Subtle population structuring found within northern long-eared bats (*Myotis septentrionalis*) inhabiting mainland Atlantic Canada

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A Thesis Submitted to
Saint Mary's University, Halifax, Nova Scotia
in Partial Fulfillment of the Requirements for
the Degree of Master of Science in Applied Science.

April, 2014, Halifax, Nova Scotia

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Date: April 24, 2014

Abstract

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Delineating the connections between seasonal sites is required to effectively manage migratory species. One method to characterize the connectivity among populations is to assess the genetic similarity between individuals sampled from various seasonal sites. Combining maternally and biparentally inherited markers can further identify how movement behaviour may differ between sexes. For this study mitochondrial and microsatellite markers were used to assess genetic connectivity between seasonal sites used by the northern long-eared bats. Subtle population structuring found across mainland Atlantic Canada was not linked to sample site locations or spatial distance, suggesting that historical or behavioural processes may be driving population structuring. Analysis conducted on seasonal sites separately, determined that movement behaviour differs between male and female bats. Females are philopatric towards summering sites while male dispersal maintains connectivity between and within seasonal sites.

April 24, 2014.

Acknowledgements

I would first like to thank Hugh Broders and Tim Frasier for the opportunity to work on this project, and providing me with a great number of skills that I will continue to practice. I would also like to thank Brenna Frasier for edits and support. I would like to acknowledge all that assisted in sample collection and offered their knowledge and expertise in the lab. This includes but is not limited to Lynne Burns and Krista Arseneault. For funding I would like to thank Saint Mary's University, Faculty of Graduate Studies, NSERC, and Nova Scotia Power.

There are a number of people for whom I am greatly appreciative to, as they provided a great amount of support throughout these past two years. I have infinite gratitude for my partner Dan Greenberg, my roommate Stephanie Béland and my family. Finally, I would like to thank the graduate society, especially Emily Walker, Logan Gray, Ciara Logar, and Alicia Irwin.

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Chapter 1:

Subtle population structuring found within northern long-eared bats (*Myotis septentrionalis*) inhabiting mainland Atlantic Canada: Introduction

Migration is a fundamental process that has evolved independently across numerous animal taxa as an adaptation to couple seasonal resource needs of individuals to seasonal and spatial patterns of resource abundance (Alerstam et al. 2003; Dingle & Drake 2007; Janmaat et al. 2009). The repeated evolution of migratory behaviour is a testament to its benefits: the ability to locate novel resources and avoid competition, increasing survival and reproductive success relative to nonmigrating individuals (Dingle 1980). Migration can be the movement of individuals between seasonal foraging grounds (e.g. wildebeest, *Connochaetes taurinus*; Wilmshurst et al. 1999, snowy owl, *Bubo scandiacus*; Shelford 1945, tuna, *Thunnus*; Miller 2007), between breeding and nonbreeding sites (e.g. humpback whales, *Megaptera novaeangliae*; Rasmussen et al. 2007, salmon *Oncorhynchus spp.*; Dickerson et al. 2005), or driven by both food and breeding demands (e.g. bull trout, *Salvelinus confluentus*; Starcevich et al. 2012, golden eagles, *Aquila chryseatos*; McIntyre and Adams 1999). The degree and form of migration is dependent on the needs of individuals within a given species. Since migration is a vital component of the population dynamics for many species, there is considerable interest in understanding the proximate and ultimate causes and consequences of migration.

Although migration is a widely studied aspect of animal behaviour, identifying migratory patterns remains challenging (Alterstam et al. 2003). In the past, funding and technological limitations have limited tracking research to economically important and large species (Hobson 1999). In recent years, technological advances such as remote sensing and radio and satellite telemetry have facilitated the tracking of animal movements over large distances (Broquet & Petit 2009). However, these methods are not

always viable, particularly for species with small body size and/or cryptic behaviour (Hobson 1999). As a result, there is much interest in developing accurate intrinsic markers that can be used to infer movement, such as isotopes and molecular genetics (Nathan 2003; Rubenstein & Hobson 2004).

Migration and breeding strategies of a species govern the patterns of genetic variation among and within populations (Chesser 1991). The analysis of DNA can provide information on individual movement by identifying regions of discontinuity in allele frequencies, inferring the process of gene flow, and thus the movement of individuals among populations (Bohonak 1999; Palys et al. 2000). In the past, studies testing for genetic structure used allele frequency data obtained through protein coding loci (e.g. Ward et al. 1992; Rossi et al. 1998; Pérez-Losada et al. 1999; Palys et al. 2000). However, coding regions are under strong selection against mutations since mutations, usually limit gene functionality and can result in fatality (Nei 1987; Metzgar et al. 2000). Coding regions, in general therefore, have low variability and cannot be used to accurately infer patterns of gene flow and population structuring, but rather selection (Glenn et al. 2002). Unlike coding regions, noncoding regions are not under strong selection and often have high levels of substitution. Noncoding markers are essential in population genetic studies because they accumulate neutral and nonfatal deleterious mutations, which will better reflect demographic history and patterns (Galtier et al. 2009). Noncoding regions can be found within both mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) (Schlötterer 2004).

