bats during swarming include chasing and vocalizations where bats often fly in circles around the entrance to underground sites prior to entering (Thomas *et al*. 1979, Furmankiewicz *et al.* 2013). I examined if such groups predictably occurred over a 5 minute interval where I assumed bats captured in the same interval were actively maintaining a spatio-temporal association with other bats whereby they were interacting. This interval represented a trade-off in being of short duration to minimize catching individuals not associating together, while being long enough to catch those presumed to be associating while also enabling efficient handling of captured bats. All bats of the same species captured in each 5-minute interval, which started with the first captured bat, were considered as a group. I considered adult females and YOY trapped together in these intervals as putative mother-offspring groups. Intervals were consecutively sampled through the night until a handling limit was reached for processing bats that depended on the nightly total of captured individuals. The mean number of intervals sampled per night was 40 (range 15-59) sampled on 26 nights.

Other work has suggested that individual males associate during swarming more than expected from chance alone over multiple nights (Rivers 2005). Therefore, my second approach was to characterize recaptures of marked male bats using the site, over multiple nights, via three recapture methods. First, in all three years, concurrent with the interval sampling trapping sessions, I actively hand-scanned all captured bats for PIT-tags and recorded the capture time. Second, in 2010 I set up a harp trap with PIT-tag antenna fitted around holes cut in the sides of the capture bags (PIT-harp trap). This customized trap facilitated bats being 'captured' and passively scanned for a tag as they escaped out through one of the two holes housing the antenna. The modified PIT-harp trap was left out over multiple nights to passively scan bats and was checked approximately every 5 days. Prior to deploying the PIT-harp trap without personnel present, trials were conducted to observe the behaviour of bats in the traps and confirm that all captured bats escaped. Third, in 2011, I installed PIT-tag antenna in a temporary mesh gate placed at the mine entrance to passively scan bats as they entered or exited the mine. The mesh allowed air flow into the mine and minimized any effect of the gates on hibernacula microclimate. Use of the PIT-harp trap was discontinued in 2011, since it had the potential to be a vector for the spread of WNS. Active trapping and scanning was conducted in autumn 2011 although trapping frequency was reduced. Detections were recorded at the 1 minute level.

3.3.4 Genetic methods: DNA extractions and genotyping

Genomic DNA was extracted for potential mother-offspring pairs (i.e., adult female and YOY captured during the same 5 minute interval) following a standard proteinase-K, phenol and chloroform procedure followed by ethanol precipitation

(Sambrook & Russell 2001). I also extracted DNA from adult and YOY not considered as a putative mother-offspring pairs (e.g., adult males and females and YOY captured in different internals) to calculate population allele frequencies for relatedness analyses. Samples were genotyped at 10 microsatellite marker loci developed for *M. lucifugus* that cross-amplify in *M. septentrionalis* (Burns *et al.* 2012; Appendix B). Loci were amplified in 4 multiplex reactions described in detail in Chapter 4. Amplified products were size-separated and visualized on an ABI 3500xL capillary electrophoresis system. Alleles were scored using GeneMarker (vs.1.95, SoftGenetics Inc., State College PA) by comparison to GeneScan 600 LIZ® internal lane size standard (Applied Biosystems). For each individual, all loci electropherograms were visually inspected for verification of allele peak size calling; allele peaks were binned for scoring after examination of frequency distributions of raw allele calls.

3.3.5 Analyses

3.3.5.1 Class Gregariousness

Bats captured during the temporal interval sampling were assigned to one of three sex and age classes for each species: adult females, adult males and YOY. To evaluate if there were distinct groups of bats at swarming sites, class-based association indices of gregariousness were used. Gregariousness is a measure of an individual's tendency to form associations and when individuals are assigned to particular classes, it is calculated at the class level (Underwood 1981; Pepper *et al.* 1999). I calculated general gregariousness (GG) as a general measure of the propensity of members of a class to associate with other bats of any class where it. This metric is the number of within-group associates averaged across all appearances. Secondly, I assessed whether members from

one class have a particular affinity for associating with members of a particular class using the pairwise affinity index (PAI). The PAI was calculated between and within classes (i.e., showing affinity for associating with members of their own class) where this metric controls for the GG of the class. This metric is the average number of members of a class that are found with each member of another class, divided by the general gregariousness of the classes being compared. To evaluate if these groups were different from expectations of bats grouping at random at swarming sites, I used a group randomization procedure (Smolker *et al.* 1992; Bejder *et al.* 1998; Pepper *et al.* 1999). Here group sizes and the number of individuals in each class were held constant to what was observed while shuffling group membership using the PopTools add-in for Excel (Hood 2010). The data were shuffled 10 000 times and each class association index was calculated for each run. The mean value of these simulated runs was taken as the expected value of association for grouping at random and the ratio of the observed value over the expected value minus 1 was calculated for the direction and magnitude of deviation (Deviation ratio, DR; Pepper *et al.* 1999). Values of > 0 indicated association with more individuals than expected at random and values < 0 indicate fewer associations than expected. A comparison of the observed value to the 2.5% tails of the distribution of the randomized index values provided a two-tailed test of the null hypothesis: all classes of bats were equivalent in their grouping behaviour (i.e., grouping at random with members of any class).

3.3.5.2 Assessment of male coalitions

Social structure can be elucidated from the characterization of the pattern of dyadic interactions of individuals (Hinde 1976). Because interactions of bats could not

be directly observed during swarming, I assumed that males recaptured closely in time on the same night at the site could be interacting in a group and were therefore associated (Whitehead & Dufault 1999). I did not examine associations with females since I re-captured fewer females to perform analyses (*M. lucifugus*$_{fem}$ n=13; *M. septentrionalis*$_{fem}$ n=26). Work from tracking males via radio-telemetry at the swarming site detected male *M. lucifugus* (n=9) spending on average 25 minutes per visit (range 0.02-3.41 hours), although duration varied among visits and among individuals (Lowe 2012). Thus, I choose the approximate average time spent per visit, 30 minutes, as a time threshold to characterize potential associations with other males. To be conservative, for all detections of tagged males, I buffered their time stamp to 30 minutes before and after the recording and considered any other bats detected in the hour-long period as a member of the individuals group. Detections were examined sequentially within a night where a new group was considered only when group membership changed (i.e., a new tagged male was recorded or one was no longer detected) or if the same solitary bat was detected >30 minutes from the previous time stamp. The associations were based on tagged males that were detected at the site ≥ 2 times; therefore inferences are based on these individuals only.

To characterize the strength of association among males at the swarming site, I calculated the half-weight association index (HWI) for tagged males of both species (Cairns & Schwager 1987). The HWI estimates the proportion of the sampled nights that dyads were recorded together using the swarming site relative to the total number of nights that each individual was recorded at the site, regardless of whether together or not. I used the HWI over other indices because it is less biased when not all individuals of a

group can be identified (Whitehead 2008a). Owing to the dynamic nature of swarming, it is likely that in this study not all interacting bats were tagged and thus not identified. Since my detection methods required bats be trapped or enter the mine, and these methods varied throughout the study, I may also not have recorded all individuals interacting at the site in groups. The HWI will therefore underestimate the strength of associations among bats making the estimates conservative. However, my interest was not in specifically estimating the degree of association among individuals but rather to assess if groups of male bats associate during swarming.

If males formed groups with preferred individuals, then one should detect non-random association patterns. If associations were different from those expected due to individuals randomly associating (i.e., forming groups), then the coefficient of variation (CV) of association indices of the sample population would be greater than those expected if associations were random (Bejder *et al.* 1998; Whitehead 2008a). To test this I first calculated the CV of the swarming association index matrix constructed of pairwise HWI for each possible pair of tagged males. I then used a permutation procedure following Bejder et al (1998) and Manly (1995) implemented with modifications in SOCPROG (vs 2.5; Whitehead 2009). The associations were permuted within samples because this test accounts for differences in gregariousness among individuals and animals leaving and returning to the study area and is therefore the preferred test for my study system (Whitehead 2009). The structure of the data was retained by holding the total number of individuals identified and number of groups constant to that found in the original data matrix. I tested the null hypothesis that there were no individuals preferentially grouping (or avoiding each other) between nights where the sampling

period, a night, was considered from dusk until dawn. The data were permuted 10 000 times and I compared the CV of the observed association matrix to the distribution of CV's generated from the permutations. If the observed CV was greater than that of the permuted data ($P > 0.95$) then the null hypothesis of randomly grouping males was rejected. As recommended by Whitehead (2008b), I estimated the level of social differentiation (*S;* using the likelihood method) and the mean number of associations per individual (*H*) in SOCPROG 2.5 to examine if the data were sufficient to reject the null hypothesis of individuals associating randomly. This should be true when $S^2 \times H > 5$ (Whitehead 2008b).

3.3.5.3 Relatedness

Owing to a low sample size of successfully genotyped putative mother-offspring pairs for *M. lucifugus* (n=2) I restricted the analyses to pairs of *M. septentrionalis* (n=11). I first estimated the reference population allele frequencies, observed and expected heterozygosities and deviations from Hardy Weinberg Equilibrium (HWE) using the program CERVUS version 3.0.3 (Kalinowski *et al.* 2007). Studies have shown that different relatedness estimators can be influenced by inherent characteristics of molecular markers (e.g., number of loci, levels of heterozygosity influencing the number and frequency of alleles) and by the coancestry of the individuals being examined (Van de Casteele *et al.* 2001; e.g., their pedigree; Blouin 2003; Csillery *et al.* 2006). This means that regardless of their actual true level of relatedness among individuals (e.g., their pedigree) there can be large variation among related individuals and among estimated relatedness measures from genetic data that uses the proportion of alleles that are identical by descent. For example, although the average proportion of identical by

descent alleles for full sibling is 0.5, it can range from 0 and 1 which can create complications in classification of estimated values to discrete categories (e.g., offspring, full siblings etc.). Therefore, caution should be used when using genetic data alone to infer relatedness (van Horn *et al.* 2008). Thus, instead of trying to identify the actual relatedness of associated pairs, I focused on testing if associated individuals (putative mother-offspring pairs) were more related than adult female-YOY pairs created at random. Second, I calculated relatedness using 4 relatedness estimators to look for concordance among the four measures. I used the moment estimators that explore allele sharing due to identity by descent of Li *et al*., (1993), Queller and Goodnight (1989), Lynch and Ritland (1999) and Wang (2002).

Relatedness was calculated for all the putative pairs using allele frequencies from the reference population data set (adult males, females and YOY; n=108). To create randomly generated pairs, I took two subsets of the reference population dataset: one of adult females (n=33) and another of YOY (n=56) and randomly generated pairs from these two pools of individuals. I ran 100 permutations sampling one individual from each pool to create a random pair, and then compared the mean relatedness of the putative pairs to the distribution of the mean relatedness calculated from the randomized pairs. All calculations were performed in R (R Development Core Team 2010) using the package *related* (available from www.frasierlab.wordpress.com/software; accessed 22 May 2014). If the observed mean relatedness is greater than that of the permuted data ($P > 0.95$) then the null hypothesis, that the mean adult-female and YOY pairs is random with respect to relatedness, is rejected.

3.4 Results

3.4.1 Class Gregariousness

Over the three autumn swarming seasons at the Rawdon mine, I captured 276 adult (96 females, 180 males) and 76 YOY *M. lucifugus* and 254 adult (100 females, 154 males) and 153 YOY *M. septentrionalis* in the interval sampling. The majority of intervals for both species had only one bat captured at 74.8% (196/262) and 66.8% (183/274) for *M. lucifugus* and *M. septentrionalis*, respectively. The range in number of bats captured per interval was 0 to 4 (mean = 1.3) for *M. lucifugus* and 0 to 7 (mean = 1.5) for *M. septentrionalis*. Variation in general gregariousness was found for *M. lucifugus* where adult males grouped with significantly fewer associates than expected by chance (GG= 0.51, DR= -0.22 $P < 0.002$: Figure 1) and YOY grouped with significantly more associates than expected by chance (GG= 1.1, DR= 0.61, $P < 0.001$). Adult female *M. lucifugus* grouped to a similar level as that predicted by randomly forming groups during swarming although had a tendency to have fewer associations (GG= 0.61, DR= -0.07, $P >0.05$). *Myotis septentrionalis* showed no significant association patterns of preferred or avoided associates than expected due to chance for all three classes. However, the direction of association tendency for each class was similar to *M. lucifugus* where adult males and adult females had slightly fewer associates (males: GG= 0.92, DR= -0.03; females: GG= 0.89, DR= -0.05) and YOY had slightly more associates (GG= 1.02, DR= 0.08).

Myotis lucifugus adult males grouped significantly more with other adult male *M. lucifugus* given their level of general gregariousness (males: PAI= 1.21, $P< 0.03$, Table 1). Although not significant, YOY showed a similar tendency to group with other YOY

(PAI= 1.61, $P < 0.08$) and to have fewer associates with adult females and males (Table 3.1). Adult females and YOY of *M. septentrionalis* grouped significantly more with members of their own classes given the general gregariousness of each class (females: PAI= 1.46, $P< 0.05$; YOY: PAI= 1.27, $P < 0.04$; Table 3.2). Adult females and YOY grouped with each other significantly less than expected (PAI= 0.72, $P< 0.03$).

3.4.2 Occurrence of male coalitions

From 2008-2011 (spring and autumn seasons), I tagged 368 *M. lucifugus* (321 males, 47 females; Table 3) and 246 *M. septentrionalis* (167 males, 79 females). In total, 113 male *M. lucifugus* were detected 685 times with a mean number of detections per individual of 6.0 ± 9.4 (mean ± SD; range 1-68) on 89 nights in the three autumn swarming seasons. The mean number of nights each individual was detected on was 2.9 ± 3.5 (mean ± SD; range 1-23) with a mean group size of 2.1 ± 1.6 (mean ± SD; range 1-11). For male *M. septentrionalis*, 75 individuals were detected 892 times with a mean number of detections per individual of 11.9 ± 12 (mean ± SD; range 1-44) on 83 nights across the three swarming seasons. The mean number of nights each *M. septentrionalis* individual was detected on was 5.7 ± 5.1 (mean ± SD; range 1-23) with a mean group size of 2.3 ± 1.9 (mean ± SD; range 1-11).

Male *M. lucifugus* showed non-random associations during swarming as the CV of the observed association matrix (5.78) was greater than the CV of randomly generated groups (5.65) for > 95% of the permutations (10 000; $P > 0.005$), despite the data having low ability to reject the null hypothesis (S^2 x $H = 0.636^2$ x 6.56 = 2.653). In contrast, male *M. septentrionalis* formed associations that were not different from randomly generated groups since the CV of the observed association matrix (3.04) was found to be

greater than the CV of the randomly generated groups (3.01) in only 78% of the permutations (10 000; P > 0.785). The data were sufficient in their ability to reject the null hypothesis of random grouping (S^2 x $H = 0.677^2$ x 13.87 = 6.357).

3.4.3 Relatedness of adult female and YOY pairs

Eight of the ten microsatellite loci successfully cross-amplified in the *M. septentrionalis* samples and were used in relatedness analyses (Table 3.4). Pairwise relatedness was variable for the putative mother-offspring pairs regardless of which relatedness estimator was used and was on average quite low (Table 3.5). One pair, (pair 8) had higher relatedness than the mean of randomly generated pairs for 3 of the 4 estimators, and two pairs (2 and 4) had higher relatedness estimates in 2 of the 4 estimators (Table 3.6). With each of the four relatedness estimators, the mean observed relatedness of the 11 putative pairs was not found to be significantly greater (i.e., $P > 0.95$) than that of randomly generated pairs of adult females and YOY. This suggests that the putative pairs are not more closely related to each other than expected by chance.

3.5 Discussion

The results of this study suggest there are predictable bat groups (i.e., bats that are actively associating with other bats) during autumn swarming composed of specific sex and age classes. Young-of-the-year had the highest gregariousness of all three age and sex classes examined. For *M. lucifugus*, young bats had a greater propensity to group than expected from random grouping. This relationship was not found to be significant for young *M. septentrionalis,* however the nature of the relationship was in the same

direction as that for young *M. lucifugus* as shown by the positive, yet smaller in magnitude deviation ratio.

The finding of higher gregariousness among YOY is interesting because it may suggest that groups of YOY associate during swarming for some as yet uncharacterized social function. Early work on autumn swarming suggested that this period may be an important time for young dispersing bats to learn of overwintering and swarming sites from conspecifics (Fenton 1969). Sachteleben (1991) extended this idea and suggested that adult females pass on information regarding swarming/hibernation sites to their young by leading them to sites during autumn migration. No evidence was found to support this hypothesis in either species where adult females had general gregariousness levels equivalent to what was expected from random grouping. Further, the pairwise affinity index for members of these two classes together showed less association among members with negative deviation ratios for both species and a significant negative relationship for *M. septentrionalis*.

The low mean relatedness of pairs of adult females and YOY trapped in intervals does not support that the pairs of *M. septentrionalis* were any more related than randomly sampled pairs of adult females and YOY from the reference population at the site. Inferences from genetic data for pairs in swarming *M. bechsteinii* did not support the maternal guidance hypothesis (Kerth *et al.* 2003). In a study of swarming European species *M. mystacina*, a joint overlap in the seasonal peak of captures of adult females and YOY was documented at a swarming site (Piksa 2008). However, the extent to which this supports the maternal guidance hypothesis is speculative since other anecdotal observations in the study of YOY and adult females captured together close in time

without assessment of the genetic relationships of these pairs means their relatedness cannot be verified.

It is possible with this study that I could have missed the capture of mother-offspring pairs if, for example, these pairs travelled together to swarming sites early in the season before the study began. However, other studies have shown that adult females leave maternity colonies prior to juveniles (Speakman & Racey 1987; Papadatou *et al.* 2008) including *M. lucifugus* (Humphrey & Cope 1976). Instead, I suggest an alternative to the maternal guidance hypothesis where YOY may travel together and co-learn of sites or routes as primarily cohort youth groups. This does not preclude the transfer of information via older conspecifics either at the maternity colony or en route via the following of other bats. My data show YOY as having the highest general gregariousness, despite this not being significantly so in *M. septentrionalis,* and I did capture some groups of YOY with adult individuals. After correcting for general gregariousness, the pairwise affinity index for both species showed YOY grouped preferentially with other YOY. *Myotis lucifugus* approached significance in the same manner, and together this supports the notion of YOY interacting with YOY.

Recently, Patriquin *et al.* (2010) demonstrated that young (although not YOY) *M. septentrionalis* in maternity colonies showed the highest level of associations among age classes examined where associations persisted across years. It is possible that young bats may form social bonds as juvenile cohorts that persist over time and may include over the swarming and wintering seasons. Work on a captive colony of *Pipistrellus kuhlii* recently demonstrated the role of spatial proximity in rearing groups of newborn pups in the development of later amicable associations (Ancillotto *et al.* 2012). They suggested

that early social interactions may be important in the development and maintenance of individual associations and social structures in bats. Further work tracking YOY from the same maternity colony and across multiple seasons and years would be required to assess the validity of the persistence and importance of early social interactions in bats, including *M. lucifugus* and *M. septentrionalis*.

In contrast to my prediction, I did not find evidence of male and female mating groups at a short temporal scale. Females of both species demonstrated negative GG deviation ratios that were not significantly different from expectations of random grouping. Adult males also showed a similar direction of GG deviation ratios with male *M. lucifugus* showing significantly fewer general associations with other bats. This is reflected in the large number of intervals where only single adult males and females were captured. Pairwise affinity indices for both species also indicated that adult males, if they were grouping, tended to group more with other adult males compared to grouping with adult females, although this was only significant for *M. lucifugus*. The formation of mating groups may occur quite transiently and spontaneously during swarming once individuals arrive at the site and thus I may not have captured this emergent grouping behaviour. Previous studies document copulations and mating vocalizations occurring inside the underground areas of swarming sites (Moffat 1922; Fenton 1969; Thomas *et al.* 1979; Furmankiewicz 2008). This may mean the flight activity that I captured was composed of bats acting largely in an individualistic nature in travelling to the site rather than actual behaviours leading to grouping for mating activity. It is also possible that mating takes place outside of the underground structures, in the greater landscape surrounding a swarming site. In either scenario, swarming sites may serve as focal points

in the landscape for individuals to initially gather at and subsequent to that, social interactions and pairings for mating occur.

Despite the general findings of males having lower GG, I did find evidence to suggest that when they were found grouping, males showed preferences for grouping with other males within nights. For male *M. lucifugus*, this extended to preferences over multiple nights. Similar findings were reported in swarming *M. nattereri* where males that were tagged together on the same night were recaptured together on a different night more than expected by chance (Rivers 2005) that lead Rivers to suggest that these male coalitions may serve some undefined social purpose. I concur with River's findings in that these male swarming groups may have been comprised of individuals that were cooperating during competitive or aggressive encounters on an opportunistic basis such as those characterized in coalitions *sensu* Möller *et al.* (2001). The social basis for coalitions may be in assisting males in gaining or maintaining access to females for copulations such as found in alliances in other highly social species such as dolphins and primates (Packer 1977; Connor *et al.* 1992; Watts 1998). Alliances are distinguished from coalitions where the cooperative relationships are of an enduring nature (Möller *et al.* 2001). Owing to the dynamic nature of swarming where transiency is high and observations of the underlying behaviours to characterize alliances is difficult, the nature of the duration of these associations has yet to be thoroughly examined in swarming bats - including this study- to classify them as alliances. The temporal patterning of associations is a key component of describing social relationships (Hinde 1976) and future work describing how associations among males change over time would provide important information on temporal persistence of swarming interactions.