Mitochondrial DNA has many favourable characteristics for inferring population structure, resulting in its widespread use as a genetic marker. The mtDNA of vertebrates typically lacks recombination and is easily amplified since multiple copies occur within a single cell (Pesole et al. 1999; Galtier et al. 2009). Genetic variability within and among species is more readily inferred through mtDNA, as its estimated mutation rate is 5 to 10X greater than single copy nDNA in mammals (Brown et al. 1979). The mtDNA "control region" is a noncoding region containing controlling elements for replication and transcription (Moritz et al. 1987; Boore 1999). It has the highest substitution rate within mtDNA (specifically within the hyper variable regions; Greenberg et al.1983; Saccone et al. 1993; Tamura and Nei 1993) and is widely used for reconstructing phylogenetic histories and inferring phylogeographic patterns (Brown Gladden et al. 1999; Nabholz et al. 2008). Since mtDNA is maternally inherited, the distribution of mtDNA haplotypes across geographic regions is often used to determine the degree of female site fidelity occurring within migratory species (Brown Gladden et al.1999; Dixon 2011). In situations where females exhibit site fidelity, female dispersal patterns will be reflected in the differences of mtDNA between geographic sites. However, such analysis cannot provide complementary information for males, and therefore provides only a partial perspective of the underlying biology (Racey et al 2007). It is therefore essential to use a combination of both mtDNA and nDNA markers when trying to infer population dynamics from molecular markers.

Microsatellites are segments of tandemly repeated nucleotides, typically ranging from mono-, di-, tri-, and tetra-nucleotide repeats, occurring throughout noncoding

regions of nDNA (Weber & Wong 1993). Through DNA polymerase slippage, during

replication, microsatellites experience high levels of mutation, resulting in increased

levels of polymorphism; specifically, variation on the number of tandem repeats (Slatkin

1995; Schlötterer 2000; Schlötterer 2004). On average, the mutation rate of

microsatellites ranges from 10^{-4} to 10^{-3} per locus per generation (Weber & Wong 1993;

Sun et al. 2012), which both creates and maintains high levels of genetic variation (Kashi

1997; Tóth et al. 2000). The high level of variation, biparental inheritance, and selective

neutrality at these regions, makes microsatellites optimal markers for inferring recent

divergence and isolation events (Kashi 1997). Because hundreds of thousands of

microsatellites occur throughout the mammalian genome (Christiakov et al. 2006), and

allelic profiles can easily be examined through polymerase chain reaction (PCR),

microsatellites are a favoured marker for delineating the genetic relationships between

populations (Paetkau et al. 1995; Luikart et al. 1998).

Bats (order: Chiroptera) are ideal candidates for using intrinsic markers to

elucidate movement patterns, as their cryptic behaviour, habitat use, and ability to move

large distances typically preclude direct measurements of movement (Burland and

Wilmer 2001). Many bat species are migratory, particularly temperate species living

within highly seasonal environments (Fleming & Eby 2003). Migration of bats is driven

by a combination of seasonal changes, resulting in resource depressions, and individual

resource requirements including humidity, and temperature requirements (Webb et al.

1995; Bauer et al. 2011). The migration patterns of temperate bat species have often

been separated into three categories of spatial behaviour: sedentary, regional, and long

distance migrators (Fleming & Eby 2003). Sedentary bats usually have the shortest migration distance, traveling fewer than 50 km between their summering and wintering sites. Examples of sedentary species in North America include the big brown bat (*Eptesicus fuscus*) and the Townsend's big-eared bat (*Corynorhinus townsendii*) (Davis & Barbour 1968; Pierson et al. 1999). Regional migrating bats typically migrate 100-500km between seasonal sites, as seen in several North American species: the little brown bat (*Myotis lucifugus*), the northern long-eared bat (*M. septentrionalis*), the gray bat (*M. grisescens*), and the Indiana bat (*M. sodalis*) (Barbour and Davis 1969; Kurta and Murray 2002). Finally, long distance migrating bats travel the greatest distance and may surpass migration distances of 1000 km (Fleming & Eby 2003). In North America, long distance migration is undertaken by the eastern red bat (*Lasiurus borealis*), the hoary bat (*L. cinereus*) and the silver haired bat (*Lasionycteris noctivagans*) (Cryan 2003). The majority of species inhabiting North America are regional migrators.

North American regional migrating bats often have three main 'seasons,' including two migration events (Fenton 1969). Throughout winter, bats hibernate in subterranean areas, such as caves or abandoned mines to survive resource depressions (Fenton 1969). Upon emergence in spring, individuals migrate from their hibernacula to summering sites such as forested regions or manmade structures (Fenton and Barclay 1980; Foster & Kurta 1999). At summering sites, females often gather to form a maternity colony in which offspring are reared, whereas males are primarily solitary (Fenton 1969; Kunz 1982; Carter and Feldhamer 2005). From late summer to mid-autumn, both sexes migrate to the entrance of a hibernaculum and undergo swarming

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prior to hibernation (Fenton 1969). Swarming occurs when large numbers of bats congregate and chase one another in and around a hibernaculum site (River et al. 2005). Studies have found evidence suggesting that swarming may be the primary mating event in several bat species (e.g. Parsons et al 2003; Rivers et al. 2005; Glover & Altringman 2008). Genetic studies at swarming sites have found high genetic diversity compared to that found in summering sites (Rivers et al. 2005; Furmankiewicz & Altringham 2007), suggesting that swarming sites represent a conglomeration of individuals from multiple summering sites. However, it is still unknown how summering and swarming sites are connected and the level of fidelity towards seasonal sites. Varying degrees of fidelity towards each seasonal site may result in the following scenarios: 1) swarming site fidelity as well as natal philopatry towards summering sites; 2) panmictic use of swarming sites while remaining philopatric towards summering sites; and 3) arbitrary use of both summering and swarming sites (Figure 1).

Several genetic studies on temperate bats have found female philopatry to maternity colonies based on the analysis of mtDNA (e.g. Kerth et al. 2002; Arnold 2007; Dixon 2011). It has been suggested that the return of females to natal summering sites is adaptive due to the benefits provided from the opportunity to form social bonds with related females. Multiple females may undergo communal rearing, which can increase offspring survival (Kerth et al 2002). Few studies have focused on male philopatry; however, in the brown long-eared bat (*Plecotus auritus*) and the Natterer's bat (*M. nattereri),* mark-recapture methods have shown evidence of male and female philopatry towards a summering region (Entwistle et al. 2000; Rivers et al. 2006). As a result of

their solitary behaviour, capturing high numbers of males is often difficult (Entwistle et al. 2000) and genetic comparison identifying the differences in population structuring for each sex is often challenging.

The genetic connectivity of summering and swarming sites has only been examined in a few temperate bat species, such as the Natterer's bat. Rivers et al. (2006) found that summering colonies of the Natterer's bat migrate toward the geographically closest hibernaculum entrance for swarming (Rivers et al. 2006). Natterer's bats have also been recorded at several swarming sites within a single night, and evidence of genetic structuring between swarming sites only occur over large geographic distances (Rivers et al. 2005). However, whether this pattern applies to all temperate bat species, which occupy a wide range of environments and vary considerably in life history strategies is still unknown.

The northern long-eared bat (*M. septentrionalis*) is broadly distributed across North America but its movement patterns and spatial ecology are not well resolved as it is highly forest dependent (Caceres & Barclay 2000). Similar to other North American *Myotis* species, the northern long-eared bat migrates between seasonal sites. As in other species, it is believed that copulation occurs during swarming in autumn (Foster & Kurta 1999). In spring, individuals migrate to forested regions where roosting occurs within tree cavities or under peeling bark (Broders & Forbes 2004). Females form maternity colonies while males roost individually or within small groups (Foster & Kurta 1999; Broders & Forbes 2004). The northern long-eared bats change tree roosts throughout a summering

season, undergoing 2.8 roost switches per bat per season (Foster and Kurta 1999).

Patriquin et al (2010) suggested that female northern long-eared bats form both short- and

long-term relationships, often forming pairs that remain together during roost switches in

a summer season and over multiple years. This study has also found evidence of female

philopatry through repeated observations of females returning to the same continuous

forested region in consecutive years. However, the degree of site fidelity to both

summering and swarming sites is still largely unknown for this species. The main

objective of this thesis was to determine the population structuring between and within

swarming and summering sites for one North American temperate bat species, the

northern long-eared bat.

Figure
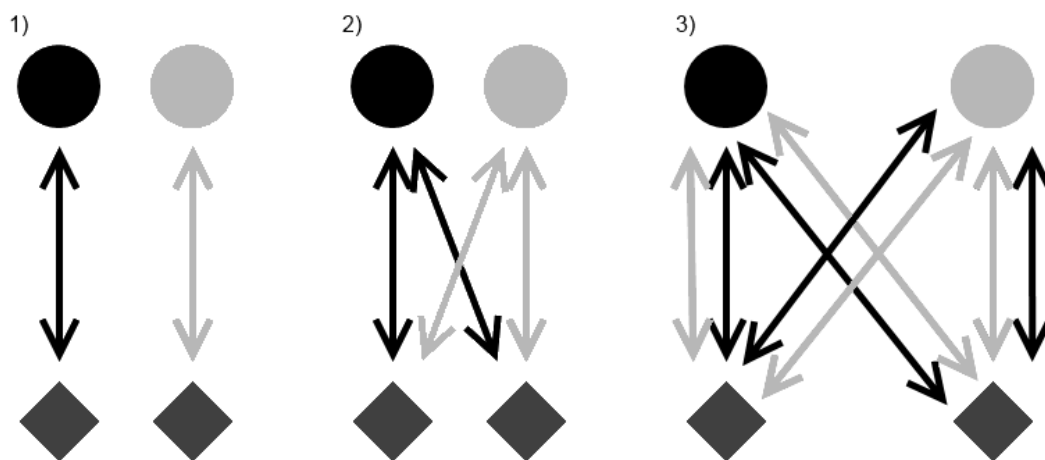
Figure 1: Various scenarios of connectivity between bat summering (circles) and swarming (diamonds) sites. 1) Natal philopatry to summering sites and fidelity to swarming sites 2) natal philopatry of summering sites with panmictic use of swarming sites 3) arbitrary use of both summering and swarming sites.

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Chapter 2:

Genetic analysis reveals subtle population structure and differences in movement behaviour between female and male northern long-eared bats (*Myotis septentrionalis*)

Abstract
            Delineating the connections between seasonal sites is crucial to effectively
manage migratory species. One method to characterize the connectivity among
populations is to assess the genetic similarity between individuals sampled from various
seasonal sites. Temperate bats occupy various seasonal sites including summering sites,
where females give birth to offspring, and swarming sites in the autumn, where it is
believed that bats congregate to mate. The degree of fidelity to specific seasonal sites
(summering sites, swarming sites) and the patterns of movement between them are still
unclear for most temperate bat species. To address this issue I characterized genetic
population structuring of the northern long-eared bat across mainland Atlantic Canada,
using mitochondrial and microsatellite markers. Low levels of population structuring
were found across all sites and structuring observed was not explained by geographic
regions. Examination of summering and swarming sites separately determined that
females are more philopatric to summering sites while males exhibit less loyalty to
summering or swarming sites and appear to be the primary dispersers within this species.

Introduction

Migration is typically considered the movement of individuals between disparate

sites that are used in different seasons. Seasonal sites often vary, both temporally and

spatially, in resource availability, which necessitates that individuals access different sites

at different times of the year, or stages of their life cycle (Alsteram et al. 2003; Janmaat et

al. 2009). Different seasonal sites often offer advantages for foraging, mating and rearing

offspring, or avoiding harsh environmental conditions. Although migratory behaviour is

taxonomically widespread, for many migratory species the underlying details remain

unclear (Webster et al. 2002). The connectivity between seasonal sites and population

structure within sites varies considerably amongst migratory species. In some species,

individuals occupy a single breeding site but migrate to separate foraging sites (e.g. grey

whale, *Eschrichtius robustus*; Frasier et al. 2011), whereas in other species a single

foraging site is comprised of individuals from several breeding sites (e.g., several species

of sea turtles; Bowen and Karl 2007). Understanding the population structure and

connectivity amongst seasonal sites can improve our ability to characterize population dynamics and develop management strategies of migratory species.