Overall, this analysis provides some interesting contrasts between species that may suggest subtle nuances in their social dynamics. In general, *M. lucifugus* had more distinct differences in the preferences of avoidances of certain sex and age classes and males were found to have preferred longer term associates. In comparison, *M. septentrionalis* showed grouping at a short temporal scale that was similar to that expected of grouping at random (although in the same directions as *M. lucifugus* as discussed previously). These differences may partly reflect the lower sample size of *M. septentrionalis* where further work may show departure from random suggested by the similarity in preferences or avoidances that to *M. lucifugus*. Male *M. septentrionalis* were not found to have significant longer term associates despite a higher mean number of times and number of nights each individual was detected. This may reflect differences in the ecology of each species where as a primarily forest dwelling species, *M. septentrionalis* may visit swarming sites more often if they are roosting close to the swarming site. Since summering females tend to roost among a network of trees (Garroway & Broders 2007; Henderson & Broders 2008; Johnson *et al.* 2012), meaning they are more diffuse on the landscape, it may be beneficial for male *M. septentrionalis* to track females primarily at swarming sites, by roosting close to the sites, to maximize mating opportunities. In contrast, *M. lucifugus* appear to have a higher degree of transiency in swarming movements (Davis & Hitchcock 1965; Norquay *et al.* 2013; this study, Chapter 2) and if males roost farther away, perhaps to be nearby maternity colonies, they may visit specific swarming sites less frequently if they achieve copulations at other sites. In general the social dynamics of male bats are poorly characterized (Safi & Kerth 2007; Safi 2008) leaving much to learned about the

ecological and other factors that underlie male social interactions, including those during swarming.

To conclude, the results of this study provide evidence of specific sex and age groups occurring during autumn swarming for two temperate *Myotis species*. These findings suggest that nightly activities occurring during autumn swarming do have underlying social functions such that bats may not simply passively aggregate at a common resource by chance. The finding of high gregariousness, that is tendency to group of YOY, particularly with other YOY, may imply that the social interactions bats experience in the first year of life may have important consequences to social associations later in life. Further, information transfer regarding migration routes and swarming/hibernation sites may occur for YOY via conspecific transfer but not necessarily via the mechanism proposed by the maternal guidance hypothesis. Male bats formed multi-male groups suggestive of male coalitions, which may indicate cooperative behaviours occur among males on a regular basis for some aspects of swarming such as mating. However, further characterization of the temporal persistence of these associations is required to fully understand the nature of these male-male associations. Many temperate bats are characterized by highly dynamic group structures in other phases of their seasonal cycles (e.g., Kerth & König 1999; Willis & Brigham 2004; Popa-Lisseanu *et al.* 2008; Patriquin *et al.* 2010) such that members join and leave groups as they seek to maximize their own fitness. This flexibility may be especially important to swarming bats where during the autumn, many activities must be balanced (i.e., fat deposition, migration, mating) such that joining and leaving groups at key times may be critical to individuals in this transitional season. Further long-term studies are needed to

fully characterize the nature of social relationships among individuals during swarming, and other phases, to better understand the role of social interactions in response to the changing environmental conditions experienced over a lifetime.

Table 3-1 Group preferences among sex and age classes, for *Myotis lucifugus* captured during autumn swarming in Nova Scotia, Canada 2009-2011. Values are expressed as the deviation ratio as observed over the expected number of associates minus 1.

	Adult males	Adult females	Young-of-the-year
Adult males	**0.22**		
Adult females	0.17	-0.04	
Young-of-the-year	-0.40	-0.16	0.66*

The deviation ratio in boldface type was identified as having significant preferences within the class
*approached significance at $P = 0.08$

Table 3-2 Group preferences among sex and age classes, for *Myotis septentrionalis* captured during autumn swarming in Nova Scotia, Canada 2009-2011. Values are expressed as the deviation ratio of observed to expected number of associates minus 1.

	Adult males	Adult females	Young-of-the-year
Adult males	0.14		
Adult females	0.01	**0.49**	
Young-of-the-year	-0.13	**-0.28**	**0.28**

Deviation ratios in boldface type were identified as having significant preferences or avoidances between classes

Table 3-3 Number of individual *Myotis lucifugus* and *M. septentrionalis* tagged by sex at a swarming site in Nova Scotia (2008-2011).

	M. lucifugus		*M. septentrionalis*	
Year	Males	Females	Males	Females
2008	52	6	27	10
2009	137	21	73	35
2010	78	11	57	27
2011	54	9	10	7

Table 3-4 Summary statistics showing the allele size range, number of alleles (NA), observed heterozygosity (HO), expected heterozygosity (HE) for each of the eight microsatellite loci used to genotype northern myotis (*M. septentrionalis*) at Rawdon, Nova Scotia (2009-2011).

Locus	Allele size range (bp)	N_A	H_O	H_E
Mluc1	114-144	12	0.740	0.818
Mluc4	144-160	5	0.375	0.518*
Mluc5	137-167	8	0.673	0.704
Mluc7	156-322	29	0.415	0.941 [ND]
Mluc8	120-204	11	0.367	0.705*
Mluc25	304-368	24	0.722	0.902
Mluc30	273-387	44	0.571	0.967 [ND]
Mluc34	363-397	33	0.682	0.961 [ND]

* significant departure from HWE at the 0.05 level
[ND] means test for significant departure from HWE not performed

Table 3-5 Observed and permuted mean pairwise relatedness using 4 estimators for adult female and young-of-the-year northern Myotis (*Myotis septentrionalis*) captured in the same five-minute interval, at Rawdon, Nova Scotia (2009-2011). *P* is the number of times the mean of the observed relatedness was greater than that of 100 simulated dataset.

Relatedness Estimator	Observed relatedness (SD)	Permuted relatedness	*P*
Queller & Goodnight (1989)	-0.110 (0.123)	0.0619	0.59
Lynch & Li (1993)	-0.131 (0.132)	0.0254	0.11
Lynch & Ritland (1999)	-0.056 (0.076)	0.0094	0.04
Wang (2002)	-0.067 (0.090)	-0.0136	0.02

Table 3-6 Observed pairwise relatedness, by estimator, of the 11 adult female and young-of-the-year pairs of northern Myotis (*Myotis septentrionalis)* captured in the same five-minute interval, at Rawdon, Nova Scotia (2009-2011).

Pair	Queller & Goodnight`	Lynch & Li	Lynch & Ritland	Wang
1	-0.273	-0.323	-0.127	-0.182
2	-0.044	-0.005	0.088 **H**	0.029 **H**
3	0.054	0.018	-0.046	-0.017
4	-0.097	-0.058	0.0344 **H**	0.106 **H**
5	-0.212	-0.270	-0.122	-0.112
6	-0.233	-0.250	-0.108	-0.133
7	-0.028	-0.071	-0.027	-0.022
8	0.099 **H**	0.063 **H**	0.022 **H**	-0.015
9	-0.233	-0.270	-0.116	-0.136
10	-0.094	-0.120	-0.128	-0.163
11	-0.154	-0.153	-0.083	-0.092

H indicates higher relatedness than the mean of 100 randomly generated pairs

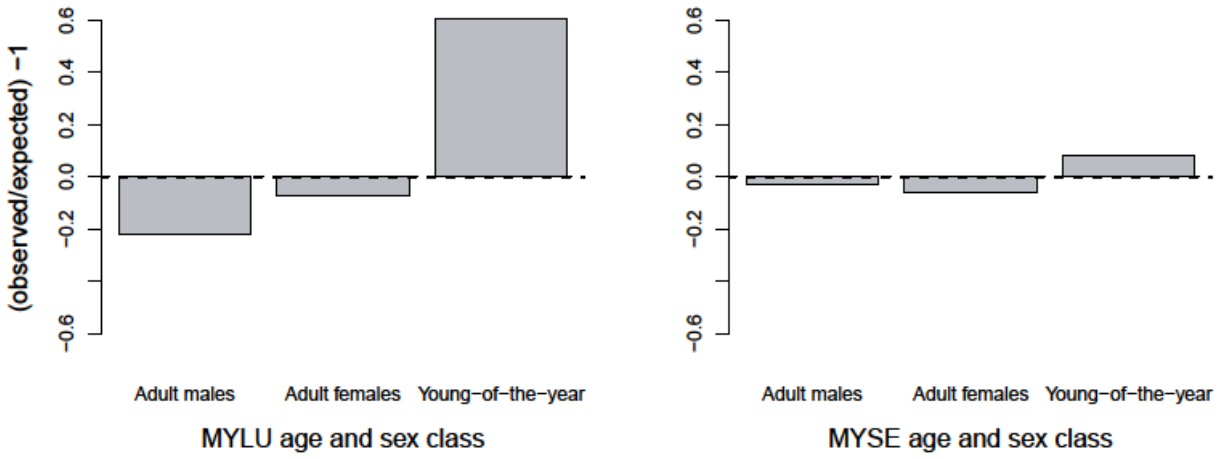

Figure 3.1 General gregariousness of age and sex classes for *Myotis lucifugus* (MYLU) and *M. septentrionalis* (MYSE) expressed as the deviation ratio of observed to expected number of associates minus 1. Expected values are the means of 10 000 randomizations. Only MYLU males and young-of-the-year had significantly less or more associates than expected from random grouping.

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CHAPTER 4 GENETIC CONNECTIVITY AMONG SWARMING SITES IN THE WIDE RANGING AND RECENTLY DECLINING LITTLE BROWN MYOTIS (*MYOTIS LUCIFUGUS*)

4.1 ABSTRACT

Characterizing movement dynamics and spatial aspects of gene flow within a species permits inference on population structuring. Since patterns of structuring are products of historical and current demographics, assessment of structure through time can yield an understanding of evolutionary dynamics acting on populations that are necessary to inform management. Recent dramatic population declines in hibernating bats in eastern North America from white-nose syndrome have prompted the need for information on movement dynamics for multiple bat species. I characterized population genetic structure of the little brown Myotis, *Myotis lucifugus*, at swarming sites in south-eastern Canada using 9 nuclear microsatellites and a 292 bp region of the mitochondrial genome. Analyses of $F_{ST,}$ Φ_{ST} and Bayesian clustering (STRUCTURE) found weak levels of genetic structure among swarming sites for the nuclear and mitochondrial genome (Global F_{ST} =0.001, P<0.05, Global Φ_{ST} = 0.045, P <0.01, STRUCTURE K =1) suggesting high contemporary gene flow. Hierarchical AMOVA also suggest little structuring at a regional level. Metrics of nuclear genetic structure were not found to differ between males and females suggesting weak asymmetries in gene flow between the sexes. However, a greater degree of mitochondrial structuring does support male-biased dispersal over the long term. Demographic analyses were consistent with past population growth and suggest a population expansion occurred from approximately 1,250 to 12,500 BP, following Pleistocene deglaciation in the region. This study suggests high gene flow

and thus a high degree of connectivity among bats that visit swarming sites whereby mainland areas of the region may be best considered as one large gene pool for management and conservation.

4.2 INTRODUCTION

Understanding the structure and dynamics of populations has long been recognized as a foundation for informing management decisions for species-at-risk. This is because it provides an essential evolutionary perspective to the conservation process by providing inference on gene flow and the evolutionary consequences of dispersal (Frankel 1974; Lowe & Allendorf 2010). Population genetics can be used in conservation efforts in delineating management units, management of captives or populations in decline, population reintroductions or supplementation, and lastly in understanding past population demographics (Frankham *et al.* 2002; Pearse & Crandall 2004). Advances in population genetic theory and statistical analyses have facilitated our ability to make inferences on historical demography yielding greater insight into the processes that have led to contemporary patterns of genetic variability and population structuring (Avise *et al.* 1988; Rogers & Harpending 1992). To incorporate these insights into management strategies, a critical first step is characterizing the patterns of genetic variation. From there, inference can be made on gene flow and extent of genetic connectivity within and among populations (Slatkin 1994; Lowe & Allendorf 2010). Characterizing population structure remains an important step for conservation planning

for many wildlife populations where detailed demographic data are limited and conservation risks are high.

The degree of connectivity within and among populations is influenced by environmental and biotic factors and traits specific to species such as dispersal. Dispersal is the movement of individuals from their natal group to a breeding group in a manner such that genetic exchange has occurred (Allendorf & Luikart 2007). Dispersal has been quantified through field studies of individuals using observation methods such as mark-recapture studies to infer movements (e.g., Lebreton *et al.* 2003; Russell *et al.* 2005b; Hassall & Thompson 2012) or telemetry (e.g., Boyd *et al.* 1998; Hoogland 2013; Schofield *et al.* 2013). However, identifying individual dispersers or the actual movements that lead to gene flow is difficult, especially for species that are highly vagile, cryptic or long-lived. Assessment of dispersal and population genetic connectivity via molecular techniques can overcome these challenges by providing evidence of genetic exchange which has been demonstrated for many vagile vertebrate taxa (e.g., Lyrholm *et al.* 1999; Petit & Mayer 1999; Wright *et al.* 2005).

As the only mammalian order capable of true powered flight, bats (Order Chiroptera) have high vagility (Fenton 1997) with many species engaging in long distance movements during seasonal migrations that may range from tens to over a thousand kilometers (Fleming & Eby 2003; Hutterer *et al.* 2005). High vagility has facilitated large distributional ranges for many species. For several species, individuals may disperse over long distances resulting in high rates of gene flow and near panmictic population structuring (McCracken *et al.* 1994; Petit & Mayer 1999; Bryja *et al.* 2009). However, interspecific variation in the degree of philopatry, social structures, resource

specializations and mating systems, cause variation in population structures (e.g., Burland *et al.* 1999; Kerth *et al.* 2002; Miller-Butterworth *et al.* 2003; Campbell *et al.* 2006; Rossiter *et al.* 2012). Because assessments of population genetic structure permit inference on population connectivity, particularly when combined with other demographic data (Lowe & Allendorf 2010), they can represent an important conservation tool for bats. This is particularly important for species considered at risk where data on movements, population dynamics and connectivity are difficult to obtain efficiently to address urgent conservation concerns. Newly emergent threats to bat populations include high mortality as they migrate through wind farms (Cryan & Barclay 2009; Voigt *et al.* 2012; Hayes 2013), rapidly spreading novel diseases such as white-nose syndrome in North America (Blehert *et al.* 2009; Frick *et al.* 2010a) and older diseases such as rabies (Bogdanowicz *et al.* 2013). Data on movements and population connectivity is needed to understand and predict population level impacts from such threats (Foley *et al.* 2011).

Many temperate dwelling bats exhibit an annual cycle consisting of a lengthy period of reduced activity during hibernation, followed by a shorter active period used for self-maintenance and reproduction. For most of the active season the sexes are segregated with females forming maternity colonies and males apparently living independently or in small groups (Safi 2008), although exceptions to complete segregation are known to exist (Altringham & Senior 2005). However, in the late summer and autumn, many species form mixed-sex aggregations composed of individuals from several colonies (Parsons & Jones 2003; Rivers *et al.* 2005;

Furmankiewicz & Altringham 2007; Norquay *et al.* 2013) within which they engage in swarming activities.

Swarming is the term used to describe the event of mass visitations by bats to underground sites prior to or just following hibernation. During swarming, bats engage in chasing and mating behaviours, and presumably gather or exchange information that may include suitability of hibernation sites or knowledge of migration routes and may include the orientation of young-of-the-year (YOY) to such sites (Davis 1964; Fenton 1969; Parsons *et al.* 2003; Piksa *et al.* 2011; Bogdanowicz *et al.* 2012a). Accumulating evidence such as copulations (Fenton 1969; Thomas *et al.* 1979), male-biased sex ratios and observations of males in sexual condition (Gustafson & Damassa 1985; Entwistle *et al.* 1998; Kerth *et al.* 2003; Parsons *et al.* 2003), combined with recent genetic evidence (Veith *et al.* 2004; Rivers *et al.* 2005; Furmankiewicz & Altringham 2007; Bogdanowicz *et al.* 2012a) suggest that swarming is likely the primary mating period for many species. Mating can also occur at summer sites late in the season, (e.g., Senior *et al.* 2005; Angell *et al.* 2013), en route to swarming sites, or during hibernation (Thomas *et al.* 1979). However, if significant mating occurs during swarming it may play an important role in maintaining gene flow among individuals segregated during the summer by providing a mechanism for genetic exchange to occur among partially discrete summer bat populations.

The little brown Myotis (*Myotis lucifugus*) is a small (6-10 g) temperate swarming species, distributed widely across North America (Fenton 1969; Schowalter 1980). It is considered a roosting and dietary generalist species that roosts in buildings and trees in the summer and hibernates in caves and abandoned mines in the winter (Fenton &

Barclay 1980; van Zyll de Jong 1985; Naughton 2012). Females form maternity colonies in the summer and evidence suggests a high degree of fidelity to summer sites. However, complete philopatry does not occur as some females switch colonies (Davis & Hitchcock 1965; Humphrey & Cope 1976; Frick *et al.* 2010b; Norquay *et al.* 2013). This view is supported by recent genetic work that found low but significant population structure among maternity colonies in Minnesota suggesting some limited movements by individuals among the colonies (Dixon 2011). During the autumn, individuals make regional seasonal migration movements (hundreds of kilometers) between summer and winter/autumn sites. Movements among swarming sites can occur within the same season and across years (Fenton 1969; Humphrey & Cope 1976). Norquay et al.'s (2013) banding data analysis found that *M. lucifugus* captured during swarming had the highest movement rates of all individuals studied (summer, winter or swarming captured) which supports the contention that autumn swarming facilitates gene flow if mating occurs as a result of these movements. Further, recent European studies showed higher genetic diversity at swarming sites compared to summering sites which supports the extra-colony hypothesis where multiple summering colonies fuse at swarming sites such that they act as mating centers (Kerth *et al.* 2003; Veith *et al.* 2004; Rivers *et al.* 2005). Taken together these studies suggest that although there may be high gene flow in swarming species, different degrees of genetic structure may occur for different species depending on the specific vagility of species (e.g., distance of migratory movements) and landscape context. Structuring at swarming sites has not been previously investigated in any North American species, including *M. lucifugus*, a known regional migrating species.

In North America, six species of bats, including *M. lucifugus*, are known to be susceptible to a white-nose syndrome, a newly emergent fungal disease of hibernating bats caused by the invasive species *Pseudogymnoascus destructans* (Blehert *et al.* 2009; Foley *et al.* 2011; Warnecke *et al.* 2012). Although the fungus is present in Europe, bats do not appear to suffer mass mortality from the disease (Puechmaille *et al.* 2011). *Myotis lucifugus* appears to have suffered significant mortality from the disease with severe population collapses reported within the affected region ranging from declines of 78 to 100 % (Dzal *et al.* 2011; Langwig *et al.* 2012). Regional population extirpation is predicted within 20 years in the north-eastern United States (Frick *et al.* 2010a) and the disease is now present in 5 provinces in eastern Canada into the north and south east regions of the United States. The degree to which the movements made during swarming contribute to the spread of the disease has not been quantified. However, owing to the rapid and unprecedented population decline of the species in the affected areas, information on the spatial extent of connectivity during this dynamic time period is needed. In addition to the summer colony study in Minnesota (Dixon 2011), previous published population genetic studies of *M. lucifugus* include a study of western putative subspecies designations using nuclear and mitochondrial markers that found little differentiation among summering areas (where the two putative groups converge), suggesting high gene flow among these groups in the sampled region (Lausen *et al.* 2008). Assessments of genetic variation among hibernacula have also found weak genetic differentiation suggesting high gene flow (Carmody *et al.* 1971; Miller-Butterworth *et al.* 2014).

The goal of this study was to determine to what degree swarming sites, or groups of sites, represent distinctive genetic clusters in the region. I quantified genetic variation in *M. lucifugus* among swarming sites in south-eastern Canada using both mitochondrial DNA and nuclear microsatellite markers to investigate population genetic structure. Under the extra-colony mating hypothesis, swarming sites encompass individuals from a catchment area of summering bats where maternity colonies show site fidelity to swarming sites (e.g., Veith *et al.* 2004; Rivers *et al.* 2005). I therefore predicted that maternally inherited markers would show a higher proportion of genetic variance within swarming sites compared to among sites, as a result of high female site fidelity, and thus some level of structuring among sites. Structuring of maternally inherited markers was further examined in the context of past demographic processes (e.g., population expansion or contraction) acting on the population to better understand any patterns observed. Previous tagging studies suggest some movements among swarming sites by at least some individuals during the autumn swarming season (e.g., Fenton 1969; Norquay *et al.* 2013). Since *M. lucifugus* can make extensive migration movements during the autumn mating season, I hypothesized there is a high degree of genetic connectivity among swarming sites even under the extra-colony hypothesis which operates with some degree of swarming site fidelity occurring. I therefore predicted that genetic differentiation among swarming sites on nuclear markers would be lower than found on maternally inherited markers, suggesting gene flow occurs among sites regularly enough that genetic clustering would be found across multiple swarming sites rather than each swarming site representing a single genetic cluster of bats.