For migratory species that are capable of long distance movement and exhibit cryptic behaviour, indirect methods provide a practical approach to infer movement patterns. Characterizing the genetic profiles of individuals across seasonal migratory sites can provide critical information on population structuring and the degree of connectivity between sites (Bohonak 1999). When populations experience restricted gene flow, genetic drift and mutation may cause divergence into distinct genetic clusters, each with unique genetic characteristics (Palys et al. 2000). Maternally inherited mitochondrial DNA (mtDNA) permits inference of female site fidelity and the extent of connectivity between seasonal sites, while the analysis of highly polymorphic nuclear microsatellite DNA (nDNA) provides complementary information for both sexes (Paetkau et al. 1995; Brown Gladden et al.1999; Brown et al. 2005; Vonhof et al. 2008; Dixon 2011; Croteau et al. 2012).

The migratory patterns of temperate bats are still largely unknown, as their nocturnal behaviour and vagility typically preclude direct measurements of migration. North American bats, which migrate on a regional scale, use at least three seasonal sites and undergo two migration events per year (Fenton 1969). To survive resource depressions, bats hibernate for up to eight months during winter in subterranean areas such as caves or abandoned mines (Fenton 1969). Upon emergence in spring, individuals migrate to summering areas where they roost in trees or manmade structures (Fenton and

Barclay 1980; Foster & Kurta 1999). During this time, females form maternity colonies where they rear offspring while males typically remain solitary (Kunz 1982; Carter and Feldhamer 2005). In late summer to mid-autumn, both sexes migrate up to 500 km, to the entrance of one or more hibernacula and undergo 'swarming' prior to hibernation (Barbour and Davis 1969; Fenton 1969; Kurta and Murray 2002). Swarming occurs when large numbers of bats congregate at the entrance of a hibernaculum (River et al. 2005). Greater genetic diversity observed during swarming in comparison to summering sites suggests that swarming sites are comprised of bats from multiple summering areas (Kerth et al. 2003; Parsons et al 2003; Veith et al. 2004; Rivers et al. 2005; Glover & Altringman 2008; Furmankiewicz & Altringham 2007). Therefore, it has been hypothesized that swarming facilitates mating and/or information transfer between individuals about hibernacula (Kerth et al. 2003; Rivers et al. 2005; Veirth et al. 2004; Furmankiewicz & Altringham 2007). However, for most temperate bat species, the population structuring across swarming and summering sites remains unknown, as a swarming site may act as a catchment area for geographically close summering sites. Similarly, whether bats exhibit fidelity towards a particular swarming site, and how the degree of fidelity varies between sexes, is also unclear.

The objective of this study was to characterize the population structuring of the northern long-eared bat (*Myotis septentrionalis*) at summering sites and swarming sites in mainland Atlantic Canada. This species is widely distributed across North America, and throughout the summering seasons is highly forest dependent (Caceres & Barclay 2000), providing an interesting contrast to the majority of studies which typically research

temperate bat species occupying manmade structures. For this study I tested the following hypotheses. First, male and female northern long-eared bats are highly philopatric to natal summering sites. Since mating does not occur at the summering sites, philopatric behaviour of males will not negatively affect the population (e.g. increase inbreeding). Male philoparty may result in an increase of survival rate, as males may become familiar with the resources available. As individuals return to their birth region annually, I expected mitochondrial DNA (mtDNA) to reflect high levels of genetic differentiation among summering sites. Second, swarming sites are catchment areas for bats, supporting mating populations comprised of multiple surrounding summering sites. If true, a greater amount of mitochondrial genetic diversity should be found at a single swarming site than a single summering site and swarming sites would have greater genetic similarity to geographically close summering regions and genetic structuring would occur between distant swarming sites. However, if males are primarily dispersing while females are loyal to swarming sites, mtDNA would be significantly different among swarming sites while nDNA would reveal little to no genetic difference among swarming sites.

Materials and Methods

Sampling

Tissue samples of adult northern long-eared bats were collected opportunistically between 2000 – 2012, from 11 swarming sites and four forested summering areas in New Brunswick and Nova Scotia Canada (n = 457; 242 males, 215 females; Figure 2; Table 1). Geographic distances between sites varied from 9-310 km. Swarming site sample

collection was conducted from mid-August to October while summering samples were collected from May to early August. Bats were captured during the swarming season using harp traps (Ausbat Research Equipment, Lower Plenty, Victoria, Australia) at the entrance of caves or abandoned mines. In the summer, tissue samples were obtained from May to early August using a combination of harp traps and mistnets (Avinet, Dryden, New York, USA) were placed along forest trails. Once captured, bats were removed from traps and placed in cloth bags to reduce stress on the animals. Following capture, bats were identified to species and were sexed and aged as either adult of young of the year (Anthony 1988). A 3-5 mm diameter sample of the patagium tissue was removed from both wings using forceps and cuticle scissors or a 3-mm diameter biopsy punch. After tissue samples were obtained from both wings all sampling equipment was sterilized with 95% ethanol. Tissues were stored at -20°C or were placed in 95% ethanol, Allprotect Tissue Reagent (Qiagen), or salt saturated 20% dimethyl sulfoxide solution with ethylenediamine tetraacetic acid (EDTA; Seutin et al. 1991) then stored at -20°C. Following sampling, bats were released at the site of capture. All bat handling and tissue collection was carried out following certification from Saint Mary's University Animal Care Committee and in accordance with the Canadian Council on Animal Care (CCAC). Genomic DNA was extracted from both wing samples following a standard phenol:chloroform technique (Sambrook & Russell 2001) followed by ethanol precipitation. DNA quantity was estimated, and DNA quality was assessed, based on electrophoresis of 2 µl of DNA through 1.5% agarose gels and visualization based on staining with SYBR Green I (Life Technologies) and UV illumination.