4.3 Materials and Methods

4.3.1 *Sample collection and DNA extraction*

During the autumns of 2009-2011 (10 August to 06 October), bats were trapped in harp traps (Austbat Research Equipment, Lower Plenty, Victoria, Australia) or mist nets (Avinet, Dryden, New York) set at 15 swarming sites in three Canadian provinces: Quebec (QC), New Brunswick (NB) and Nova Scotia (NS; Table 1; Figure 1). Sites were situated from 15 to 860 km from each other. Sites in NS and NB were selected as they were known swarming/hibernation sites. I included samples from QC to assess if NS and NB were effectively one breeding group given the close proximity of sites to each other in these provinces. Precautionary WNS decontamination protocols provided by the US Fish and Wildlife Service were followed for all sampling using the most current protocol for each sampling season (available from http://whitenosesyndrome.org/topics/decontamination). Methods for the capture and handling of bats were approved under permits from each provincial jurisdiction. White-nose syndrome was detected in winter 2009/10 in southern counties of Quebec close to the sampling sites, and no further sampling was conducted. Detection of WNS in the winter of 2010/11 in New Brunswick at one of the sites restricted sampling to one site that was in a different county and did not have WNS detected at the time of sampling. I also reduced trapping efforts in Nova Scotia in autumn 2011 to only sample sites where sample sizes were exceptionally low to reduce the risk of spreading the disease via the capture and handling of bats since WNS was not detected at those sites.

For all captures, sex was identified, and age was determined as young-of-the-year (YOY) or adult based on the degree of ossification and fusion in the epiphyseal growth plates of the fourth metacarpal (Anthony 1988). Two small tissue samples (≈ 9mm^2 each) were collected from each of the wings of individuals (plagio or uropatagium; Faure *et al.* 2009; Broders *et al.* 2013) and then bats were released. Tissue samples were placed in either Allprotect Tissue Reagent (Qiagen) or 20% salt saturated DMSO solution with 0.25M EDTA (Seutin *et al.* 1991), and stored frozen at -20°C. Tissues collected in Quebec were placed in 95% ethanol and stored at -20°C. In total, tissue samples were collected from 768 adults and 174 YOY. High molecular weight genomic DNA was extracted following a standard proteinase-K, phenol and chloroform procedure followed by ethanol precipitation (Sambrook & Russell 2001). Extracted DNA was resuspended and diluted to approximately 5 ng/µL in $TE_{0.1}$ buffer (10 mM TRIS-Cl (pH 8), 0.1 mM EDTA, pH 8).

4.3.2 Mitochondrial DNA sequencing

An approximate 300 base pair (bp) fragment of the mitochondrial control region, hypervariable II domain (HV II), was amplified in 356 individuals (Table 1) using the previously described primer L16517 (Fumagalli *et al.* 1996) and primer KAHVII 5'-GTAGCGTGAATATGTCCTG-3' (developed in-lab) which is internal to primer sH651 of Castella et al. (2001). Amplifications were carried out in 20 µL reaction volumes containing 1X PCR Buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl; Invitrogen), 0.2 mM of each dNTP (Invitrogen), 1.5 mM $MgCl_2$, 0.16 mg/mL Bovine Serum Albumin (Sigma Aldrich), 0.05 U/ µL *Taq* DNA polymerase and approximately 10 ng of template DNA. The PCR amplification conditions were as follows: an initial denaturing cycle of 95°C for

5 minutes; followed by 30 cycles of 95°C for 30 seconds, an annealing temperature of 55°C for 1 minute, 72°C for 1 minute; with a final extension period of 64°C for 45 minutes. Approximately 80 ng of amplified product was visualized on a 2% agarose gel then 600-800 ng of amplified product was purified of unused primers and dNTP's using the Antarctic Phosphatase/Exonuclease I protocol (New England Biolabs). Sequencing was performed with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies) with primer KAHVII using approximately 15 ng of purified product as template. Following optimization of the sequencing protocol on a subset of samples, I carried out sequencing using the Macrogen INC., Seoul, Korea Sequencing Service. All base calls were verified manually through visual examination of electropherograms and sequences were trimmed to a common 292 bp segment using 4Peaks (v1.7) DNA sequence editing software (Griekspoor & Groothuis 2006).

4.3.3 Tests of assumptions and genetic structuring on mtDNA

Mitochondrial DNA (mtDNA) sequences were aligned using Clustal W (Thompson *et al.* 1994) in the software MEGA (Tamura *et al.* 2011) using the default parameters following confirmation of congruence among alignments produced by doubling and halving the parameter settings. Sequences were then collapsed into haplotypes and formatted for downstream analysis using FaBox (Villesen 2007). To assess levels of genetic variation in the HV II domain, haplotype diversity (*h*; Nei 1987) and nucleotide diversity (π; Tajima 1983; Nei 1987) were calculated within each swarming site, on the whole data set and for each sex separately using Arlequin v3.5.1.2 (Excoffier 2010).

To examine genetic differentiation among all swarming sites, Φ_{ST} values were calculated using Arlequin. Partitioning of genetic variation at a regional level was examined using provinces as a proxy for putative regional groups in a hierarchical analysis of molecular variance (AMOVA) on adult females, adult males and all adults. To determine what substitution model was most appropriate for the mitochondrial data, I ran ModelGenerator (Keane *et al.* 2006) on the mtDNA sequence data using the BIC and AIC selection criterion. This approach identified a version of the Kimura 2-parameter model (K80+G; Kimura 1980) with a gamma distribution shape parameter estimate (α) of 0.11 and estimated transition/transversion rate ratio of 11.91. To assess if swarming sites cluster as maximally differentiated genetic groups in relation to maximal geographic separation on mitochondrial data, I performed a spatial analysis of molecular variance (SAMOVA; Dupanloup *et al.* 2002). The genealogical relationships among mtDNA haplotypes were explored using a median-joining network (Bandelt *et al.* 1999) in the program Network v4.6.1 (http://www.fluxus-engineering.com). Networks allow for alternative potential evolutionary relationships to be shown as internal cycle. Median-joining networks incorporate median vectors which represent unsampled sequences or ancestral sequences that can allow for greater inference of genealogical relationships despite "missing" intermediary haplotypes.

4.3.4 Population History

Variation in the HV II domain of the mtDNA control region was used to investigate historical demography after a pattern of expansion was suggested from the haplotype network analysis. Since nuclear microsatellite data suggested weak population structure (see Results), I analyzed the mtDNA HV II data as the full dataset from all 15

swarming sites. First, I examined the distribution of the number of pairwise differences, the mismatch distribution, of samples to infer if a recent and sudden expansion occurred (Rogers & Harpending 1992). The observed mismatch distribution was plotted against the expected values of a stable population (i.e., a population with constant population size). I also conducted 3 neutrality tests including Fu's F_S (Fu 1997) and Fu and Li' F* and D* statistics (Fu & Li 1993). All of these analyses were calculated in DnaSP (Librado & Rozas 2009). The F_S test has been shown to be a powerful test to detect population expansions (Ramos-Onsins & Rozas 2002) and is based on examination of the haplotype distribution where large negative values are expected under the scenario of expansion or alternatively from genetic hitchhiking resulting in a selective sweep. The statistical significance of this metric was evaluated by running 5000 coalescent simulations in DnaSP to create an expected distribution, and then comparing the observed value to these expected values. Comparing different neutrality tests can distinguish between the processes of a population expansion, genetic hitchhiking or background selection. If Fu's F_S is significant but Fu and Li's F* and D*are not significant then a population expansion is inferred over background selection where the former is observed as an excess of recent mutations and the later as a deficiency of recent mutations (Fu 1997).

To infer the timing of the expansion, a Bayesian skyline plot was constructed using the coalescent model in the program BEAST v1.8.0 (Drummond *et al.* 2005). In trial runs I initially tested three substitution models (HKY, GTR, TN93) with 4 variants of site heterogeneity parameters for a total of 12 models within BEAST. I then used a Bayes Factor assessment in TRACER (Rambaut & Drummond 2007) to find the best fit

substitution model for the data. For the Bayesian skyline plot analysis I subsequently used the TN93 +G model (Tamura & Nei 1993), with a lognormal relaxed clock run for 3.0×10^8 steps, sampling every 1000 steps. Two independent chains were run and the results were combined in LogCombiner as offered with the BEAST package. These parameters were found to be sufficient for convergence in trial runs since ESS parameters were >200 as viewed in TRACER. I used the range of divergence rates estimated for the HV II region in another temperate bat, *Nyctalus noctula* (6.5% -25.2%; Petit *et al.* 1999) to estimate the rate in BEAST for the *M. lucifugus* sequences since no estimates exist for this species.

4.3.5 Microsatellite Genotyping

All samples were genotyped at 10 microsatellite loci previously described for this species (Table S1; Burns *et al.* 2012; Appendix B). Briefly, loci were amplified in four multiplex reactions with optimized primer concentrations and polymerase chain reaction (PCR) annealing temperature. Reaction volumes were 10 µL containing reagents as described above for mitochondrial work with primer concentrations varying per locus in each multiplex (Table S1, Supporting Information). Each forward primer was labeled with one of four fluorescent dyes (NED, 6-FAM, VIC, PET©; Life Technologies). Amplification conditions for PCR were as follows: an initial denaturing cycle of 95°C for 5 minutes; followed by 30 cycles of 95°C for 30 seconds, annealing temperature for 1 minute, 72°C for 1 minute; with a final extension period of 64°C for 45 minutes. Cycling was carried out on Applied Biosystems 96 Well Veriti Thermal Cyclers and amplified products were size-separated and visualized on an ABI 3500xL capillary electrophoresis system. Alleles were scored using GeneMarker (vs.1.95, SoftGenetics

Inc., State College PA) by comparison to GeneScan 600 LIZ® internal lane size standard (Life Technologies). For each individual, all loci electropherograms were visually inspected for verification of allele peak size calling; allele peaks were binned for scoring after examination of frequency distributions of raw allele calls. A negative and positive sample control (i.e., the same individual) was used on each 96 well plate run to ensure typing consistency among runs.

4.3.6 Tests of assumptions and genetic structuring on nuclear DNA

Microsatellite loci were tested for departure from Hardy-Weinberg equilibrium (HWE) across each locus and within each swarming site using the Markov chain method in GENEPOP v4.1.3 (Raymond & Rousset 1995). Loci were checked for linkage disequilibrium in GENEPOP. Observed (H_O) and expected heterozygosities (H_E), number of alleles observed (N_A) per population, and null allele frequency per locus were assessed using CERVUS v3.0.3 (Kalinowski *et al.* 2007) ; F_{IS} and allelic richness were calculated using FSTAT v2.9.3 (Goudet 1995). To complement adult nuclear analyses and assess temporal stability of genetic differentiation estimates, I calculated F_{ST} for two cohort sets of YOY for samples collected in 2009 (n=69; 7 swarming sites) and 2010 (n=102; 11 swarming sites).

To examine genetic differentiation among all swarming sites, F statistics were obtained by calculating overall and pairwise F_{ST} (FSTAT; Goudet 1995). A test for heterozygote deficiency was performed in GENEPOP. A Bayesian model-based clustering analysis was implemented using program STRUCTURE (v2.3.4) to infer the number of distinct genetic clusters within the nuclear dataset (Pritchard *et al.* 2000; Falush *et al.* 2003). Simulations were run without any *a priori* population information

incorporated, using the admixture model with correlated allele frequencies among groups (Falush *et al.* 2003). Ten replicate runs were performed for $K = 1$ to 15 (the maximum number of swarming sites) with a burn-in of 500,000 steps and 2,000,000 recorded for the Markov chain Monte Carlo (MCMC) steps. I examined the ln probability, ln[P(X|*K*)], of the ten runs for each value of *K* (Pritchard *et al.* 2000) to evaluate the most probable number of genetic clusters (i.e., subpopulations) in the data.

Spatial analyses of genetic structure were conducted by performing an isolation-by-distance analysis (IBD) to test for correlation between geographic distance and genetic differentiation using a Mantel test implemented in the web-based IBDWS (v3.23; http://idbws.sdsu.edu/~idbws/). Pairwise F_{ST} values were converted to (F_{ST} /(1- F_{ST})) following Rousset (1997) and the log of the geographic distances (straight-line linear distances) were used where geographic coordinates were determined at each site using a global positioning system (GPS). Similar to the mitochondrial data, I conducted an AMOVA for the full nuclear dataset on all adults.

In addition, I examined population structure by examining relatedness within swarming sites implemented in the program STORM (Frasier 2008). This program calculates the pairwise relatedness coefficient of Li et al. (1993) with the weighting by locus scheme of Lynch and Ritland (1999) and Van de Casteele et al. (2001). A relatedness coefficient was calculated for all pairs within each swarming site and the average was calculated within and across all swarming sites. To test if the average relatedness at swarming sites differs from expectations of random grouping (i.e., from individuals from any swarming site), individual genotypes were shuffled 999 times

between swarming sites keeping sample sizes the same to create a distribution of expected relatedness values from randomly associating individuals to estimate P-values.

Lastly, I tested for sex-biased dispersal by investigating F_{ST} and relatedness within each sex class using the method described by Goudet *et al* . (2002). I considered females to be the more philopatric sex fitting with the generalized pattern of mammals and previous work on temperate bats including specifically on *M. lucifugus* (Greenwood 1980; Kerth *et al.* 2002; Chen *et al.* 2008; Dixon 2011). Therefore, F_{ST} and relatedness are expected to be larger for females, the sex with the greater tendency to be philopatric. To test if these metrics statistically differed between the sexes I used the randomization approach implemented in FSTAT (10,000 permutations) where sex was randomly assigned to individuals within each subpopulation holding the number of each sex constant.

4.4 Results

4.4.1 mtDNA genetic variation

Ninety-five haplotypes were obtained from 356 adults sampled across the fifteen swarming sites. Fifty-three polymorphic sites defined the haplotypes arising from 45 transitions, 8 transversions and two insertion/deletion events. Many haplotypes were found in only single individuals (56.8%). After correcting for sample size, I found that at the regional level Quebec had the highest proportion of unique haplotypes (37.7%, n=69) followed by New Brunswick (27.3%, n=55) and Nova Scotia (17.7%, n=226). Four haplotypes were found in high frequency (n=30 or greater), with one found at 13

swarming sites (MYLU002, n=77) and two found at 12 swarming sites (MYLU006, n=35; MYLU007, n=30). Both of these haplotypes were found in all three provinces. The remaining high frequency haplotype (MYLU018, n=48) was found at 10 swarming sites, 9 of which were in Nova Scotia and at one site in New Brunswick where only 1 individual with this haplotype was found. Haplotype diversity (*h*) was relatively high averaging 0.8523 ± 0.0981(SD), and ranging from 0.8552 ± 0.0804, 0.8857 ± 0.1731, and 0.9073 ± 0.0285 for Nova Scotia, New Brunswick and Quebec, respectively. Nucleotide diversity (π) was generally low and similar across all swarming sites averaging 0.0150 ± 0.0028 (SD) with a range of 0.0094 to 0.0184, although by province it was highest in Quebec followed by Nova Scotia and New Brunswick at 0.0155 ± 0.0029, 0.0154 ±0.0025 , 0.0132 ±0.038, respectively.

In analyses of each sex, 57 haplotypes were found in 160 females and 71 haplotypes were found in 196 males. Females exhibited a trend of higher variation in haplotype diversity among provinces whereas males exhibited more similar haplotype diversity among provinces (Table S2, Supplementary Information) although these differences do not appear to differ greatly in magnitude; I did not test for significant differences. Also, these values were not corrected for sample sizes of each sex at each site. The pattern of variation in π among provinces was consistent for females but for males it was highest in Nova Scotia followed by Quebec and New Brunswick.

4.4.2 mtDNA population structure and demographic history

Structure inferred from mitochondrial data was an order of magnitude stronger than that from nuclear data (see below), but still indicative of low levels of population differentiation. Twenty-seven of the 105 pairwise comparisons (25.7%) of Φ_{ST} were

significant, after correction for multiple tests, and the global Φ_{ST} estimate for all adults was 0.045 ($P < 0.001$). Analyzed separately, males and females had similar global Φ_{ST} estimates of 0.045 and 0.052 (both $P < 0.001$), respectively. Hierarchical analysis by AMOVA found that the majority of mitochondrial genetic differences were within swarming sites (91.4%) and only 3.05% ($P <0.0001$) of the variation among provinces suggesting low regional structuring. Spatial analysis of molecular variance (SAMOVA) estimated two groups as the most likely scenario ($F_{CT} = 0.162$). However, this approach cannot test the probability of just one group and therefore this analysis could not differentiate between the hypotheses of one or two primary clusters. The median-joining network (Figure 2) demonstrated the lack of strong structuring by swarming site or by region (province) where many haplotypes were shared among sites and provinces with no distinct clustering of haplotypes by site. Low structuring within the network is suggestive of high historical gene flow. I defined an overall pattern of 7 haplogroups radiating off of an unsampled intermediate haplotype in the centre with several smaller haplogroups showing a star-like pattern of many single nucleotide substitution haplotypes off of these larger central, high frequency haplotypes; this pattern is indicative of a population expansion (Avise 2000).

The pairwise comparison of all samples yielded a mismatch distribution of a unimodal peak that fits with a model of population expansion (Figure 3). Fu's F_S statistic was statistically significant at $F_S = -99.87$ ($P < 0.001$) and ($P = 0.11$) and Fu and Li's F* and D* were not significant (F* = -2.052, $P > 0.10$; D* = -2.077, $P > 0.10$). The shape of the Bayesian skyline plot (BSP) suggests a similar population history to that of the mismatch distribution with an inferred population expansion (Figure 4). The mean

estimated divergence rate for the sequences was 15.7 %/Myr with a mean likelihood of -1322.98. The BSP suggests *M. lucifugus* experienced a demographic expansion between 1,250 and 12,500 before present, in the spatial region of the sampling.

4.4.3 Nuclear DNA genetic variation

Of the 10 microsatellite loci genotyped, one locus (*Mluc*30) was removed from subsequent analysis because of significant deviations from Hardy-Weinberg equilibrium (HWE) across all swarming sites. Eight of remaining nine loci (without inclusion of comparisons of *Mluc*30) generally met the assumptions of HWE (Table S3) with Mluc5 showing deviations from HWE in six of the 15 sites and a null allele frequency estimation of 6.3%. Locus Mluc21 showed deviations from HWE in 13 of 15 sites and a null allele frequency estimate of 31.5% but I chose to keep it to retain more loci for analyses after initial tests with it removed were generally concordant. Although null alleles can reduce genetic diversity resulting in increased F_{ST} estimates (Paetkau *et al.* 1997; Chapuis & Estoup 2007), my calculation of F_{ST} averaged the estimate over all loci which should reduce this bias. The assumptions of linkage equilibrium were generally met with only 4 of the 40 comparisons deviating from linkage equilibrium, after Bonferroni correction. The genotyping error rate for the 9 loci, calculated from duplicate runs and analysis of 61 individuals (8% of the dataset) ranged from 0% to 3.3% per locus with a mean error rate of 1.6% although I did not perform a blind test of this. I retained 735 adults and 168 YOY for analyses that were successfully genotyped at ≥ seven of the nine loci. Mean observed heterozygosity was moderately high for adults (Table 2), generally similar across swarming sites (0.686 ± 0.018 SD) and was similar to levels found in YOY sampled in 2009 (0.716 ± 0.065 SD; Table S4, Supporting Information)

and 2010 (0.730 ± 0.067 SD). Similar allelic richness values were observed across all three provinces for adults (6.15-7.15).

4.4.4 Nuclear DNA population structure

Metrics of population structure on nuclear markers indicate weak population differentiation. Of the 105 pairwise F_{ST} values from microsatellite data, 104 were non-significant (Table 3) and the global F_{ST} estimate was 0.001 ± 0.001 SE ($P = 0.02$). When analyzed separately, female and male adult bats also displayed a similar magnitude of global F_{ST} at 0.003 ± 0.002 SE ($P = 0.003$) and 0.001 ± 0.001 SE ($P = 0.002$), respectively. Despite the smaller sample sizes, the two cohort groups composed of YOY displayed a similar order of magnitude of global F_{ST} estimates to that found for the adult dataset (2009: $F_{ST} = 0.004 \pm 0.004$ SE; 2010: $F_{ST} = 0.002 \pm 0.003$ SE). These low estimates suggest high contemporary gene flow among swarming sites or shared recent ancestry for each sex and age class. Estimates of F_{IS} for all swarming sites were positive (Table 2) with a global estimate of $F_{IS} = 0.135 \pm 0.049$ (SE) and the global test for heterozygote deficiency was significant ($P < 0.005$). This suggests non-random mating may occur within sites although the presence of null alleles at some loci could also explain these positive F_{IS} estimates.

Low variance in the ln[P(X|*K*)] from the STRUCTURE analysis, across the replicates demonstrated convergence of the chains and indicated that $K = 1$ (mean ln[P(X|*K*)] = -24912.98 ± 0.175 (SD)) was the most likely number of genetic clusters represented in the data (Figure S1, Supplementary Material). No evidence of correlation between geographic distance and genetic differentiation (isolation by distance) was found ($r = 0.200$, $P = 0.877$). Hierarchical analysis by AMOVA of microsatellite data indicated

that the majority of genetic variation was found within swarming sites at the individual level (84.8% $P < 0.001$; Table 4) with low but significant variation found among swarming sites within provinces (15.0% $P < 0.001$) and very low variation found among provinces (0.15%, not statistically significant). Analyzed separately, adult males and adult females showed similar patterns to all adults together with only 0.11% and 0.08% of the variation found among provincial regions for males and females, respectively supporting the lack of isolation by distance pattern.

Average pairwise relatedness among individuals within swarming sites was low with a mean *r* of -0.015 (range: -0.061 to 0.033 per site) within all sites. The mean expected within swarming site pairwise relatedness in 1000 permutations was -0.015 and therefore the observed mean was not significantly different from simulated values of random groupings of bats ($P = 0.532$). Relatedness was similarly low for males and females when analyzed separately where no observed mean within-swarming sites estimates were significantly different from random expectations after Bonferroni correction (Table S5; Supporting Information). In testing for sex-biased dispersal in FSTAT, I found stronger differentiation for females compared to males (F_{ST} females = 0.0033, F_{ST} males = 0.0004) and higher relatedness for females compared to males (females: 0.0059; males: 0.0008). However, these differences were not significantly different (F_{ST} $P = 0.31$; relatedness $P = 0.30$).

4.5 Discussion

4.5.1 Population structure

Fitting with expectations of the high movement capabilities of the species, all lines of evidence from analysis of nuclear microsatellite data suggest weak population genetic structuring for *M. lucifugus* in south-eastern Canada consistent with high gene flow. I found low global and pairwise F_{ST} among swarming sites with only one significant pairwise comparison suggesting that some weak structuring does occur. The significant comparison occurred between site 8 and site 9 where aside from this comparison, most other comparisons involving site 8 had higher estimates of F_{ST}. This may reflect that this site was sampled more frequently than all other sites owing to concurrent studies being conducted there which could have influenced the estimates of allele frequencies at the site. Regardless, the single significant comparison suggests high gene flow. Further support of high genetic connectivity comes from the STRUCTURE results which did not detect any genetic clusters within the data, and from the low estimates of pairwise relatedness among individuals that did not differ from expectations of free mixing of bats among swarming sites (i.e., random). Similar low estimates of relatedness at swarming sites were also found in three species of whiskered bats (genus *Myotis*) despite the presence of some pairs that may be full siblings (Bogdanowicz *et al.* 2012a). Genetic variation was higher within swarming sites compared to among sites which is consistent with the extra-colony hypothesis and may suggest some swarming site fidelity albeit with high genetic exchange among swarming sites. Lastly, an AMOVA did not detect large structuring at a regional spatial scale nor did I detect a significant isolation-by-distance (IBD) pattern.

In an analysis of summer captured individuals along riparian corridors, Lausen (2007) detected a significant IBD pattern in *M. lucifugus* which contrasts with a recent

study of *M. lucifugus* maternity colonies where no significant IBD was detected (Dixon 2011); both studies occurred over a similar spatial scale (550-600 km) which were slightly smaller than mine (869 km). The extent to which an IBD pattern is displayed in bats tends to be stronger for more sedentary species compared to migratory species and depends on the spatial scale of sampling (Altringham 2011). However, it may also depend on landscape structure and context since availability and connectivity of habitat resources (e.g., foraging, roosting or commuting, swarming sites) can influence movements, dispersal and ultimately gene flow as shown in other mammals (Coulon *et al.* 2004; e.g., Broquet *et al.* 2006). The study by Lausen (2007) occurred in a prairie/agricultural landscape with sampling along river systems. This context may have restricted movements of individuals along these linear riparian features such that an IBD pattern was detected albeit within a larger framework of extensive gene flow within the species similar to that characterized in ours and the Dixon (2011) study. This current study occurred primarily in the Atlantic Maritime Ecozone which is characterized by extensive forest cover (76%; McAlpine & Smith 2010), and also occurred during the autumn swarming and migration period where movements are expected to be greater. This contrasts to the timing and landscape of Lausen's study.

The results from the mitochondrial DNA analyses also showed low levels of genetic structuring and further suggest a recent history of high gene flow in the sampled region. No structuring was detected based on geography in the SAMOVA analysis nor was there strong support from the hierarchical AMOVA to detect structure associated with geography at multiple spatial scales. The median joining network displayed little structuring of haplotypes by swarming site with many high frequency haplotypes found at

multiple sites and no clustering in any areas of the network by individual sites. In examining the network at the provincial level, there is still little evidence for strong structuring with 6 of the 7 haplogroups containing sequences found in ≥ 2 provinces with the exception of the singleton found in Nova Scotia. Site 7 stands out as having some of the highest Φ_{ST} values with other close by swarming sites in Nova Scotia and Quebec. This site was sampled on only two nights in one year, 2010, whereas most other sites were sampled more frequently (multiple nights in multiple years). If bats on a given night represent a small proportion of those swarming over the season and these bats are from the same summering colony/area that share common ancestry, this could explain these results.

Taken together, my results are consistent with weak genetic structuring as has been observed in other bat species known to swarm such as *M. nattereri* (Rivers *et al.* 2005), the three species of the *M. mystacinus* species complex (Bogdanowicz *et al.* 2012a) and *Plecotus auritus* (Furmankiewicz & Altringham 2007). Consistent with the extra-colony hypothesis, swarming appears to facilitate gene flow among segregated behavioural summer groups with recent work demonstrating greater genetic diversity and lower relatedness at swarming sites relative to summer maternity colonies (Veith *et al.* 2004; Furmankiewicz & Altringham 2007; Kerth *et al.* 2008). This suggests bats from multiple colonies meet at swarming sites but may not necessarily mate there. However, using simulations, Rivers *et al*. (2005) found that in the swarming *M. nattereri*, the levels of observed population structure were most consistent with a model with effective mating occurring at swarming/hibernation sites rather than within summer colonies. In conjunction with behavioural studies documenting mating activities at swarming sites

(Barclay & Thomas 1979; McGuire *et al.* 2009; Furmankiewicz *et al.* 2013), this supports the contention that swarming sites are 'hot spots' for gene flow (Kerth *et al.* 2003).

Bat species that make extensive migratory movements or have large dispersal capacities can be characterized by near panmictic genetic structures such as *Tadarida brasiliensis* (McCracken *et al.* 1994; Russell *et al.* 2005a), *N. noctula* (Petit & Mayer 1999) and *Pipistrellus pipistrellus* and *P. pygmaeus* (Bryja *et al.* 2009). It is important to note that regional differences in the magnitude of population genetic structure can also be found in some migratory species owing to different landscapes and resultant migratory behaviour (Bryja *et al.* 2009; Sztencel-Jablonka & Bogdanowicz 2012). In *N. noctula* and another long distance migratory Pipistrelle species (*P. nathusii*), mating takes place during migration (Petit & Mayer 2000; Petit *et al.* 2001; Hutterer *et al.* 2005) which may largely explain many of the low genetic structures observed in migratory species over great distances. I suggest that for *M. lucifugus* in my study area, the evidence of weak genetic structuring is likely due to a combination of swarming behaviour, which facilitates dispersal among segregated winter and summer groups, and the high movement capability of *M. lucifugus* due to migration during this period. Although mating may occur outside of the swarming period for *M. lucifugus* (Fenton 1969; Thomas *et al.* 1979), and recent work in the swarming *M. daubentonii* has shown mating to occur at summer sites (Senior *et al.* 2005; Angell *et al.* 2013), further work would be required to assess the importance of mating activities occurring away from swarming sites to the overall mating strategies and contributions to gene flow in *M. lucifugus*. In a study of *M. nattereri*, Rivers *et al.* (2005) found that swarming sites show genetic distinctiveness over a smaller geographic range than my study area and suggested that bats from a given

summer colony show high swarming site fidelity. This differs from *M. lucifugus,* which appears to show less swarming site fidelity being more transient in their swarming movements (Humphrey & Cope 1976; Norquay *et al.* 2013) compared to *M. nattereri.*

Myotis lucifugus is thought to display the typical mammalian pattern of male natal dispersal (Greenwood 1980) with males generally not returning to their natal maternity colonies to associate closely with females in subsequent years (Davis & Hitchcock 1965; Fenton 1969; Frick *et al.* 2010b). Since swarming in bats may function as a form of temporary dispersal facilitating gene flow among individuals segregated during the summer, characterizing the extent of sex-biased dispersal in determining biases in sex-directed gene flow should take place at swarming sites when most mating is thought to occur. I did not find evidence to suggest strong asymmetries in gene flow between the sexes on bi-parentally inherited nuclear markers. However, it is important to keep in mind that the method I used detects recent differences in dispersal (Prugnolle & de Meeus 2002). Further, it makes many assumptions such as sampling after dispersal has occurred, which is problematic in species with overlapping generations, and works best under scenarios of strong sex-biases in dispersal (Goudet *et al.* 2002) which may not be the case for *M. lucifugus*.

Movement data from recapture studies during the swarming period are scarce but there are occurrences of large movements by both males and females. Work from Manitoba and Ontario (Canada) for *M. lucifugus* showed 2 females and 3 males were captured visiting multiple swarming sites (Norquay *et al.* 2013). This and other studies have shown both sexes swarming and hibernating at different sites within and among years (Davis & Hitchcock 1965; Fenton 1969; Humphrey & Cope 1976). Autumn

swarming appears to be a complex time for bats with an individual's activity including migrating to overwintering sites, increasing fat stores for hibernation and mating. Thus, individuals may have multiple motivations impacting their decisions on their activities during this time stemming from differences in energy allocation (Kunz *et al.* 1998). For males, movements may primarily reflect mating choices in trying to maximize mating opportunities during swarming but may also represent movements made in selecting an optimal hibernation site. Females may more strongly select the later scenario over securing many mating opportunities compared to males. Regardless, if regular movements by both sexes ultimately contribute to gene flow, then this occurs among sites by both sexes during this temporary dispersal period and my data support this assertion. Inference of sex-biased dispersal can also come from comparisons of structure on markers with different modes of inheritance (i.e., mtDNA) which tend to reflect more long-term patterns of gene flow. I found stronger structuring on mtDNA compared to nuclear DNA which may suggest there is a male-bias in gene flow long-term since stronger differentiation on mtDNA is expected when females are more philopatric (Prugnolle & de Meeus 2002). However, regular movements by both species during swarming may reduce the magnitude of the bias as detected in the short term. Future work quantifying sex specific-demographic parameters to estimate the magnitude of the male-biased dispersal should be undertaken to better understand these dynamics such as was done for *N. noctula* (Petit *et al.* 2001).

4.5.2 Population history

The data suggest that *M. lucifugus* in my study area experienced a population expansion since the last glaciation. This interpretation is supported by several lines of

evidence including the star-like topology of the median joining network, neutrality tests, mismatch distribution and Bayesian skyline plot analysis. A significant neutrality test can suggest multiple scenarios including a population expansion, genetic 'hitchhiking' by an advantageous mutation or background selection. However, some of these various explanations can potentially be differentiated from each other by comparing different neutrality tests. For example, Fu and Li's F* and D* statistics (Fu & Li 1993) are more strongly affected by background selection relative to Fu's F_S statistic (Fu 1997) which is more strongly affected by population expansion or selective sweeps. In comparing the two, a significant F_S and non-significant F*and D* indicate an excess of singleton haplotypes which favours a scenario of a population expansion or a selective sweep rather than background selection which the data show. I cannot rule out the possibility of a past selective sweep that replaced all mtDNA haplotypes which was then subsequently followed by an accumulation of neutral variants from that haplotype (Maruyama & Birky 1991). However, the concordance of this expansion scenario with other supporting analyses strongly supports a population expansion as does additional information from the molecular diversity indices of the mtDNA data.

Haplotype diversity (*h)* in the HV II region was relatively high and nucleotide diversity (π) was low, a pattern which is consistent with a population expansion. A similar pattern of exceptionally high *h* and low π was described in the tropical Brazilian free-tailed bat (*T. brasiliensis)* which is thought to have undergone an expansion within the past 3000 years (Russell *et al.* 2005a). The levels of *h* and π that I found are more similar to those found in the temperate common noctule bat (*N. noctula*) where the inferred expansion followed the Younger Dryas period (12,900 -11,500 BP; Petit *et al.*

1999). From the BSP with an estimated divergence rate of 15.7 % / Myr, I estimate a population expansion occurring from approximately 12,500 to 1,250 BP which broadly correlates to recolonization of forests in the region that occurred following Pleistocene glaciation in North America. It is now thought that during the last glacial maximum (LGM; approximately 18 ka), the ice sheet in south-eastern Canada extended close to the present day off-shore continental shelf with ice free areas extending south of the region along the coast in the United States with glacial refugia on present day George's Bank just south of Nova Scotia (Shaw *et al.* 2006). Although other off-shore refugia have been proposed and debated under alternate models of glacial reconstruction (Pielou 1991; Davis & Browne 1996), their occurrence may have been after the LGM (Shaw *et al.* 2006), or were short in duration following changing sea levels (Holland 1981; Shaw *et al.* 2002) such that they may not have supported extensive forest ecosystems to act as suitable refugia for bats. Following glacial retreat, forest recolonization is thought to have occurred from the south by 13 ka (summarized in Miller 2010) and the estimated population expansion for *M. lucifugus* follows this shortly thereafter. The high vagility of bats and the behavioural flexibility in roosting exhibited by *M. lucifugus* (Fenton & Barclay 1980) may mean that they could have closely tracked forest recolonization including use of early open-stand forests through to their replacement by closed-stand forests. The presence of caves containing fossil Quarternary mammals in an area just north of the study area (Gaspé, Quebec; Harington 2011) suggest that some underground sites have a long history of existence in the region that could facilitate the hibernation requirements of bats as they recolonized forested areas.

4.5.3 Genetic connectivity and conservation implications

My findings suggest a high degree of genetic connectivity in *M. lucifugus* with gene flow occurring from dispersal by both males and females, although it may be male-biased. Along with mutation and selection, gene flow is only one of the major forces that shape the genetic structure of species and the role of genetic drift must also be considered (Hartl & Clark 1997). Gene flow can counteract the effects of genetic drift by opposing the divergences that strong genetic drift reinforces. However, the effects of drift depend on effective population size (N_e) where large N_e reduces the role of genetic drift. Key factors that influence N_e include population size and patterns of reproductive success (Allendorf & Luikart 2007). Bats can exhibit social structures and mating systems that can result in non-random mating leading to variation among individuals in reproductive success and potentially N_e despite large population abundances (Storz 1999). Although N_e has not been quantified for *M. lucifugus*, I expect it to be quite large (at least prior to WNS) based on large historical hibernating population estimates (Trombulak *et al.* 2001; Frick *et al.* 2010a; Turner *et al.* 2011) and linkages between many of the sites from banding work (Davis & Hitchcock 1965; Fenton 1970; Humphrey & Cope 1976) such has been shown in *T. brasiliensis* (Russell *et al.* 2005a). With a large N_e, low levels of genetic structure are expected even with low genetic exchange and future work that characterizes this parameter would provide valuable insight into the genetic structuring of *M. lucifugus*, particularly in light of the large population declines from WNS.

The implications of high genetic connectivity in managing populations under the epizootic of WNS remain complicated and largely unknown. High genetic connectivity may imply dispersal, whether permanent or temporary, over extensive spatial scales.

Since recent work has shown bat-to-bat transmission in a laboratory setting (Lorch *et al.* 2011), it is possible that these movements, if the bats are infectious at the time, could be contributing to the rapid spread of the disease. Although this has not been tested, bats have many opportunities for direct contact with other bats during swarming due to mating and potentially information sharing activities occurring within and around underground sites. These activities could facilitate transmission of spores among bats if these sites act as environmental reservoirs of *P. destructans* (Lindner *et al.* 2011). Taken together with evidence of large movements from recapture data from swarming and among hibernation sites (Humphrey & Cope 1976; Norquay *et al.* 2013), this high level of connectivity may partially explain the rapid spread of the disease. Recent work has shown correspondence among genetic structure and the spread of WNS in Pennsylvania in *M. lucifugus* which may reflect movement patterns of bats (Miller-Butterworth *et al.* 2014). In my study area, WNS was detected first in Quebec followed by detection in the neighbouring provinces of New Brunswick and Nova Scotia a year later with the total spread of the disease in North America from discovery in 2006 in excess of 2000 km. With no effective means to control the spread of the disease thus far, further work assessing connectivity, both genetic and demographic, within other areas of the species range may be able to provide information in the short term on transmission dynamics and spread. However, this knowledge may also be used to inform pertinent demographic questions on survival, immigration and emigration rates as they relate to future population persistence and connectivity of local populations (Lowe & Allendorf 2010).

In summary, my findings suggest high gene flow and therefore high genetic connectivity among swarming sites of *M. lucifugus* in south-eastern Canada. I did not

find evidence to suggest a strong signature of structure but rather found evidence of a demographic expansion following deglaciation of the region. Although my study suggests dispersal over a large spatial scale in the recent past, predicting how the dynamics of dispersal will contribute to the trajectory of population persistence in the future is not a straightforward process. Since the emergence of WNS, many local hibernating populations have been dramatically reduced in the eastern portion of the range (Frick *et al.* 2010a; Ingersoll *et al.* 2013), including my study area (Burns & Broders, unpublished data). Future work should incorporate other approaches to characterize dispersal and other demographic parameters in addition to genetic data to allow predictions to be made on population viability in light of WNS. Though it was not an initial goal of this study, my data will provide a valuable baseline for future comparative studies of genetic structure and connectivity before and after a large mortality event. An understanding of the patterns of connectivity prior to such an event may enable such information to be incorporated into management plans for other regional populations prior to the arrival of WNS in those regions and in this region in a post-WNS setting.

Table 4-1 Sampling site locations and numbers of individual *Myotis lucifugus* included in mitochondrial and nuclear microsatellites analyses. Young-of-the-year were nuclear microsatellites only. NS= Nova Scotia, NB = New Brunswick , QC = Quebec.

	Number of adults					Young-of-the-year	
Site	Province	mtDNA		microsatellites		2009	2010
1	NS	18	(3 F/ 15 M)	25	(5 F/ 20 M)	5	7
2	NS	22	(11 F/ 11 M)	60	(25 F/ 35 M)	3	15
3	NS	29	(14 F/ 15 M)	70	(22 F / 48 M)	-	2
4	NS	23	(15 F/ 8 M)	126	(70 F / 56 M)	27	9
5	NS	30	(16 F/ 14 M	47	(24 F/ 23 M)	4	7
6	NS	30	(14 F/ 16 M)	54	(15 F/ 39 M)	8	8
7	NS	14	(5 F/ 9 M)	15	(5 F/ 10 M)	-	9
8	NS	31	(15 F/ 16 M)	88	(26 F/ 62 M)	17	23
9	NS	29	(14 F/ 15 M)	70	(23 F/ 47 M)	-	3
10	NB	27	(6 F/ 21 M)	29	(6 F / 23 M)	-	3
11	NB	28	(15 F/ 13 M)	40	(16 F/ 24 M)	-	15
12	NB	6	(3 F/ 3 M)	11	(4 F/ 7 M)	-	-
13	QC	28	(13 F/ 15 M)	60	(24 F/ 36 M)	-	-
14	QC	27	(10 F/ 17 M)	26	(7 F/ 19 M)	3	-
15	QC	14	(6 F/ 8 M)	14	(6 F/ 8 M)	-	-

Table 4-2 Genetic variation descriptors at 9 microsatellite loci and a 292-bp fragment of the mitochondrial DNA control region in adult *M. lucifugus* in south-eastern Canada including the mean number of alleles per locus (A/locus), allelic richness (AR), observed heterozygosity (H_O),within site inbreeding coefficient (F_{IS}), expected heterozygosity (H_E), haplotype diversity (h) and nucleotide diversity (π).

	Nuclear microsatellite data					Mitochondrial control region		
Site	A/locus	AR	H_O	H_E	F_{IS}	Number haplotypes	h	π
1	9.3	6.86	0.653	0.812	0.145	9	0.869	0.0173
2	11.2	6.83	0.670	0.804	0.105	10	0.788	0.0140
3	12.2	6.81	0.692	0.792	0.081	18	0.958	0.0184
4	13.4	7.02	0.710	0.822	0.080	10	0.850	0.0152
5	10.6	6.72	0.671	0.807	0.084	14	0.897	0.0171
6	11.4	7.05	0.687	0.817	0.093	15	0.897	0.0177
7	8.0	6.87	0.674	0.823	0.133	6	0.681	0.0117
8	12.2	6.67	0.682	0.800	0.099	15	0.908	0.0153
9	11.9	6.74	0.697	0.805	0.079	16	0.850	0.0117
10	10.1	6.74	0.709	0.803	0.077	17	0.940	0.0169
11	10.7	7.15	0.697	0.828	0.111	13	0.825	0.0134
12	6.6	6.15	0.667	0.779	0.106	3	0.600	0.0094
13	12.2	7.06	0.696	0.827	0.095	14	0.892	0.0123
14	9.6	6.76	0.714	0.824	0.056	16	0.940	0.0183
15	8.0	7.12	0.667	0.801	0.090	10	0.890	0.0158

Table 4-3 Pairwise F_{ST} estimates for 15 swarming sites for *M. lucifugus* based on nuclear microsatellite variation (above diagonal), and pairwise Φ_{ST} estimates based on mtDNA control region (below diagonal).