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Sequencing the Mitochondrial DNA

Approximately 400 bp of hypervariable region II of the mtDNA control region was amplified using oligonucleotide primers L16517-forward (Fumagalli et al. 1996) and KAHVII-reverse (designed in lab, 5' GTAGCGTGAATATGTCCTG 3'). Polymerase chain reaction (PCR) amplification was carried out in a 20 µL reaction volume containing template DNA (20 ng), 1X PCR buffer (Promega Inc.), 0.2 mM for each dNTP (Invitrogen), 1.5 mM $MgCl_2$ (Promega Inc.), 0.3 µM of each primer, 0.16 mg/mL of bovine serum albumin (Sigma Aldrich), and 0.05 U/µL of GoTaq® Flexi DNA polymerase (Promega Inc.). PCR cycling conditions consisted of an initial denaturing step of $95^{o}C$ for 5 min; 30 cycles of denaturation at $95^{o}C$ for 30 s, annealing at $48^{o}C$ for 1 min, $72^{o}C$ for 1 min; and a final extension period at $64^{o}C$ for 45 min. Cycling was carried out on Applied Biosystems 96 well Veriti Thermal Cyclers. In preparation for DNA sequencing, dNTPs and excess primers remaining in the amplified solution were degraded. This reaction was carried out by adding 5.78 µL cocktail containing 1.29X Antarctic phosphatase buffer (50 mM Bis-Tris-Propane-HCl, 1 mM MgCl2, 0.1 mM ZnCl2, pH 6.0), 0.1 U/µl Antarctic phosphatase (New England Biolabs), and 0.123 U/µl exonuclease I to each solution containing 5 µl amplified DNA,. Samples were then incubated for 15 min at 37°C, and then 15 minutes at 80°C. PCR products were then sent to Macrogen Inc. (Seoul, Korea), along with the L16517-forward primer, where Sanger Sequencing was conducted (Sanger et al. 1977).

Sequences obtained from the HVII control region were manually edited using

MEGA v5.1 (Tamura et al. 2011). This included trimming sequences to a region of clean

florescent peaks and identifying that nucleotides were properly assigned; if not

assignments were manually corrected. After editing, sequences were aligned using

CLUSTAL W (Thompson et al. 1994) implemented in MEGA. Individual sequences

were then imported into FaBox 1.41 to identify individuals with identical sequences and

assign haplotypes to samples (Villesen 2007). Modelgenerator v85 was used to determine

the most appropriate model of molecular evolution (Keane et al. 2006). Using the

molecular model of Tamura and Nei (Tamura & Nei 1993), as determined by

Modelgenerator. Gene diversity (H) and nucleotide diversity (π) were estimated using

Arlequin v 3.5 (Excoffier et al. 2010). For Arlequin v 3.5, TREE-PUZZLE 5.2 (Schmidt

et al 2002) was used to calculate the transition/transversion rates and parameter α of the

gamma distribution.

Microsatellite Genotyping

Ten of 11 tetranucleotide microsatellite loci developed for *M. lucifugus* were used

to amplify samples (Burns et al. 2012). Samples were amplified using 4 PCR multiplex

reactions (Table 2). PCR amplification was carried out in a 20 µL reaction volume

containing 10 ng template DNA, 1X PCR buffer (GoTaq® Flexi, Promega Inc.), 0.2 mM

of each dNTP (Invitrogen), 1.5 mM $MgCl_2$ (Promega Inc.), 0.08 - 0.2 µM of each primer

(see Table 2), 0.16 mg/mL of bovine serum albumin (Sigma Aldrich), and 0.05 U/µL of

GoTaq® Flexi DNA polymerase (Promega Inc.). PCR cycling conditions were the same

as those described for mitochondrial amplification. Samples were combined with HiDi[TM]

formamide and GeneScan-600 LIZ size standard (Life Technologies) and size-separated.

Samples were then visualized on an Applied Biosystems 3500xL Genetic Analyzer.

Each locus within the four multiplexes was examined by eye and allele sizes were

calculated using GeneMarker software 2.0 (SoftGenetics). Analyses were only conducted

on individuals where alleles for five or more loci were obtained. The number of alleles,

null allele frequencies, observed and expected heterozygosities, and deviation from

Hardy–Weinberg Equilibrium (HWE) were calculated using Cervus 3.0 (Kalinowski et

al. 2007). The inbreeding coefficient (F_{Is}) was calculated using SPAGEDI 1.3 (Hardy &

Vekemans 2002). Genepop 4.2.1 (Rousset 2008) was used to conduct global test across

loci for heterozygote excess/deficiency (Rousset and Raymond 1995).

Analysis of Population Structure

Patterns of genetic characteristics within and among sites were analyzed using a

combination of "classical" assessments and clustering approaches. Classical assessments

of population structure were conducted based on genetic differentiation between sites,

where individuals are assigned *a priori* to the site where the sample was collected

Clustering methods do not require *a priori* definition of groups, rather, the number of

genetically distinct clusters is estimated from the sample set and individuals are

probabilistically assigned to each group.

Assessment of Philopatry and Site Fidelity

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Classical methods were used to assess population structuring of mtDNA and nDNA. For this study datasets were separated by sample sites so that summering individuals and swarming individuals could be examined separately. Differentiation of mitochondrial haplotypes among sites was assessed based on an analysis of molecular variance (AMOVA) as implemented in the program Arlequin v 3.5 (Excoffier et al. 2010). Overall F_{ST} of the microsatellite data was assessed using the methods described in Weir & Cockerham (1984), as implemented in Genepop 4.2.1 (Rousset 2008). Significance values for F_{ST} of mtDNA and nDNA were obtained by using 1000 permutations.