Site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1		0.000	0.000	-0.001	-0.001	-0.002	-0.005	-0.001	0.000	-0.001	-0.003	-0.004	0.001	-0.004	0.001
2	0.035		0.002	-0.001	-0.001	-0.002	-0.001	0.001	0.000	0.003	0.002	0.000	0.006	-0.005	0.008
3	0.049	**0.113**		0.003	0.002	-0.001	0.003	0.003	0.001	-0.003	0.003	0.000	0.010	-0.001	0.009
4	-0.009	-0.002	0.064		0.003	-0.002	-0.003	0.001	-0.001	-0.002	-0.002	-0.004	0.001	-0.002	0.001
5	-0.006	0.018	0.017	-0.021		0.001	0.001	0.002	0.004	0.007	0.009	0.001	0.009	0.001	0.010
6	0.011	0.011	0.007	0.006	-0.016		-0.006	0.003	-0.002	-0.003	0.000	-0.004	0.005	-0.005	0.004
7	0.148	**0.297**	0.124	0.156	0.121	**0.162**		0.005	-0.004	-0.003	0.000	-0.006	-0.003	0.000	-0.001
8	0.074	0.116	-0.010	0.062	0.014	0.007	0.094		**0.005**	0.000	0.001	0.000	0.003	-0.001	0.008
9	0.097	-0.015	**0.195**	0.066	**0.087**	**0.076**	**0.420**	**0.215**		-0.004	0.000	0.001	0.005	0.001	0.003
10	0.047	-0.057	0.189	0.020	0.055	0.051	0.432	**0.218**	-0.081		-0.003	-0.003	0.001	-0.012	0.004
11	0.006	0.010	0.036	0.001	-0.003	-0.003	**0.193**	0.051	0.063	0.024		-0.005	0.000	-0.004	0.000
12	**0.090**	-0.016	**0.138**	0.065	0.070	0.032	**0.372**	**0.151**	-0.002	-0.029	0.036		0.004	-0.007	0.001
13	0.063	0.085	**0.086**	0.077	0.060	0.062	**0.271**	**0.128**	**0.107**	0.064	0.022	**0.090**		0.009	0.004
14	0.068	**0.153**	0.094	**0.130**	0.106	**0.101**	**0.344**	**0.179**	**0.172**	0.145	0.060	0.151	0.007		0.008
15	0.062	-0.003	**0.134**	0.053	0.056	0.039	**0.379**	**0.164**	-0.004	-0.038	0.015	-0.009	0.036	0.083	

Swarming site codes are given in Table 1. Bold numbers indicate significant after Bonferroni corrections

Table 4-4 Hierarchical analysis of molecular variance (AMOVA) among mtDNA control sequences (Φ_{ST}) and 9 nuclear microsatellite loci (F_{ST}) of *M. lucifugus* with regional (provinces) groupings. Percentage of the variation is for the three hierarchical levels.

Source of variation	Φ_{ST} Sum of Squares	Variance components	Variation (%)	F_{ST} Sum of Squares	Variance components	Variation (%)
All adults						
Among provinces	25.455	0.075	3.05	11.548	0.005	0.15
Among swarming sites w/n provinces	64.746	0.136	5.53	3079.456	0.550	15.02
Within swarming sites	763.67	2.240	91.42	2283.5	3.107	84.83
Total	853.871	2.450		5374.504	3.662	
Females						
Among provinces	14.594	0.090	3.75	9.19	0.003	0.08
Among swarming sites w/n provinces	43.16	0.138	5.74	1174.826	0.597	16.24
Within swarming sites	314.284	2.167	90.51	855.5	3.077	83.67
Total	372.038	2.395		2039.516	3.678	
Males						
Among provinces	17.078	0.076	3.02	9.9	0.004	0.11
Among swarming sites w/n provinces	48.676	0.141	5.62	1891.176	0.520	14.26
Within swarming sites	413.941	2.287	91.36	1428	2.125	85.63
Total				3329.075	3.649	

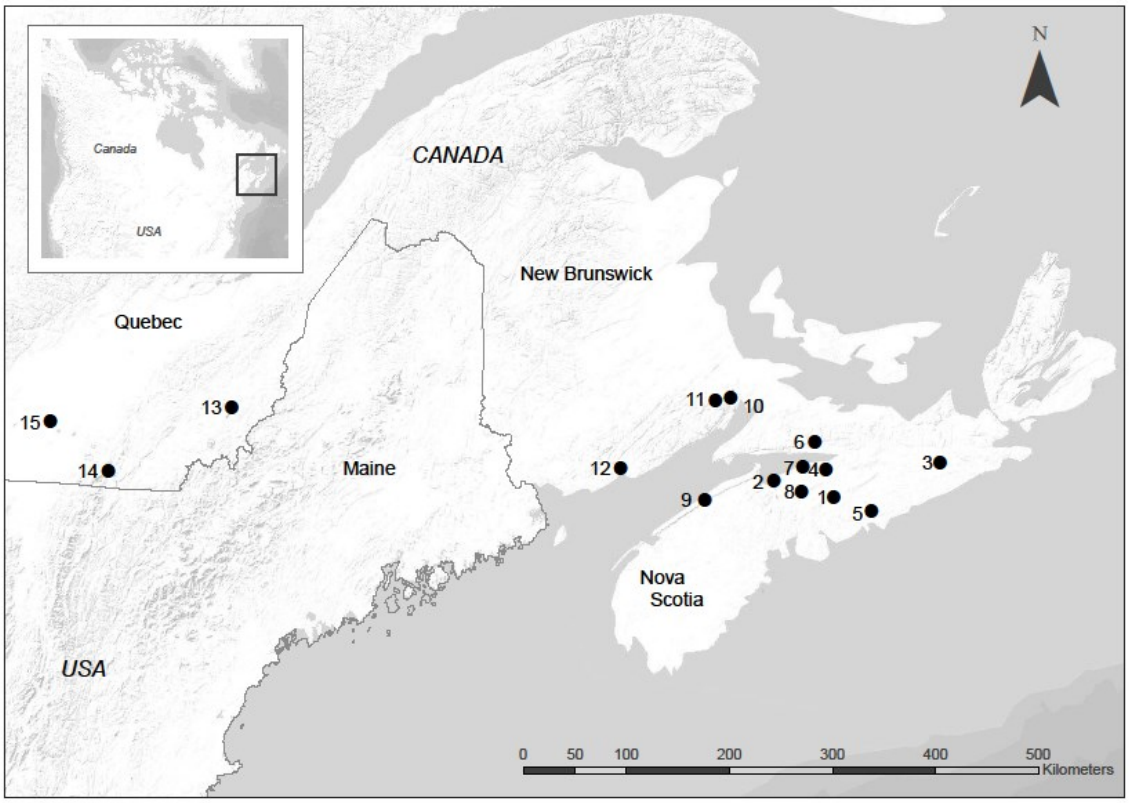

Figure 4.1 Sampling locations for *M. lucifugus* captured at swarming sites in south-eastern Canada to assess population genetic structure. Geographic coordinates and names are not used due to the sensitive nature of swarming and hibernation sites; numbers correspond to site numbers in tables.

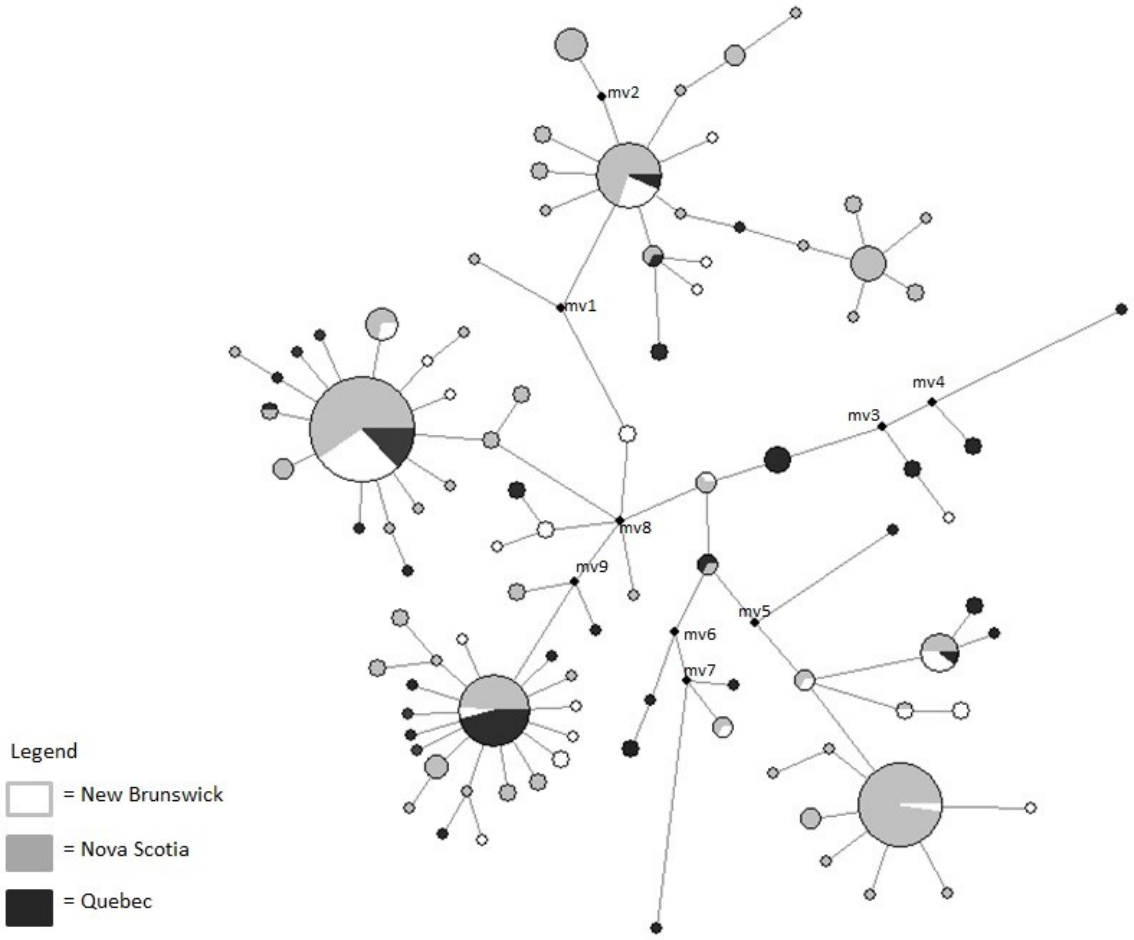

Figure 4.2 A median-joining network for *M. lucifugus* based on a 292 base pair mitochondrial DNA segment of the control region coded by province. Circle size corresponds to haplotype frequency with inferred hypothetical haplotypes (mv) not sampled in the current study shown.

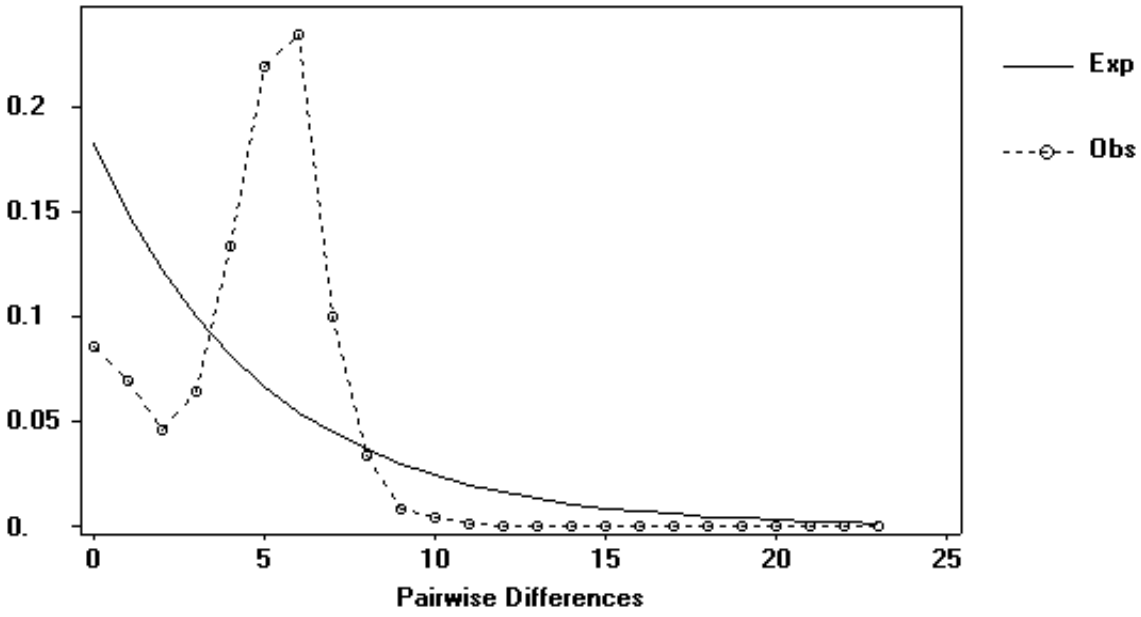

Figure 4.3 Mismatch distribution of *Myotis lucifugus* based on a 292 base pair segment of the mitochondrial control region showing the observed frequency of pairwise differences among sequences (hatched line). The expected distribution (solid line) is for a population of constant size.

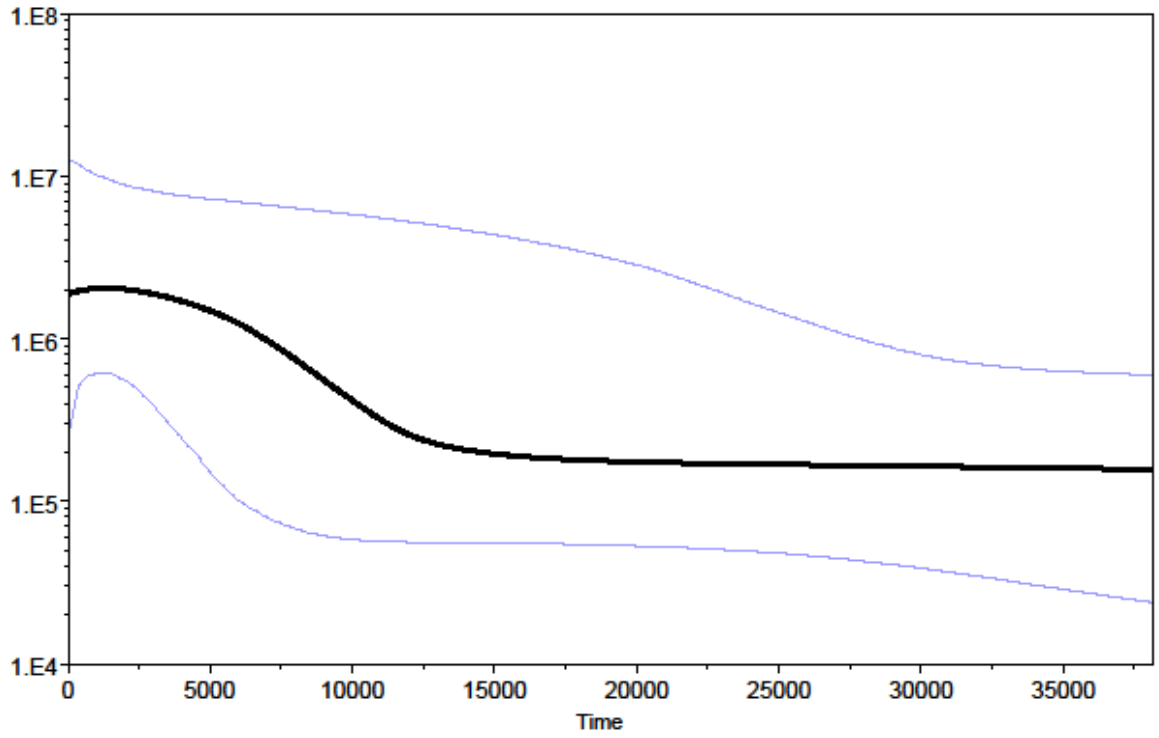

Figure 4.4 Bayesian skyline plot of the changes in effective population size backwards in time for *M. lucifugus* sampled from swarming sites in south-eastern Canada. The x-axis represents time measured in years and the y-axis the population size (logarithmic) expressed as the product of the effective population size and the generation time in years ($N_e\tau$).

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4.7 SUPPLEMENTARY MATERIAL

Table 4S 1 Multiplex PCR conditions, loci specific fluorescent dye, observed number of alleles and allele size ranges (base pairs) for microsatellite loci used in genotyping *Myotis lucifugus* bats from south-eastern Canada.

Locus	Fluorescent dye	Multiplex Reaction	Annealing temperature (°C)	Primer concentration [μM]	Number of alleles	Allele sizes (bp)
*Mluc*1	6-FAM	1	60	0.08	10	115-151
Mluc4	VIC	2	60	0.10	8	141-169
Mluc5	6-FAM	2	60	0.10	10	132-172
Mluc7	NED	3	60	0.10	31	140-260
Mluc8	PET ®	2	60	0.20	28	145-285
Mluc11	6-FAM	4	55	0.10	10	220-256
Mluc21	6-FAM	1	60	0.20	5	303-319
Mluc25	PET ®	4	55	0.15	27	298-402
Mluc30	6-FAM	3	60	0.15	59	266-402
Mluc34	PET ®	3	60	0.20	15	328-384

Table 4S 2 Genetic variation in a 292-bp fragment of the mitochondrial DNA control region in adult male and female *M. lucifugus* in south-eastern Canada as haplotype diversity (*h*) and nucleotide diversity (π).

Site	Males (*n* =196)			Females (*n* =160)		
	Number of haplotypes	*h*	π	Number of haplotypes	*h*	π
1	9	0.876	0.0179	3	1.000	0.0160
2	7	0.873	0.0162	5	0.709	0.0115
3	10	0.924	0.0194	12	0.967	0.0167
4	5	0.857	0.0166	9	0.876	0.0154
5	7	0.879	0.0159	11	0.933	0.0182
6	10	0.900	0.0186	8	0.901	0.0174
7	5	0.722	0.0130	3	0.700	0.0110
8	9	0.908	0.0155	11	0.933	0.0153
9	8	0.733	0.0087	10	0.934	0.0139
10	14	0.938	0.0160	6	1.000	0.0192
11	9	0.923	0.0148	7	0.724	0.0113
12	2	0.667	0.0115	2	0.667	0.0092
13	9	0.905	0.0135	8	0.859	0.0105
14	11	0.934	0.0184	8	0.956	0.0155
15	5	0.786	0.0133	6	1.000	0.0206

Table 4S 3 *P* values for deviation from HWE for each locus and swarming site sampled in genotyping *Myotis lucifugus* bats from south-eastern Canada.

	Swarming site															
Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Null allele frequency
Mluc1	0.569	0.029	0.478	0.295	0.337	0.214	0.250	0.434	0.893	0.790	0.760	0.012	0.471	0.739	0.415	0.011
Mluc4	0.022	0.156	0.892	0.067	0.867	0.588	0.785	0.965	0.604	0.290	0.522	0.886	0.768	0.007	0.233	0.032
Mluc5	0.605	0.577	0.145	0.014	0.029	0.217	0.000	0.221	0.011	0.740	0.079	0.443	0.000	0.046	0.361	0.063
Mluc7	0.754	0.231	0.310	0.723	0.180	0.071	0.854	0.525	0.644	0.761	0.000	0.867	0.361	0.170	1.000	0.006
Mluc8	0.972	0.426	0.900	0.069	0.053	0.157	0.485	0.138	0.116	0.122	0.500	0.012	0.544	0.595	0.010	0.024
Mluc11	0.272	0.262	0.184	0.423	0.879	0.002	0.499	0.038	0.103	0.618	0.873	0.477	0.006	0.762	0.583	0.071
Mluc21	0.000	0.000	0.000	0.000	0.001	0.002	0.002	0.000	0.000	0.001	0.000	0.090	0.000	0.103	0.015	0.315
Mluc25	0.364	0.659	0.607	0.431	0.270	0.804	0.115	0.082	0.923	0.718	0.416	0.986	0.809	0.244	0.590	0.002
Mluc34	0.124	0.646	0.009	0.853	0.789	0.218	0.277	0.081	0.916	0.463	0.089	0.981	0.279	0.953	0.914	0.014

Table 4S 4 Genetic variation descriptors at 9 microsatellite loci in young-of-the-year *M. lucifugus* bats in south-eastern Canada. Measure include the mean number of alleles per locus (A/locus), observed heterozygosity (H_O), expected heterozygosity (H_E) and within site inbreeding coefficient (F_{IS}).

	2009 Juveniles (n= 67)				2010 juveniles (n =101)			
Site	A/locus	H_O	H_E	F_{IS}	A/locus	H_O	H_E	F_{IS}
1	4.67	0.627	0.777	0.213	6.11	0.714	0.812	0.129
2	3.44	0.778	0.807	0.053	7.78	0.772	0.755	-0.023
3	-	-	-	-	3.00	0.833	0.796	-0.083
4	9.22	0.722	0.787	0.084	6.67	0.750	0.818	0.089
5	7	0.775	0.728	0.155	6.56	0.812	0.853	0.055
6	4.33	0.630	0.832	0.074	7.00	0.749	0.797	0.067
7	-	-	-	-	6.11	0.658	0.781	0.167
8	8.22	0.713	0.807	0.120	8.78	0.765	0.800	0.045
9	-	-	-	-	3.67	0.685	0.811	0.196
10	-	-	-	-	3.44	0.611	0.759	0.25
11	-	-	-	-	8.11	0.686	0.787	0.134
14	3.67	0.767	0.741	0.036	-	-	-	-

Table 4S 5 Average pairwise relatedness coefficients for individual *M. lucifugus* from swarming sites and the average across all swarming sites. No coefficients were found to be significantly different from random groupings of bats across all swarming sites after Bonferroni correction.