Genetic Connectivity and Isolation by Distance

A Welch's t-test was performed to test differences between summering and swarming gene diversity. To further determine genetic differentiation between collection sites, a pairwise F_{ST} of mitochondrial haplotypes between summering and swarming sites was implemented in the program Arlequin v 3.5 (Excoffier et al. 2010). Complementary pairwise F_{ST} of the microsatellite data was implemented in Genepop 4.2.1 (Rousset 2008). Significance values for mtDNA and nDNA were obtained by using 1000 permutations, then adjusted using Bonferroni correction (Hochberg 1988). Additionally, to determine if movement was restricted by spatial scale, a spatial analysis of genetic structure was conducted by performing an isolation by distance analysis (IBD) on both mitochondrial and microsatellite data using a Mantel test implemented in the web-based IBDWS v 3.32 (Jensen et al. 2005). IBD is used to determine the relationship between

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calculated pairwise F_{ST} and pairwise geographic distances (km) between collection sites. Analysing summering and swarming sites separately allowed us to also determine if a greater amount of genetic differentiation occurs over a large spatial scale in swarming sites. To further determine the movement patterns between summering and swarming sites, separate regression analyses were conducted to examine the relationship between a single summering site to the 11 swarming sites.

Analysis of Relatedness

Philopatry should result in high levels of relatedness of individuals within a particular summering site. Therefore, to determine if summering sites were comprised of highly related individuals I analyzed relatedness (*r*) within and among sites using the program STORM v. 2.0 (Frasier 2008). STORM estimates the average relatedness value within clusters, and then generates "expected" values (under the hypothesis of no patterns) by randomly shuffling individuals across clusters, while keeping each cluster size constant. This generates a distribution of expected relatedness values if the observed clusters represent random groups of individuals from the population. Comparison of observed and expected values then provides a means for hypothesis testing.

Overall Population Structuring

STRUCTURE 2.3.4 (Falush et al. 2003) was used to characterize population structuring occurring across all individuals. STRUCTURE implements a Bayesian approach to estimate the number of clusters (*K*) and to assign individuals to those clusters

(Pritchard et al. 2000). For this study, values of *K* considered were 1-11 (total number of swarming sites). STRUCTURE was run allowing for admixture and for allele frequencies to be correlated across clusters. For the Markov Chain Monte Carlo steps, 50,000 steps were used as the burn-in period, with a subsequent 2,000,000 steps of recorded data. The program was run for 10 iterations of each *K*, and the average probability across iterations was taken as the estimated probability for that *K*. As well, the probability that each individual belongs to each cluster was then visually examined using the program Distruct 1.1 (Rosenberg 2004).

Additionally, structuring across all individuals was characterized using discriminant analysis of principal components (DAPC) conducted using the R package 'adegenet' (Jombart et al. 2010; R Core Development Team, 2013). This program first identified the optimal number of clusters using Bayesian Information Criterion. The dataset was then transformed using principal component analysis (PCA). Subsequently, discriminant analysis was used retain principal components, where optimal number of principal components were identified using α–score (optimal PC= 13; Jombart et al. 2010). Additionally, DAPC assigns membership probability for each individual to different clusters. Since the multivariate analysis implemented by adegenet does not rely on assumptions of HWE, absence of linkage, or specific models of molecular evolution, it provides a robust estimate of the number of clusters in wild populations (Jombart et al. 2008). To determine if the observed clusters differed significantly from what we would expect by chance I simulated 500 data sets of unrelated individuals using the software Family-Sim (Frasier 2009). These random datasets were then run through the previously

stated analyses, the results of which were compared to the observed data to determine its significance.

Results

Mitochondrial Variation

Mitochondrial DNA control region sequences were successfully obtained from 415 individuals. Once sequences were edited, aligned and trimmed to equal length (297 base pairs), haplotypes were assigned. Thirty- two variable sites were identified, representing 98 unique haplotypes. Thirty of the 98 haplotypes were identified from individuals captured at summering sites and 85 were identified from individuals captured at swarming sites (Table 3). Of the 98 haplotypes, seven haplotypes had a frequency over ten individuals, while most haplotypes were found at low frequencies. Analysis determined that for both summering and swarming sites, genetic variation was greater among individuals within a sample site rather than between sampled sites (Table 4).

Microsatellite Variation

Analyses were conducted on genotypes obtained for 449 of the 457 samples. Allele diversity varied from 7-62 across loci. Assessment of loci found that four loci significantly deviate from Hardy-Weinberg Equilibrium (Table 5) and all loci, excluding Mluc1 (p=0.06), had significant heterozygote deficiency ($p<0.05$). From the analysis of all individuals and loci, the global inbreeding coefficient (F_{IS}) was 0.19.

Assessment of Philopatry

The AMOVA analysis of mitochondrial data from summering sites suggested that these sites have significant genetic differentiation (F_{ST}=0.12, p<0.0001; Table 4). Overall F_{ST} of nDNA revealed low but significant genetic differentiation among summering sites (F_{ST}=0.007, p<0.0001). This was further supported through the analysis of relatedness, which suggested that observed relatedness within summering sites was not significantly higher than expected by chance, suggesting that bats inhabiting summering sites are not necessarily close relatives (r= -0.017, p>0.1).

Assessment of Seasonal Site Connectivity

After combining all sites according to site type, swarming sites were found to have significantly higher gene diversity (H = 0.940) than summering sites (H = 0.920) ($t_{6.5}$=16.303, p<0.0001). Overall F_{ST} calculated for both mitochondrial (F_{ST}=0.043, p<0.0001) and nuclear (F_{ST}=0.001, p=0.02) data revealed low but significant genetic differentiation among swarming sites. Only three of mtDNA pairwise comparisons of F_{ST} conducted between a summering and swarming site, were significant (each including the summering site, Kejimkujik National Park (KNP); Table 6). The remaining summering sites had high levels of genetic similarity towards all swarming sites. In the pairwise analyses of microsatellite data, significant genetic differentiation was found between KNP and eight swarming sites, Fundy National Park (FNP) and six swarming sites, Nuttby and three swarming sites; Dollar Lake and Rawdon Mine. Although many of the summering to swarming site comparisons resulted in significant values, all excluding three had a F_{ST} value below 0.01 (Table7). No significant correlation was found between

F_{ST} and geographic distance for either mtDNA (r=0.05, p=0.65) or nDNA (r=0.01, p=0.535). When sites were separated according to season and analyzed, there was still no correlation in both markers (mtDNA: r_{SUMMER}= -0.783, p= 0.171, r_{SWARM}= 0.023, p= 0.593; nDNA: r_{SUMMER}= 0.435, p= 0.775, r_{SWARM}= 0.108, p= 0.695) The regressions conducted between one summering site and all swarming sites revealed that the pairwise F_{ST} calculated from mitochondrial data are positively correlated with geographic distances at both Dollar Lake and FNP (Figure 3), no significant correlation was found. Comparing the regressions conducted between mtDNA and nDNA reveals a greater relationship between mtDNA pairwise F_{ST} and geographic distances than nDNA (Figure 3).

Overall Population Structuring

Through the characterization of genetic structuring occurring across all sites, it was determined, using Bayesian modelling, that three genetically distinct clusters had the highest probability. This is evident as there is a large slope increase from K=2 to K=3 shows greater probability that K=3 (Figure 4). Genetic assignments of individuals to K=3 revealed that a single sample site is comprised of individuals from all three genetic clusters (Figure 5). As well, strong assignments only occurred when individuals were assigned to cluster 1.

From my data set, DAPC found five genetically distinguishable clusters, where three clusters have a high degree of genetic similarity (Figure 6). These observed clusters were found to be significant as all 500 simulated datasets analyzed resulted in a single

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cluster. From assignment of individuals to one of the five genetic clusters, it was determined that clusters were comprised of individuals obtained from all 15 samples sites excluding cluster five, which was comprised of individuals from 14 sample sites.

Discussion

These results indicate that the northern long-eared bat shows some degree of philopatry to summering sites. Both maternally and biparentally inherited DNA markers revealed genetic differentiation among summering sites, but despite significant differentiation in nDNA, the overall level of differentiation was low suggesting weak structuring. Similarly, this finding was further supported by the lack of nDNA relatedness observed within summering sites. The moderate levels of mtDNA structuring, along with absent or weak nDNA structuring, is consistent with other *Myotis* species (e.g. Kerth et al. 2002; Castella et al. 2001; Dixon 2011). Weak nDNA structuring is expected when males are the primary dispersers between mating sites from year-to-year. In comparison to other *Myotis* species (e.g., Castella et al. 2001; Kerth et al. 2002; Castella et al. 2001; Dixon 2011), the northern long-eared bat has weaker mtDNA structuring between summering sites. However, in this study the summering site samples were comprised of both males and females. This may be a result of females show higher degrees of philopatry towards summering sites; while degrees of male philopatry are lower. Comparisons of mtDNA and nDNA revealed that there is a greater correlation between genetic distance and geographic distance through mtDNA than nDNA, which may further support that females show a greater amount of site fidelity than males. Female philopatry

within the northern long-eared bat is consistent with direct observations that females form short- and long-term relationships with each other (Patriquin et al. 2010). In several species of bats, females cooperate to defend pups, undergo social grooming and food sharing (Carter and Wilkinson 2013). Forming close bonds are likely to increase an individual's opportunity to undergo cooperative behaviour. Philopatry is likely adaptive, as females will be more inclined to assist in rearing another individual's pup if the favour is returned in future years.

In comparison to summering areas, swarming sites had greater genetic diversity, suggesting that bats from various summering areas congregate at a swarming site, as seen in other temperate bat species (Kerth et al. 2003; Veith et al. 2004; Rivers et al. 2005). Individuals appear to exhibit less fidelity towards swarming sites than summering sites. As genetic differentiation was higher in mtDNA than in nDNA, structuring is likely a result of females having greater fidelity than males towards swarming sites. However, mtDNA differentiation was relatively low, and suggests that swarming fidelity is likely variable amongst individuals.

Overall nDNA genetic structuring was found to be weak, suggesting high levels of gene flow occurring across mainland Atlantic Canada. Therefore, migration of the northern long-eared bat does not appear to be restricted at this spatial scale. This pattern was further supported by the absence of isolation by distance which was revealed in both mtDNA and nDNA, markers. This suggests that the northern long-eared bat is not restricted by distances up to 350 km. Subtle structuring through DAPC analysis detected

five genetically distinguishable clusters. Out of these five clusters, three appear to show high amounts of genetic similarity, which may explain why Bayesian modeling revealed the greatest probability of three clusters. In both DAPC and Bayesian modeling, the genetic clusters were not found to have a relationship to the location of sample sites; therefore this subtle structuring is likely driven by some other factor.

The unexpected genetic structuring within the northern long-eared bats in this region may be caused by historical or behavioural factors, rather than geographic processes. The presence of multiple apparent lineages within a geographic region may result from the secondary contact of formerly separate populations that diverged in the past. Given the importance of glaciation events in the genetic structuring of many temperate species (Hewitt 2000), it is possible that the genetic clusters observed represent different allopatric lineages that diverged at the last glacial maximum and since became sympatric. Elucidating the phylogeography and historical demography of the northern long eared bat across its broad range in North America may be useful in explaining contemporary patterns of population structuring by identifying historical bottlenecks, vicariance events and/or range expansions (Hoffman and Blouin 2004).