Site	All adults	Females	Males
1	-0.033	-0.066	-0.027
2	-0.014	0.006	-0.033
3	-0.001	0.060	-0.029
4	-0.031	-0.038	-0.024
5	-0.030	-0.125	-0.003
6	-0.006	0.004	-0.021
7	-0.061	0.005	-0.057
8	0.020	0.039	0.009
9	0.000	0.010	-0.011
10	0.001	0.026	-0.005
11	-0.051	-0.050	-0.052
12	0.033	-0.029	0.028
13	-0.030	-0.076	0.002
14	-0.010	-0.067	0.017
15	-0.018	-0.042	-0.039
Average	-0.015	-0.023	-0.016

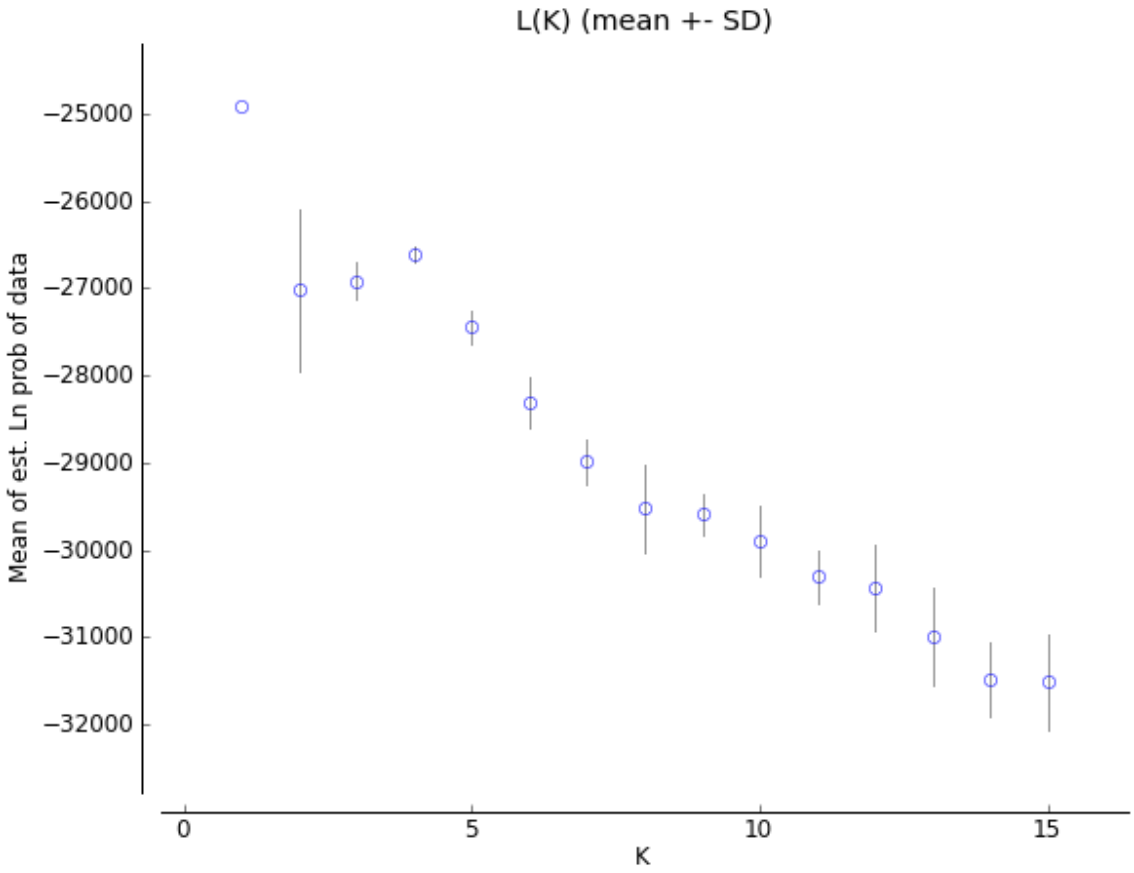

Figure 4S 1 Mean posterior probability of the data (Ln*P*(*D*) against the number of *K* genetic clusters within the data for 735 *M. lucifugus* sampled at 15 swarming sites in south-eastern Canada.

CHAPTER 5 CORRELATES OF DISPERSAL EXTENT PREDICT THE DEGREE OF POPULATION GENETIC STRUCTURING IN BATS

5.1 ABSTRACT

Dispersal is essential for maintaining demographic and genetic connectivity. For bats, correlates of dispersal extent such as morphology and movement dynamics are reported as having an influence on population genetic structure although these traits exhibit co-variance which has not been previously examined. I used a principal components framework with phylogenetically independent contrasts to compare five dispersal extent predictors (wing loading, aspect ratio, geographic range size, migratory status and median latitude) with population genetic structure among bats. I found that high wing loading values and migration negatively correlate with genetic structure after accounting for co-variance. These findings suggest that bats that can achieve higher flight speeds and migrate seasonally have higher gene flow and resultant genetic connectivity relative to bats that fly slower and do not migrate. These results represent a step towards understanding factors that shape the genetic structure of bat populations.

5.2 INTRODUCTION

Dispersal plays a key role in population demographic and genetic connectivity and is critical for long-term persistence of species (Slatkin 1985; Lowe & Allendorf 2010). Directly quantifying dispersal for mobile organisms (e.g., identifying dispersing individuals and actual dispersal movements) remains a challenge for most species, but the

identification of traits correlated with dispersal via comparative methods has been valuable for indirectly assessing dispersal for many taxa (Paradis *et al.* 1998; Bowman *et al.* 2002; Whitmee & Orme 2013). Since dispersal influences the extent and patterns of gene flow it can be an important determinant of population genetic structure (Bohonak 1999), and over time it can influence patterns of genetic divergence and ultimately speciation (Doebeli & Dieckmann 2003).

Broadly, interspecific variation in vagility correlates with the degree of genetic structuring of populations within their respective distributions. This was demonstrated early on in Barrowclough's review (1983) of allozyme-based studies that compared structure in amphibians, reptiles, non-volant mammals, and birds. There, the highest degree of structure was found in amphibians and the lowest in birds although there was variability within taxa. Thus, a species' propensity to disperse broadly correlates with the degree of population structuring where species that have a strong dispersal capacity and therefore high gene flow, have resultant low structure (Bohonak 1999; Bradbury *et al.* 2008). For flying species that in turn have high vagility, morphological traits that correlate with dispersal ability include measures of wing shape as they relate to flight efficiency (Bowlin & Wikelski 2008; Sekar 2012; Taylor *et al.* 2012). Dispersal ability is generally considered as taxon specific as it is related to a suite of inter-related morphological, behavioural and life history traits. However, intra-specific variation in the propensity to disperse, or in distance dispersed may also be important (Clobert *et al.* 2009; Stevens *et al.* 2010).

Bats, order Chiroptera, are a diverse taxon with a potential capacity for high dispersal owing to their ability to sustain flight. Several morphological, behavioural and

ecological traits have been hypothesized to explain variation in the degree of population structure observed in this order. Wing morphology metrics such as the wing loading and wing aspect ratio have been found to correlate with maneuverability, foraging efficiency and the tendency to migrate (Aldridge & Rautenbach 1987; Norberg & Rayner 1987). Wing loading is a measure of wing size relative to body size ([body mass x gravity] / wing area) where higher wing loading is positively correlated with flight speeds and therefore may influence dispersal distance. Aspect ratio is a metric of wing shape ([wing span area]^2/ wing area) where higher aspect ratios are positively associated with flight efficiency over long distances. Using these metrics, several authors have been able to explain some of the interspecific variation in the degree of population structuring among bats (i.e., demographic and genetic structuring; Norberg & Rayner 1987; Entwistle *et al.* 2000; Olival 2012; Taylor *et al.* 2012). Since foraging strategies also place selection pressure on wing characteristics related to the aerodynamics needed to forage in a specific way, this may favour traits different than migration. Thus the most suitable wing shape parameters for one aspect of a bats life history may not be as well suited to other aspects (Altringham 2011) and wing shape alone likely cannot fully explain the differences in population structuring among bats.

Understanding how differences in the propensity to migrate relate to various differences in life history traits among bats has become a topic of interest in the literature (McGuire & Ratcliffe 2011), including how differences may relate to population structuring (Moussy *et al.* 2012; Olival 2012). Migration propensity in bats can be characterized using three categories: short-range (50-500 km) , long-range (>500 km) or non-migratory (< 50 km; Hutterer *et al.* 2005) with those species that migrate comprising

<3% of all species although migratory tendencies remain poorly characterized for many species (Fleming & Eby 2003; McGuire & Ratcliffe 2011). Further, migratory tendency can be specific to populations (e.g., Brazilian free-tailed bats; Russell *et al.* 2005), or specific to classes within populations, such as where females migrate longer distances or to different areas than males (e.g., hoary bats; Cryan *et al.* 2004). Since migration is associated with living in seasonal environments, migration is thought to be more common in temperate than tropical species; however, some species may track seasonally available food resources in the tropics and migration may be more prevalent in tropical species than currently documented (Fleming & Eby 2003). Because mating and gestation/rearing are spatially and temporally decoupled events for many temperate bats, mating while migrating may be an important strategy to maximize encounters with potential mates while also tracking seasonal resources (Hutterer *et al.* 2005). Since migration typically involves long-distance movements, in the range of hundreds to thousands of kilometers (Fleming & Eby 2003), this may translate to higher gene flow over larger spatial scales and less genetic structuring.

Recent work suggests the independent evolution of migration in both temperate and tropical lineages (Bisson *et al.* 2009). These generalized biogeographic distribution relationships have not been examined with respect to genetic population structure but it is predicted that temperate species will have less structure than tropical species given the highly variable nature of the seasons in temperate areas favouring migration. Other species traits related to movement dynamics such as geographic range size have been linked to dispersal ability in other taxa (Holt *et al.* 1997; Laube *et al.* 2013), and may also influence dispersal extent in bats. Work from the conservation literature has found that

movement range variables such small home range size, geographic range and aspect ratio are linked to extinction risk in many groups of species including bats (Purvis *et al.* 2000; Jones *et al.* 2003). Therefore, exploring relationships between these variables may provide a better understanding of broad-scale linkages between population structuring and conservation approaches via intermediary dispersal correlates. For example, Safi and Kerth (2004) found wing morphology, as it relates to foraging behaviours, to be a good proxy of resource specialization, which could in turn predict extinction risk in insectivorous bats. This demonstrates that many attributes of flight efficiency and movement potential are likely interrelated since evolution can favour suites of traits that provide adaptive strategies to specific environmental pressures. Therefore, correlations between wing morphology attributes, migration behaviour, resource specialization and geographic range are all likely.

Interest in exploring predictors of demographic and genetic population structuring in bats has long permeated the literature (Norberg & Rayner 1987; Entwistle *et al.* 2000; Burland & Worthington Wilmer 2001; Moussy *et al.* 2012) with Olival (2012) recently providing the first quantitative assessment of 18 ecological and evolutionary correlates of population genetic structure in bats. This later study found strong support for wing morphology metrics as important correlates of population genetic structuring across the order. However, the paucity of ecological and life history trait data for many bat species precluded a single synthetic analysis that controlled for the covariance of many of the predictor traits. The goal of this current study was to identify key predictors of the extent of bat dispersal and quantitatively describe how these relate to population genetic structure across the order, using a comparative approach, while accounting for the

interrelated nature of these measures. I focused on five metrics of dispersal extent and predicted that wing loading, aspect ratio, geographic range size and median latitude should all display negative relationships with population genetic structure. Further, migratory species should display less genetic structure than non-migratory species.

5.3 Materials and Methods

5.3.1 Data collection

I compiled bat population genetic studies using previous review papers (Barrowclough 1983; Moussy *et al.* 2012; Olival 2012) and searches of the Web of Science database. Studies included in the analysis had to meet several criteria including a minimum total sample size of ≥30 with no fewer than three geographic areas (presumed subpopulations) sampled. For species with multiple studies published, I selected the study that best maximized meeting the following criteria: spanned the largest distance between sample sites (range wide vs. small regional study) or had the largest overall sample size. I included protein-based (allozymes) and DNA-based molecular markers (microsatellites or sequencing of mitochondrial DNA). Allozyme, microsatellite and mitochondrial studies represented 17%, 67% and 16% of the data set respectively. Because island systems could have confounding effects on the genetics of island populations, owing to historical patterns of connectedness (Peterson & Heaney 1993; Muscarella *et al.* 2011), we excluded studies that only included multiple island populations. If studies contained island and mainland populations, we recalculated estimates of population genetic structure over the mainland populations only if they met previous criteria. Island studies carried out wholly on one island (e.g. Madagascar, Taiwan) were included in the analysis.

I used Wright's F_{ST} (1951) as an estimate of population genetic structure in bat populations because of its widespread use as a measure to summarize genetic differentiation among populations (Whitlock 2011). Many estimates of F_{ST} were directly reported in studies and used as such in the analysis. Alternatively, I calculated these values from genetic data presented in papers where we calculated pairwise per locus estimates of F_{ST} from allele frequency tables following Nei's approach (1977): $F_{ST} = (H_T - H_S)/H_T$. H_T is the total heterozygosity in the population and was calculated as $H_T = 1 - \sum_{i-1}^{k} \bar{p}_i^2$ where $\bar{p}_i$ is the frequency of allele i averaged over the subpopulations and k is the number of alleles in the total population. H_S is the heterozygosity in the subpopulation s and H_S was calculated as $H_S = 1 - \sum_{i-1}^{h} \bar{p}_{i,s}^2$ where $\bar{p}_{i,s}$ is the frequency of the *i*th allele in the subpopulation. Per locus F_{ST} estimates were then averaged over all loci for a global F_{ST} estimate (Burland & Worthington Wilmer 2001).

To facilitate comparison among all types of studies, I converted F_{ST} estimates to bi-parentally inherited, diploid gene flow, assuming Wright's island model estimates *sensu* Bradbury et al. (2008), using the formula of Kinlan and Gaines (2003). This conversion assumes there is an equal sex ratio and equal migration occurring. First, mitochondrial estimates of gene flow were calculated as: $(Nm_{mt}) = 0.5\left[\left(\frac{1}{F_{st,mt}}\right) - 1\right]$. These values were then multipled by 2 to account for biparental inheritance of nuclear markers, and were recalculated assuming an island model of migration with diploid gene flow as: $F_{st,diploid,biparental} = \left[\frac{1}{(4(2[Nm]_{mt})+1}\right]$. Mitochondrial studies which presented Φ_{ST} were not included in the analysis because this measure incorporates additional

information on substitution differences (Excoffier *et al.* 1992) that cannot be corrected for in the same manner.

When comparing F_{ST} across multiple species and studies, the extent of geographic sampling should be accounted for as it can strongly influence the measure of F_{ST} (Burland & Worthington Wilmer 2001; Stevens *et al.* 2010). This was done by calculating the maximum distance (D_{MAX}) between sampled sites for each study. Location coordinates were taken directly from the studies or were approximated from maps and subsequently put into Google Earth © vs. 7.0.2.8415 to estimate the coordinates. Straight line maximum distances were then calculated from coordinates and maximum distances were log transformed to meet normality assumptions, and then regressed against estimates of F_{ST} using simple linear regression with the residuals used in further analysis.

I examined five variables (wing loading, aspect ratio, geographic range size, migratory status, median latitude) predicted to impact the extent of dispersal in bats from previous work (Jones *et al.* 2003; Moussy *et al.* 2012; Olival 2012). For a study to be included, measures of all variables had to be obtained to be included in the multivariate analysis (see Supplementary Material for species and variables included). Although this may have reduced the overall sample size compared to the previous exploratory study of (Olival 2012), I hoped to achieve a trade-off in favour of a greater ability to detect trends across the order in a single focused, multivariate analysis.

Wing loading and aspect ratio values were obtained from Norberg and Rayner's morphology correlate analysis (Norberg & Rayner 1987). If values could not be located in the literature, they were calculated from body mass taken from family-specific power

equations of Norberg and Rayner (1987; Table 5.2). Body mass (*M*) was entered in kilograms, and calculated wing loading units are $N\ m^{-2}$, where *N* = Newtons (mass times the gravitational constant). Body mass values were averaged over all specimens listed in the CRC handbook of Mammalian Body Masses (Silva & Downing 1995) or from other primary publications for species as required. Aspect ratio is a unit-less measure derived from the formula wing span area squared divided by wing span area. Migration status for species was classified as short-distance (movements >100 km and <1000 km), long-distance (movements >1000 km) or non-migratory following categories from recent reviews (Bisson *et al.* 2009; McGuire & Ratcliffe 2011; Moussy *et al.* 2012) rather than Hutterer *et al*.(2005). Other primary sources were used if species were not listed in these reviews. Since the proportion of species that are non-migratory is higher relative to migratory species and often is not explicitly stated as such we followed the convention of McGuire and Ratcliffe (2011) where if migration was not specifically mentioned and lack of migration was otherwise characterized for the species we classified them as non-migratory.

The size of the geographic range and the median of the latitudinal range (absolute value), as a measure of biogeographic distribution were calculated for each species from IUCN distribution maps (IUCN 2012) using a Geographic Information System (ArcMap 10.1; ESRI, Redlands, California). Shape files of mapped areas were downloaded from the IUCN database (IUCN 2012), polygons with a presence value of 1(Extant) were extracted, and the areas were calculated and summed to represent the range in square kilometers. Geographic range sizes were log transformed to meet normality assumptions for analysis. To characterize the biogeographic range of each species, the maximum and

minimum latitudes were calculated from the shape files and the median value (absolute value) was calculated. I chose to use median latitudinal range to evaluate latitude as a continuous variable rather than use a binary categorical variable (e.g. tropical/temperate) where selecting a criteria to classify the location of majority of the distribution in one or the other was arbitrary. However, to aid interpretation I considered species with a median latitude of >23.5 N or S as temperate and those between 23.5° N and 23.5° S as tropical.

5.3.2 Comparative analyses

To account for correlation among predictors, multivariate components (dimensions) were constructed using a correlation matrix-based form of principal components analysis (PCA) called factor analysis of mixed data (FAMD; Pagès 2004) using package FactoMineR (Lê *et al.* 2008) in R (R Development Core Team 2010). A FAMD was used because it can handle both continuous and categorical data whereas a traditional PCA can only handle continuous data. The generated FAMD coordinate scores were used as the independent variables in a multiple regression on phylogenetically independent contrasts (PIC). Since species that are close relatives may be more similar for a trait due to shared ancestry rather than by chance, non-independence is introduced into correlative or regression modelling which must be accounted for using PIC (Felsenstein 1985). Contrasts were generated using the CRUNCH algorithm of the R package Caper (Orme 2012), and a composite phylogeny was constructed for the 43 species examined (representing nine families) founded on the supertree of Miller-Butterworth et al. (2007) for describing inter-familial relationships (Figure 1). When resolution was needed within families we used Baker et al. (2012) for

the Phyllostomidae, Ammerman et al. (2012) for the Molossidae, Almeida et al. (2009) for the Pteropodidae, Hoofer & Van den Bussche (2003) for the Vespertilionidae with Stadelmann et al. (2007) for relationships among *Myotis* species. Contrasts of the dependent variable were permuted and parameter estimates (PEs) calculated 999 times to calculate significance; if the PE was >97.5% or <2.5% of the permuted values (2-tailed test) and if the confidence interval (CI) did not overlap zero. Variables with loadings >0.4 were considered important and interpreted based on interpretation guidelines of Tabachnick and Fidell (2006). To visualize the multi-dimensional relationships among the variables of importance in predicting population genetic structuring, I plotted the two dimensions that maximally separated the clustered variables.

5.4 Results

As expected with only five predictor variables, three multivariate dimensions explained 71.4% of the variation demonstrating strong correlation among the dispersal extent predictor variables. Dimension 1 (explained 31.4% of the variation) grouped variables associated primarily with migration where it had the strongest loadings of median latitude and migration tendency. Short-distance migrants grouped at high latitudes, which are temperate areas, as demonstrated by the negative loadings on the dimension (Table 5.1). Long-distance migrants grouped at more tropical lower latitudes as shown by the positive loadings on this dimension. Non-migratory species overlapped both long-distance and short-distance migrants although was slightly skewed to lower latitudes as shown by the weak negative loadings and corresponding box plot of the range of median latitude by migration category (Figure 5.2). Aspect ratio also grouped on this

dimension with species with higher aspect ratios grouping with long-distance migrants as demonstrated by the positive loadings.

Dimension 2 (explained 21.0% of the variation) had the strongest loadings of aspect ratio, geographic range size and wing loading, and migration to a lesser extent. This dimension shows the strong correlation between the two morphometric variables which was not unexpected since both use measures of wing area in deriving these metrics. Dimension 3 (explained 19.0% of the variation) was most heavily loaded with the variables wing loading and migration category, with species with high wing-loading grouping with migrating species (short- and long-distance). All three dimensions were included as independent variables in the multivariate regression on phylogenetically independent contrasts.

Only Dimension 3 was found to have significant effects in the model on population genetic structure (Dimension 3: PE = -0.0454, 95% CI: -0.0784, -0.0012, P <0.02). The negative parameter estimate for this dimension indicates that wing loading and migration tendency were negatively associated with population genetic structure (Figure 5.2, Table 5.1). This shows that after accounting for the interrelated nature of the dispersal extent predictors where dimensions 1 and 2 collectively explained 52.4% of the variation, approximately 20% of the remaining variation exhibited by bats in their migration tendency and wing loading values could still be used to explain some of the variation in population genetic structuring. Long-distance migrants showed the greatest range in wing loading values and non-migratory species showed the greatest variation in the range of degree of population genetic structuring (F_{ST} residual) compared to either short or long-distance migrants (Figure 5.2). The largest variation in wing loading was

shown in long-distance migrants followed by non-migratory and short-distance migrants. Clustering of migration categories with wing loading values was maximally visualized in relation to their median latitudinal range by plotting dimension 1 against dimension 3 (Figure 5.3). Clustering of dimension 2 against dimension 3 is shown in the Supplementary Material (Figure 5.4).

5.5 Discussion

These results suggest that wing loading and migration tendency can predict the magnitude of population genetic structure in bats. Specifically, migratory species with high wing loading have less genetic structure than those with low wing loading and are non-migratory. Since powered flight is a defining feature of the order it is not surprising that a feature of the wing, as it relates to movement and dispersal capabilities is an important predictor. These results support recent work which has also suggested wing loading was an important predictor of population structure in Molossid bats (Taylor *et al.* 2012). Olival's (2012) analysis across the order found that another wing morphology metric, aspect ratio, was the best predictor in univariate analyses of genetic structure but within a multivariate framework, wing loading was the best morphological predictor. Strong correlations between these two morphometric measures are known (Norberg & Rayner 1987; Olival 2012) and in this analysis these two did strongly correlate on one dimension; however this dimension was not significant in predicting population genetic structure. I suggest that the congruence of this analysis with these other works may mean that wing loading has a broad applicability as a dispersal correlate as it relates to higher achievable flight speeds and inferred larger dispersal distances. Thus it may have the

potential to be a proxy measure for predicting genetic structure in conservation planning for species where dispersal dynamics or genetic structure are not well characterized.

Similarly, these results support the hypothesis that migration tendency can also influence the degree of population genetic structure found in bats (Burland & Worthington Wilmer 2001; Moussy *et al.* 2012). In classifying migration as a dichotomous variable, Olival's recent analysis (2012) also found that in univariate analysis, species classified as migratory had lower F_{ST} values compared to those that were non-migratory. In this analysis, I classified migration into three categories to incorporate a spatial component of migration - distance class - into the model. After accounting for the spatial extent of sampling, the residual genetic structure of both migratory categories displayed smaller variance compared to non-migrants and had overall lower values thus supporting previous work. In the factor analysis we found that short-distance migrants clustered with temperate species and long-distance migrants clustered more closely with tropical species supporting a recent contention of repeated independent evolution in migration in bats where long-distance migration evolved independently form short-distance migration and from a tropical lineage (Bisson *et al.* 2009). Although it is tempting to consider migration as categorical it can be specific to populations and individuals, so as data accrues we may find that migration extent is more continuous (Fleming & Eby 2003). Nevertheless, the negative relationship between migration and population structure has been shown in a diverse suite of volant taxa (Paar *et al.* 2004; Miller *et al.* 2012) supporting its utility to predict population structure across taxa.

Although I have shown that two correlates of dispersal extent predict population genetic structure in bats, I emphasize the correlative nature of the analysis. A suite of other factors may also explain the genetic structure of populations including social structure, mating system, and environment (past and present; Anthony & Blumstein 2000). Bats exhibit a diverse range of social structure and mating systems (McCracken & Wilkinson 2000; Kerth 2008) and if either alters demographics by introducing behaviourally segregated groups then these can impact gene flow and population genetic structure as has been suggested for other mammals (van Staaden 1995; Storz 1999) but has not been thoroughly investigated in bats. Overall, less attention has been paid to tropical bat species compared to temperate species of late. This is despite the greater diversity of social structures and mating systems in the tropics compared to the temperate regions where there is less pressure for groups to divide seasonally (but see McCracken & Bradbury 1981; McCracken 1984; Wilkinson 1985). This analysis found that the greatest variance in population genetic structures observed was in the non-migratory species which may be more abundant in tropical areas. Comparative studies on tropical species may therefore provide a way to examine the effects of these factors on population genetic structure in the future such as was recently demonstrated in a study of seven Malaysian species (Rossiter *et al.* 2012).

Past geological and/or climate events can also affect gene flow. For example, historical colonization patterns of islands can impact population genetic structure and obscure current dispersal patterns (Hewitt & Butlin 1997) hence the exclusion of island studies. Other events such as glaciation can also have marked impacts on the genetic structure of bats (Ruedi & Castella 2003; Dool *et al.* 2013). I did not account for this

effect on temperate species and although it may have impacted the population structure of some of the species I believe the broad trends across the order should still hold true. Studies of several species from the same region, subject to the same glacial history, could provide insight into the relative roles of past events and species traits on population genetic structure. Lastly, the type of molecular marker employed can also influence the level of genetic differentiation owing to differences in selection pressures and mutation rates (Hendry 1999). With high mutation rates in microsatellite markers, this may have impacted the magnitude of genetic differentiation assessed compared to allozymes. However, this effect may be most prevalent where mutation plays a dominant role over migration (i.e., gene flow; Rousset 1997; Hedrick 2005) and with a generally high degree of gene flow in many bat species, this may be of minimal importance for many of the species in my analysis.

The cryptic nature of bats means that much remains unknown about their dispersal habits and patterns. Assessment of trends using predictive modelling may permit inference for management of lesser known species. Since there is conservation concern for approximately 20% of bat species (Jones *et al.* 2003), understanding which traits relate to population structure may inform conservation strategies and management directives at a broad level. Further, characterization of population connectivity is becoming increasingly important for understanding transmission of disease such as white-nose syndrome (Frick *et al.* 2010), rabies (Bogdanowicz *et al.* 2013) and other potential zoonotic diseases (Turmelle & Olival 2009). Because population structure is influenced by multiple physiological, behavioural and environmental factors that influence gene flow, future focused and hypothesis-driven comparative studies will be

invaluable for understanding the factors that explain the diverse population structures of bats.

Table 5. 1 Mean (SD) value or count of independent variables and component loadings of dimensions assessed to describe population genetic structuring in bats. Only Dimension 3 was found to have significant effects.

Variable	Mean (SD) or count	Dimension 1	Dimension 2	Dimension 3[a]
Wing loading(Nm^{-2})	12.95 (8.56)	0.3143	0.4799	**0.4715**
Aspect ratio	6.74 (1.05)	0.4850	-0.5414	-0.0498
Geographic range (km^2)	1.33 x10^{11} (1.04 x10^{12})	0.0710	0.5175	0.2104
Latitudinal Median (DD)	25.97 (17.16)	-0.5687	-0.2457	0.2446
Migration category				
Short-distance	9	-1.1494	-0.3587	**1.1391**
Long-distance	8	1.3870	-0.6976	**1.0144**
Non-migratory	26	-0.0289	0.3388	**-0.7064**

[a] Loadings in boldface type are those identified as having significant effects on population genetic structuring in the model.

Table 5. 2 Family or subfamily specific equations for calculating wing loading and aspect ratio for bats from body mass (M) in kilograms. Equations are from Norberg and Rayner (1987).

Family/subfamily	Wing loading	Aspect ratio
Family Pteropodidae , subfamily Pteropodinae	$45.94M^{0.339}$	$5.40M^{-0.111}$
Family Rhinolphidae	$91.41M^{0.541}$	$11.14M^{0.128}$
Family Phyllostomidae, subfamily Glossophaginae	$63.13M^{0.540}$	$13.32M^{0.160}$
Family Vespertilionidae, subfamily Vespertilioninae	$108.1M^{0.540}$	$16.30M^{0.191}$
Family Miniopteridae	$803.0M^{0.985}$	$38.35M^{0.362}$

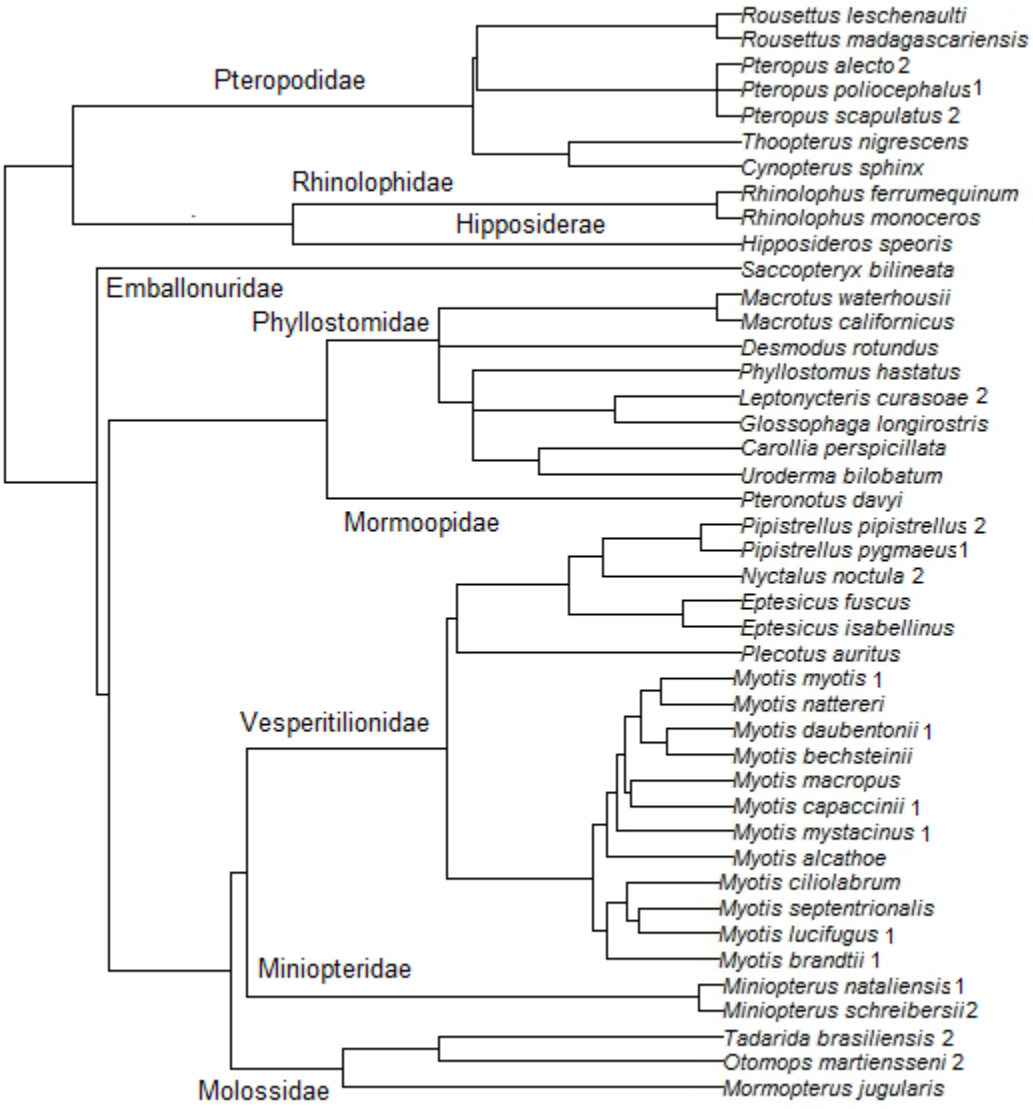

Figure 5.1 Bat composite phylogeny for 43 species used to generate the phylogenetically independent contrasts (PICs). Migration categories are shown as short-distance (1); long-distance (2) and non-migratory as the remaining unlabelled species.

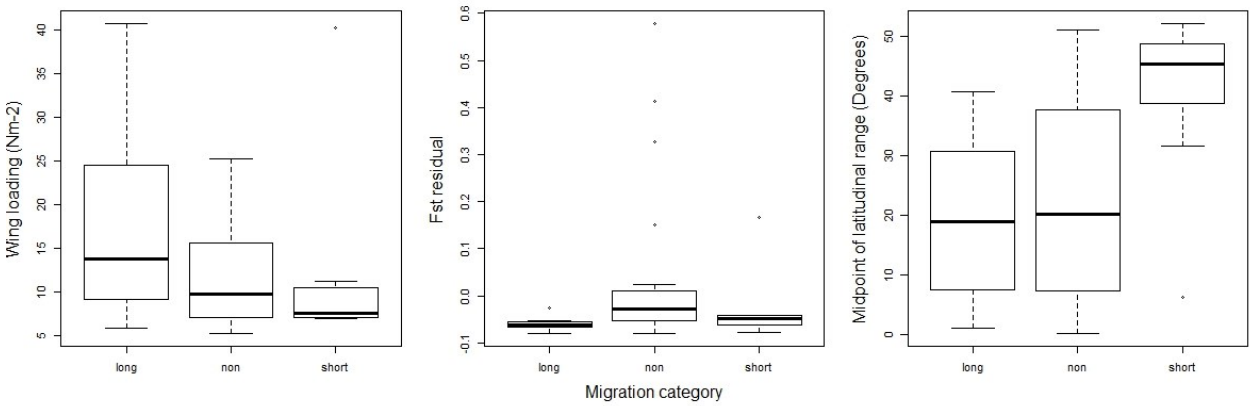

Figure 5.2 Variation among dispersal extent correlates in predicting population genetic structure of bats by migration category. The bold line indicates the median, the box plot encompasses the 25-75 percentiles of the data and the whiskers extend to 1.5 times the inter-quartile range.

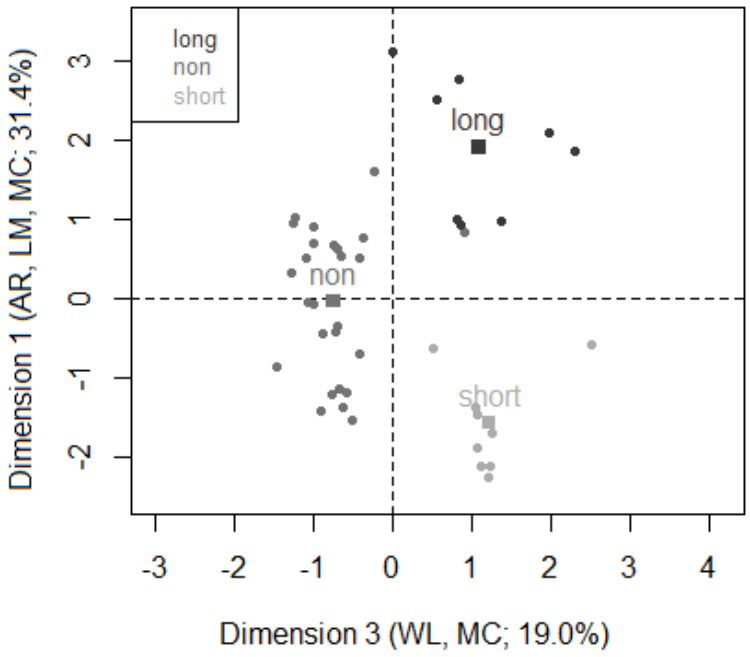

Figure 5.3 Factor analysis of mixed data showing the correlation between dispersal extent predictors of dimension 3 (WL: wing loading; MC: migration category) and dimension 1 (AR: aspect ratio; LM: latitudinal median; MC: migration category). Species are coloured by migration category (dark grey- long-distance migrants; medium grey- non-migratory; light grey- short-distance migrants) and the centroids of each migration category are shown (squares).

5.6 References

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5.7 SUPPLEMENTARY MATERIAL

Table 5S 1 Species, sample size, genetic marker, F_{ST} used in the analysis (directly reported or calculated), maximum distance of sampling in study (Dmax; km), Geographic range size (km^2), median of the latitudinal range (median latitude; DD), migration category, wing loading (Nm^{-2}) and aspect ratio for the 43 species in our comparative study.

Family	Species	*N*	Marker	F_{ST}	Dmax (km)	Range size (km^2)	Median Latitude (DD)	Migration	Wing loading (Nm^{-2})	Aspect ratio	References
Emballonuridae	*Saccopterx bilineata*	58	allozyme	0.013	38.71	1.26×10^7	0.223	non	5.9	6.1	[1-3]
Hipposideridae	*Hipposideros speoris*	186	microsatellites	0.651	1175	1.22×10^6	18.520	non	8.9	6.5	[1, 4, 5]
Miniopteridae	*Miniopterus nataliensis*	307	mtDNA	0.241	1440	3.49×10^6	6.246	short	7.4	6.2	[6-8]
	Miniopterus schreibersii	407	microsatellites	0.038	488	3.71×10^6	26.788	long	10.2	7.0	[1, 9, 10]
Molossidae	*Mormopterus jugularis*	50	mtDNA	0.009	912	2.35×10^5	19.147	non	13.4	7.9	[11, 12]
	Otomops martiensseni	31	mtDNA	0.016	4249	7.58×10^6	7.349	long	16.2	8.9	[11, 13]
	Tadarida brasiliensis	412	allozyme	0.008	1010	1.38×10^7	1.236	long	11.5	8.2	[1, 9, 14]
Mormoopidae	*Pteronotus davyi*	105	mtDNA	0.097	2394	3.47×10^6	11.029	non	8.0	8.3	[1, 4, 15]
Phyllostomidae	*Carollia perspicillata*	81	mtDNA	0.015	12.5	1.38×10^7	4.152	non	19.9	5.7	[16, 17]
	Desmodus rotundus	40	allozyme	0.050	2252	1.77×10^7	7.443	non	14.0	6.7	[1, 2, 18]
	Glossophaga longirostris	41	mtDNA	0.397	824	1.57×10^6	6.949	non	11.2	6.7	[4, 19, 20]
	Leptonycteris curasoae	42	mtDNA	0.015	953	8.41×10^5	7.631	long	5.9	10.6	[1, 2, 19]
	Macrotus californicus	100	allozyme	0.090	590	6.43×10^5	29.564	non	10.2	6.4	[1, 17, 21]
	Macrotus waterhousii	69	allozyme	0.051	935	8.03×10^5	21.204	non	7.3	9.0	[1, 21]
	Phyllostomus hastatus	172	allozyme	0.031	10.3	1.26×10^7	3.872	non	25.2	7.6	[1, 2, 22, 23]
	Uroderma bilobatum	151	mtDNA	0.002	12.5	1.28×10^7	3.203	non	21.5	6.1	[16, 17]
Pteropodidae	*Cynopterus sphinx*	218	microsatellites	0.024	3915	6.46×10^6	12.182	non	15.6	6.7	[1, 4, 24]
	Pteropus alecto	114	allozyme	0.023	2961	1.35×10^6	14.713	long	40.7	5.6	[17, 20, 25]
	Pteropus poliocephalus	156	allozyme	0.014	721	2.49×10^5	31.558	short	40.2	5.6	[2, 20, 25]
	Pteropus scapulatus	117	allozyme	0.028	2625	3.04×10^6	23.159	long	32.8	7.3	[1, 2, 26]
	Rousettus leschenaulti	157	microsatellites	0.007	3828	6.76×10^{12}	13.144	non	23.1	6.0	[1, 4, 24]

Family	Species	*N*	Marker	F_{ST}	Dmax (km)	Range size (km^2)	Median Latitude (DD)	Migration	Wing loading (Nm^{-2})	Aspect ratio	References
Pteropodidae	*Rousettus madagascariensis*	193	microsatellites	0.004	1366	2.93×10^5	18.541	non	18.2	7.3	[4, 20, 27]
	Thoopterus nigrescens	37	microsatellites	0.48	620	1.83×10^5	0.578	non	17.6	6.7	[28, 29]
Rhinolophidae	*Rhinolophus ferrumequinum*	516	microsatellites	0.043	9670	9.75×10^6	37.653	non	12.2	6.1	[1, 2, 20, 30]
	Rhinolophus monoceros	455	microsatellites	0.009	176	2.40×10^4	23.600	non	5.2	5.7	[4, 20, 31]
Vespertilionidae	*Eptesicus fuscus*	271	microsatellites	0.003	473	1.32×10^7	28.625	non	9.4	6.4	[1, 9, 32]
	Eptesicus isabellinus	200	mtDNA	0.039	839	7.02×10^5	32.791	non	12.2	6.5	[1, 9, 33]
	Myotis bechsteinii	175	microsatellites	0.041	150	2.52×10^6	46.302	non	9.0	6.0	[1, 9, 34]
	Myotis brandtii	128	microsatellites	0.012	400	7.66×10^6	52.103	short	7.1	6.0	[35-37]
	Myotis capaccinii	36	microsatellites	0.00	1650	1.25×10^6	38.691	short	10.5	6.8	[1, 36, 38]
	Myotis ciliolabrum	427	microsatellites	0.010	473	1.39×10^6	45.332	non	6.7	6.1	[1, 32, 39]
	Myotis daubentonii	671	microsatellites	0.017	345	5.80×10^6	45.365	short	7.0	6.3	[11, 20, 40]
	Myotis lucifugus	401	microsatellites	0.002	473	1.20×10^7	48.703	short	7.5	6.0	(11, 20, 32)
	Myotis macropus	173	microsatellites	0.221	883	1.33×10^6	22.708	non	9.0	6.8	[10, 15, 41]
	Myotis myotis	480	microsatellites	0.035	2786	3.87×10^6	43.623	short	11.2	6.3	[11, 20, 42]
	Myotis mystacinus	182	microsatellites	0.004	100	4.97×10^6	47.620	short	7.1	6.0	[11, 45, 37]
	Myotis nattereri	282	microsatellites	0.017	131	5.87×10^6	47.902	non	6.1	6.4	[11, 20, 43]
	Myotis septentrionalis	88	microsatellites	0.002	240	4.95×10^6	46.871	non	6.8	5.8	[11, 20, 44]
	Nyctalus noctula	264	microsatellites	0.006	4015	8.03×10^6	40.700	long	16.1	7.4	[11, 20, 45]
	Pipistrellus pipistrellus	274	microsatellites	0.005	651	1.15×10^7	34.853	long	8.1	7.5	[11, 20, 46]
	Pipistrellus pygmaeus	233	microsatellites	0.006	761	1.95×10^6	49.742	short	8.1	7.5	[15, 35, 46]
	Plecotus auritus	195	microsatellites	0.019	100	6.53×10^6	51.039	non	7.1	5.7	[11, 17, 47]

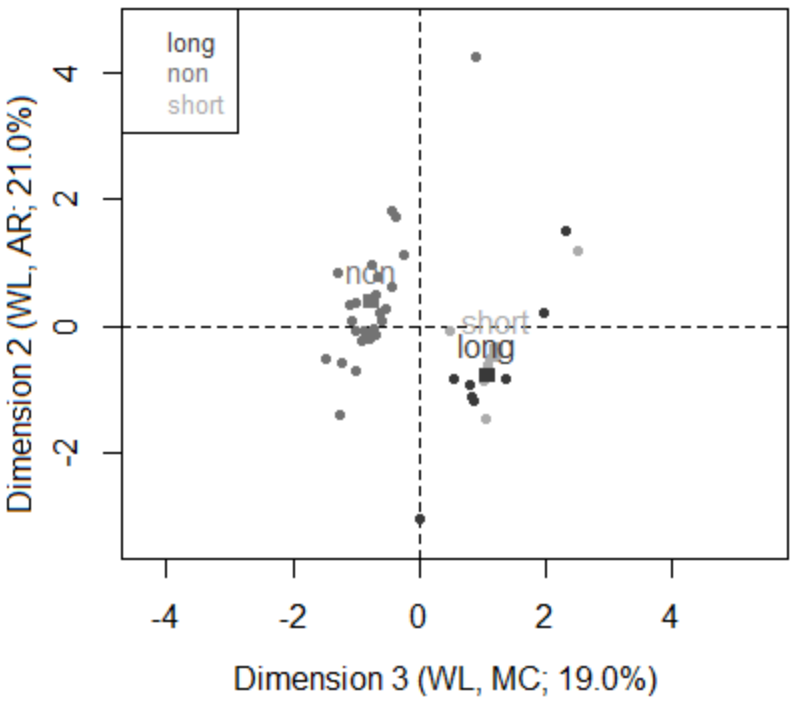

Figure 5S 1 Factor analysis of mixed data showing the correlation between dispersal extent predictors of dimension 2 (WL: wing loading; AR: aspect ratio) and dimension 3 (WL; wing loading; MC: migration category). Species are coloured by migration category (dark grey- long-distance migrants; medium grey- non-migratory; light grey- short-distance migrants) and the centroids of each migration category are shown (squares).

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CHAPTER 6 SYNTHESIS & CONCLUSIONS

6.1 SUMMARY: DYNAMICS OF AUTUMN SWARMING AND POPULATION STRUCTURE

The primary goal of this thesis was to characterize the population dynamics of temperate bats during the autumn swarming period to better understand population structure. By using a multi-faceted approach that explored the activities, associations and resultant cohesion of individuals via two approaches (Waples & Gaggiotti 2006), a more comprehensive understanding of this dynamic period for bats was achieved.

Using a direct approach that examined the potential for different classes of individuals (e.g., sex and age classes) to be documented during autumn swarming, thus indicating demographic population structure, I characterized the intersexual differences in autumn swarming activities of bats to test predictions on intersexual variation in behaviours to maximize fitness. I found that relative to females, males occurred in larger numbers at swarming sites and had a longer swarming season that wholly overlapped that of females. Males accounted for a disproportionately large proportion of the number of the recaptures suggesting they returned to swarming sites more frequently. Male mammals can maximize fitness by securing more mating opportunities (Andersson 1994; Becker *et al.* 2013). Female mammals are limited by the number of offspring they can produce, and thus maximize their fitness by investing energy into fewer offspring thus do not need to secure as many mating opportunities as males (Andersson 1994). My data show variation in the swarming behaviour of male and female *M. lucifugus* and *M. septentrionalis* with males likely spending more time devoted to swarming activities to

maximize mating opportunities. Although the activities of male and female bats in this study overlapped, the differences suggest sex-specific activity budgets where individuals of each group may reconcile energetic constraints differently-via different activity levels-to maximize fitness.

Despite these intersexual behavioural differences, in tracking recaptures of bats at swarming sites over three seasons, I did not detect any occurrences of individuals of either sex visiting more than one swarming site. Some individuals were recaptured at the same site > 20 times (both within and across years) which suggests that at least some of these bats exhibit a degree of swarming site fidelity, at least over the time period that I examined. Recapture data showed that *M. lucifugus* were more transient in the first few weeks of swarming where swarming activity was high but recapture rates were low. A higher degree of transiency early in the season has been noted in other studies despite some individuals showing a high degree of fidelity to swarming and hibernation sites (Davis & Hitchcock 1965; Fenton 1969; Norquay *et al.* 2013). For both species, I had an overall low recapture rate (<20%) where many tagged individuals were never detected again. These individuals may be engaging in non-swarming behaviours, or be visiting swarming sites I did not sample resulting in lower site fidelity at the sites I did monitor. Given that these animals are very long lived, and some individuals are known to make long distance movements using multiple sites, we may find with more research that the degree of site fidelity varies among individuals and may change over the lifetime of an individual.

In maintaining a high degree of site fidelity, some individuals may develop preferred associations with others that they regularly interact with and collectively may

show social structuring. In exploring the social dynamics of swarming bats, I found that within a night, young-of-the-year (YOY) associated more often with other bats and associated most often with other YOY than adults. Adult male and female bats associated less frequently with each other but tended to be most often captured alone. When males were captured in groups, these groups were more likely to be composed of multiple males and in *M. lucifugus*, males had preferred male associates they grouped with over multiple nights. Taken together with the intersexual differences in swarming site visits, my work suggests that during swarming, complex behaviours between age and sex groups adds an additional level of behavioural structure of swarming populations. From the perspective of directly characterizing demographic groups, bats swarming at a site appear to be composed of predictable classes of individuals such that the focused activity at swarming sites may warrant management consideration at a local level.

Using an approach that indirectly examined the reproductive cohesiveness of individuals, I characterized population genetic structure in *M. lucifugus* among swarming sites. Weak levels of genetic structuring found on bi-parentally inherited nuclear markers suggested that high contemporary gene flow occurred among swarming sites over a range of at least 850 kilometres. Further, assessment of asymmetries in gene flow between the sexes did not find significant differences in the short-term. However, differences in the magnitude of structuring between maternally inherited and bi-parentally inherited markers suggested some degree of structuring and male-biased gene flow over the long-term. A signature of a population expansion was found for these sites corresponding to the time following Pleistocene deglaciation in the region. The genetic data are suggestive of high gene flow and thus a high degree of connectivity among bats that visit swarming

sites. Thus, my findings suggest that *M. lucifugus* in the study area may potentially be considered as one gene pool for management at a larger scale concurrent with management at a local scale. Further work that estimates the genetic divergence found in my study population in relation to that found within the entire species range would be required to evaluate the most appropriate management units for the species using population genetic data (Palsboll *et al.* 2007).

Collectively this work provides insight into the complex swarming period for temperate bats and how this may influence their population structure. The finding of quantitative differences among classes of bats in the autumn activities they engage in and who they interact with provides knowledge of the intricacies that occur among individuals at a swarming site. Where different behaviours lead to variation in other traits of individuals (e.g., movement dynamics or social interactions) such that they impact breeding and gene flow, these differences can scale up to change properties of populations (Mills 2013). As such, knowledge of these characteristics is important for interpreting the results of genetic studies that characterize population genetic structure; that which is the result of the integration of the behaviours and traits of many individuals.

Lastly, subtle differences between the two species were noted in their swarming dynamics despite many generalities of the two. Low recapture rates of both males and females early in the swarming season for *Myotis lucifugus,* showed a higher degree of transiency; although not due to reduced swarming activities. Compared to *M. lucifugus*, *M. septentrionalis* reached a peak in swarming activity earlier in the season and appeared to have a shorter swarming season whereas *M. lucifugus* had a bi-modal temporal distribution of swarming activity. *Myotis septentrionalis* showed grouping behaviours

that were similar to expectations of grouping at random within a night. Male *M. lucifugus*, on the other hand, had preferred long-term associates. As discussed, differences in the ecology of each species, where *M. septentrionalis* has a strong forest association (Sasse & Pekins 1996; Jung *et al.* 2004; Henderson & Broders 2008), or differences in behaviours may explain these differences. The movements of *M. septentrionalis* may be of a smaller spatial scale and they may remain closer to swarming sites such that swarming visits are frequent, occurring over a short time span, meaning that they interact overall with more individuals and have few preferred associates. In my analysis of dispersal correlates across the order, I used data from the literature to examine metrics related to the magnitude of dispersal movements including previous work on *M. lucifugus* and *M. septentrionalis*. This work classified *M. lucifugus* as a short-distance migrant and *M. septentrionalis* as non-migratory and therefore we may predict to see differences in the genetic structures of these two species based on my broad scale analysis.

6.2 Conclusion and Future research

In conclusion, this study provides information on the nature of the timing and interactions of bats that take place during the autumn swarming period. This work contributes to the body of knowledge that characterizes the complex transition time of autumn swarming by demonstrating that: males engage in more swarming visits compared to females which is consistent with mating being a primary function of swarming; bats associate with other specific age/sex classes that are non-random suggesting swarming is not entirely just the passive aggregation of bats at sites; genetic connectivity among swarming sites is high for *M. lucifugus* suggesting movements that

contribute to gene flow occur regularly among sites. However, there are many aspects of autumn swarming that remain to be addressed if we are to fully understand the importance of this time period to the annual cycle of swarming species.

1. This study focused on the swarming period alone and many questions remain as to the connectivity of bats from summering through swarming to hibernation. Tracking of individuals year-round would provide valuable information on how populations remain connected throughout the entire season. Research that characterizes resource use of bats during the swarming period (roosting, foraging, movement/migration routes) would be of value to land managers to ensure their populations are adequately provided for year-round.
2. On the whole, information remains lacking on population demographics for many species, including the two study species, such that many population vital rates (survivorship, recruitment etc.) are not well known. Long-term studies during the swarming period may facilitate gathering data on many individuals of both sexes, and potentially of cohorts, which is not easily obtained from summer maternity colony work or during hibernation where disturbance to bats is an issue.
3. This study provided information on the genetic connectivity of swarming sites of *M. lucifugus*. Future work that characterizes genetic connectivity for *M. septentrionalis* should also be investigated and at multiple spatial scales for both species given their large ranges. Further, information on demographic connectivity, that is how population vital rates are effected by the movement of individuals to or from subpopulations is urgently needed for conservation

planning of these and other species due to large declines from white-nose syndrome.

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APPENDIX A

PUBLICATIONS

The work presented in Chapter 2 has been submitted as:

Burns, LE and HG Broders. Submitted May 25, 2014. Maximizing mating opportunities; Higher autumn swarming activity in male Myotis bats. Animal Behaviour.

The work presented in Chapter 4 has been submitted as:

Burns, LE, Frasier, TR and HG Broders. Submitted June 16, 2014. Genetic connectivity among swarming sites in the wide ranging and recently declining little brown bat (*Myotis lucifugus*). Molecular Ecology

The work presented in Chapter 5 also appears in:

Burns, LE and HG Broders. 2014. Correlates of dispersal extent predict population genetic structuring in bats. Conservation Genetics DOI:10.1007/s10592-014-0623-y

The work presented in Appendix B also appears in:

Burns LE, HG Broders, and TR Frasier. 2012. Characterization of 11 tetranucleotide microsatellite loci for the little brown bat (*Myotis lucifugus*) based on *in silico* genome sequences. Conservation Genetics Resources 4: 653-655

APPENDIX B

CHARACTERIZATION OF 11 TETRANUCLEOTIDE MICROSATELLITE LOCI FOR THE LITTLE BROWN MYOTIS (*MYOTIS LUCIFUGUS*) BASED ON *IN SILICO* GENOME SEQUENCES

ABSTRACT

Using an *in silicio* approach, I identified tetranucleotide microsatellite loci from an existing whole genome shotgun DNA sequence of the little brown Myotis (*Myotis lucifugus*). Eleven loci were polymorphic, and exhibited a range from 4 to 25 alleles per locus (mean = 11). Observed heterozygosities ranged from 46% to 94%. Primers for the 11 loci were also tested for cross-species amplification in *M. septentrionalis*. These microsatellites will be useful for genetics-based studies of *M. lucifugus*, which are of increasing importance due to their conservation concern regarding the spread of white-nose syndrome.

INTRODUCTION AND METHODS

The little brown Myotis, *Myotis lucifugus*, is a small bat, widely distributed throughout North America(Fenton & Barclay 1980). *Myotis lucifugus* are generally described as common and abundant throughout their range. However, in 2006, white-nose syndrome (WNS) appeared in the northeastern United States causing mass mortality

of *M. lucifugus* (Blehert *et al.* 2009; Gargas *et al.* 2009), and the species is predicted to face regional population collapse in this area (Frick *et al.* 2010) . Due to the recent emergence of WNS, genetic studies would be invaluable for investigating population structure and connectivity as the disease spreads. Currently, no primers for the amplification of microsatellite loci have been developed for the species. Here, we describe primers for the amplification of 11 novel tetranucleotide microsatellite loci, and provide data on the variability and cross-species amplification success in *M. septentrionalis*.

Potential microsatellite loci were identified via an online search for tetranucleotide repeats in available *M. lucifugus* genomic sequences. Sequences originated from a whole genome shotgun DNA sequence of *M. lucifugus*, generated by the Broad Institute at Harvard (GenBank master record accession no. AAPE00000000). Sequence contigs of this genome (size 5 to 150 kb) were evaluated for microsatellites using the program WebSat (Martins *et al.* 2009) until a set of microsatellites were identified. WebSat parameters were set for a motif length of 4 with a repeat minimum of 6 units. WebSat and the associated Primer3 program (Rozen & Skaletsky 2000) were used to design primers. Primers were developed for 40 loci and amplification was tested using DNA extracted from tissue from two individuals. Genomic DNA was extracted following a generalized phenol/chloroform procedure (Sambrook & Russell 2001). PCR amplification was carried out in 20 µL reactions containing 1X PCR Buffer (20 mM Tris-HCl ph 8.4, 50 mM KCl; Invitrogen), 0.2 mM each dNTP (Invitrogen), 1.5 mM $MgCl_2$, 0.16 mg/mL Bovine Serum Albumin (Sigma Aldrich), 0.3 µM of each primer, 0.05 U/µL *Taq* DNA polymerase and 10 ng of template DNA. Each locus was initially investigated

for amplification at annealing temperatures of 50°C, 55°C and 60°C. PCR cycling conditions were 1 cycle of 95°C for 5 minutes; followed by 30 cycles of 95°C for 30 seconds , annealing temperature for 1 minute, 72°C for 1 minute; followed by a final extension period of 64°C for 45 minutes. Cycling was carried out on Applied Biosystems 96 Well Veriti Thermal Cyclers with PCR products visualized on 2.0% agarose gels stained with ethidium bromide. Allele sizes were estimated by comparison to a Low DNA Mass Ladder size standard (Invitrogen) loaded on each gel.

Twenty-four loci (60%) displayed consistent amplification and polymorphism. These loci were subsequently amplified in four individuals with fluorescently-labeled primers (Applied Biosystems). PCR products were size-separated and visualized on an ABI 3500xL capillary electrophoresis system. Alleles were scored using GeneMarker (vs.1.95, SoftGenetics Inc., State College PA) by comparison to GeneScan 600 LIZ® internal lane size standard (Applied Biosystems). Primer pairs were tested on 83 *M. lucifugus* sampled from Atlantic Canada. Since samples were collected from six geographic locations, loci may have shown deviations from Hardy-Weinberg equilibrium (HWE) stemming from underlying population structure. Therefore, we analyzed the genotypes with program Structure (Pritchard *et al.* 2000) to identify genetic clusters (subpopulations) within the data. Two genetic clusters were identified, and descriptions of microsatellite characteristics are based on analyses of both clusters.

Eleven loci were easily scored and demonstrated moderate to high polymorphism (Table A.1). The number of alleles per locus ranged from 5 to 25 (mean = 12.5) in cluster 1, and 4 to 17 (mean=9.5) in cluster 2 (Table A.2). Observed heterozygosities ranged from 58% to 94% (mean =75%) and 46% to 92% (mean=72%) for cluster 1 and 2,

respectively. After Bonferroni correction (Hochberg 1988), loci *Mluc11* and *Mluc30* deviated from HWE for cluster 1, as calculated using Genepop verson 4.0 (Raymond & Rousset 1995). For cluster 2, only locus *Mluc30* deviated from HWE. The estimated null allele frequencies of *Mluc11* was 0.1083, and *Mluc30* was 0.1150 (cluster 1); and for cluster 2, 0.0605 and 0.1411 respectively. Other loci with null allele frequency estimates >0.05 included *Mluc21* at 0.0999 and 0.1062 (cluster 1 and 2, respectively), and *Mluc29* 0.1836 (cluster 1). No loci pairs showed signs of linkage disequilibrium, in either cluster, as calculated in Genepop after Bonferroni correction. Cross-species amplification was successful for 9 microsatellite loci in *M. septentrionalis,* another species that is susceptible to WNS. These microsatellite loci should be useful in studying population genetic structure in *M. lucifugus* across the wide range of this species.

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Table A.0-1. Locus name, primer sequences, repeat motif, allele size range (AR), annealing temperature (Ta) and the GenBank Accession number for the sequence contig the locus was identified from for the 11 *M. lucifugus* microsatellite loci.

Locus	Primer sequence (5'-3')	Repeat motif	AR (bp; *n*=61)	Ta (°C)	Contig Accession No.
*Mluc*1	F: ATCATAGCGAGCGATCAAACTC	ATAG	115-143	60	AAPE02000298
	R: GCTCTTCTTTTGGTACAGGTGG				
*Mluc*4	F: AACTAACCGAGCTACCCAATCA	TGTA	145-161	60	AAPE02000018
	R: CTTTCCTTTCTCCCTTCCACTC				
*Mluc*5	F: CTAAGAAAGGGTTGCACTCTGG	ATGG	133-169	60	AAPE02014486
	R: TTGTTTACATCAGGCTTTGTGC				
*Mluc*7	F: AATACCCTTGCCTTTCTTCCTC	TCCT	139-237	60	AAPE02012111
	R: ATGTTTTCCTCAAAGTCCCTCA				
*Mluc*8	F: CCACTCAAGCACCAGATGAATA	CTTC	149-229	60	AAPE02000118
	R: AGGAATGAGGGAAGAAAGGAAG				
*Mluc*11	F: CATAAGCTGAATGAGAGGAGGG	TAAA	225-301	55	AAPE02025533
	R: TCGAATAAATACCTGGGAATGG				
*Mluc*21	F: CACTGGTATAGTTCTTTGTAGGTCTG	TGAA	304-320	55	AAPE02023048
	R: AATTTGAATGCTATGGCGAC				
*Mluc*25	F: TACACCCTCTCCAGTTCATGTG	TATC	302-382	55	AAPE02001397
	R: GAGATTACCATAGGCTCACCAAA				
*Mluc*29	F: GGAGGTGGAGAGATTGAGAAAA	AAAG	268-368	50	AAPE02004554
	R: GACACAATGAAGTCCCAAACAA				
*Mluc*30	F: CACACACACAACAGAGAGAGAGAG	GAAA	268-360	60	AAPE02000083
	R: AAAAGCTGGAAAGAAACACTGC				
*Mluc*34	F: ACAAAACACATAGATCCACCCC	AAGA	342-398	55	AAPE02035231
	R: GCCAACTTCAAAGAGAAAGGAA				

Table A.0-2. Number of alleles, observed (H*o*) and expected heterozygosities (H*e*), and Polymorphic Information Content (PIC) for 11 tetranucleotide microsatellite loci tested on 2 clusters of *M. lucifugus* and in *M. septentrionalis* in Atlantic Canada.

	M. lucifugus, cluster 1 [a] (*n*=34)			*M. lucifugus*, cluster 2 [a] (*n*=27)			*M. septentrionalis* (*n*=6)	
Locus	No. Alleles	H*o*/H*e*	PIC	No. Alleles	H*o*/H*e*	PIC	No. Alleles	H*o*/H*e*
*Mluc*1	6	0.724/0.662	0.599	6	0.731/0.702	0.639	[b]	
*Mluc*4	5	0.586/0.642	0.575	4	0.600/0.567	0.483	3	0.167/0.591
*Mluc*5	9	0.719/0.703	0.668	8	0.731/0.796	0.752	3	0.667/0.682
*Mluc*7	18	0.926/0.943	0.920	13	0.947/0.925	0.892	6	0.750/0.929
*Mluc*8	14	0.852/0.912	0.886	12	0.917/0.863	0.827	5	0.600/0.844
*Mluc*11 [c]	10	0.667/0.830*	0.794	7	0.667/0.779	0.733	5	0.333/0.788
*Mluc*21	5	0.613/0.777	0.727	4	0.458/0.552	0.486	[b]	
*Mluc*25	18	0.939/0.875	0.849	12	0.800/0.858	0.823	9	1.00/0.978
*Mluc*29 [c]	14	0.563/0.823	0.787	12	0.615/0.667	0.633	3	0.400/0.600
*Mluc*30	25	0.741/0.947*	0.925	17	0.696/0.936*	0.910	7	0.600/0.867
*Mluc*34 [c]	13	0.813/0.893	0.854	10	0.786/0.899	0.853	5	0.667/0.933
Mean	12.5	0.740/0.819	0.780	9.5	0.723/0.777	0.730	5.1	0.576/0.801

*Significantly different after Bonferroni correction for Hardy-Weinberg equilibrium[a] Structure parameters to detect the 2 clusters were: 3 iterations, for *K* ranging from 1 to 10, consisting of 50,000 steps as the "burn-in", and 500,000 steps with the recorded data, using the admixture model and allowing for allele frequencies to be correlated among clusters. Individuals were "assigned" to a cluster if they had an estimated membership value (*q*) of 0.70 or higher in one cluster and 73% of the individuals were assigned to one of the two clusters

[b] Loci that did not amplify.[c] Loci that showed allelic variation of 2 and 4 bp.

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