Alternatively, the structuring observed may indicate that female insemination does not occur at swarming sites. Studies have suggested that swarming may serve as a means for adults to inform young-of-the-year of suitable hibernacula, and that mating congregations may occur elsewhere (Fenton 1969; Veith et al. 2004). In some species, such as the noctule bat (*Nyctalus noctula*), copulation occurs along migration routes

during late summer and autumn (Petit et al. 2001). In other temperate species, evidence suggests that copulation may occur after swarming (Veith et al. 2004). In some species such as the brown long-eared bat (*P.auritus*), the percentage of inseminated females increases while epididymis size of males decreases from November to spring emergence in April (Stelkov 1962; Entwistle et al. 1998). This suggests that males may inseminate female during periodic arousal throughout the hibernation season (Veith et al. 2004). If insemination occurs during hibernation, future studies should aim to estimate genetic differentiation between northern long-eared bat hibernacula and determine if individuals swarming at a given hibernaculum subsequently hibernate there. Further research could also use direct observations to determine the time of year where epididymis size decreases and number of inseminated females increase.

For the northern long-eared bat, females appear to be driving genetic differentiation of both summering and swarming sites as is evident in the higher levels of genetic differentiation found between sites using mtDNA. Although males may show some degree of philopatry towards their natal summering sites, the low levels of differentiation found among swarming sites suggests that males feely disperse across swarming sites. This has several important consequences: if males are the primary dispersers, this would suggest they are also the main vectors for the transmission of parasites and pathogens between sites. The recent emergence of the *Pseudogymnoascus destructans* fungus has been implicated in causing widespread bat mortality (Blehert et al. 2009), and this study suggest that this pathogen may be able to spread rapidly and freely across the landscape due to male-biased dispersal. As well, male vagility may

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increase their susceptibility to mortality from both natural events and anthropogenic causes, particularly wind turbines (Arnette et al. 2008). This may have important consequences for population dynamics, as heightened male mortality will alter the population sex ratio within this species with subsequent effects on vital rates including maximum population growth (Lindström and Kokko 1998). As no geographic population structuring was detected, management strategies should treat the northern long-eared bats in this region as one population and therefore conservation management should be coordinated across local jurisdictions. Much remains unknown regarding the migratory patterns, behaviour, and population structure of temperate bats. While temperate bat species face dire circumstances, understanding these aspects of their biology is crucial to the successful management of populations in this region.

Figures and Tables
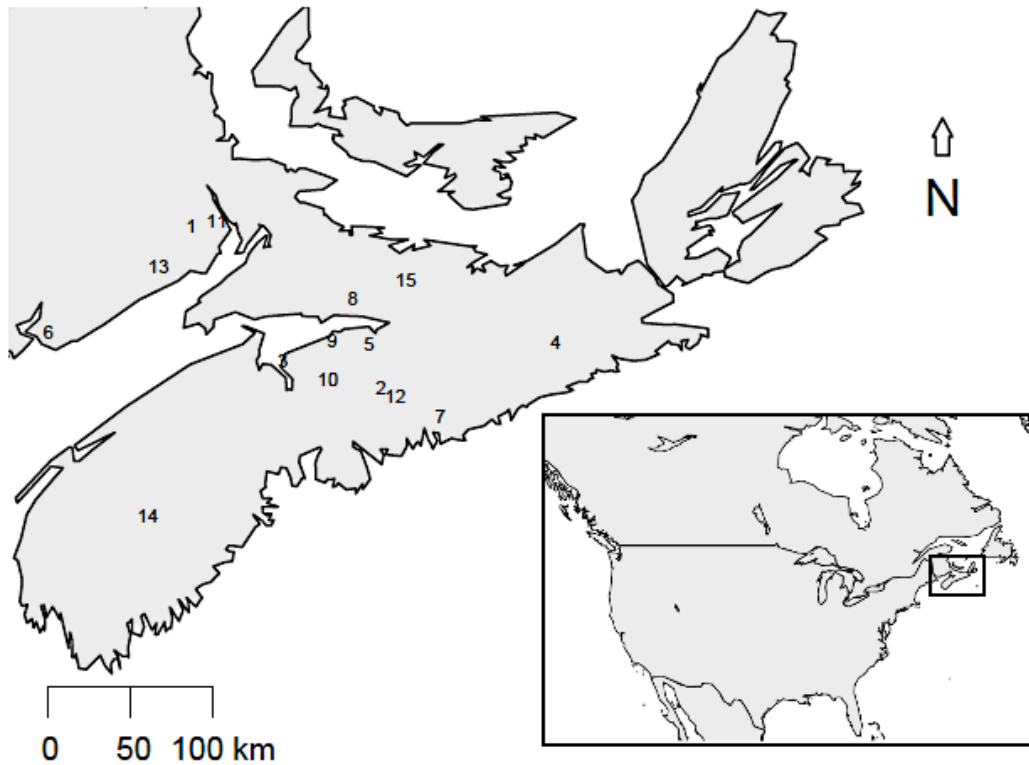
Figure 2: Summering and swarming sites where tissue samples were collected between 2000-2012 across New Brunswick and Nova Scotia, Canada.
 1: Berryton Cave, 2: Cave of Bats, 3: Cheverie Cave, 4: Glenelg Mine, 5: Hayes Cave, 6: Howes Cave, 7: Lake Charlotte Mine (LCM), 8: Lear Shaft, 9: Minasville Cave, 10: Rawdon Mine, 11: Whites Cave, 12: Dollar Lake*, 13: Fundy National Park (FNP)*, 14: Kejimkujik National Park (KNP)* 15: Nuttby*.
* denotes summering collection sites
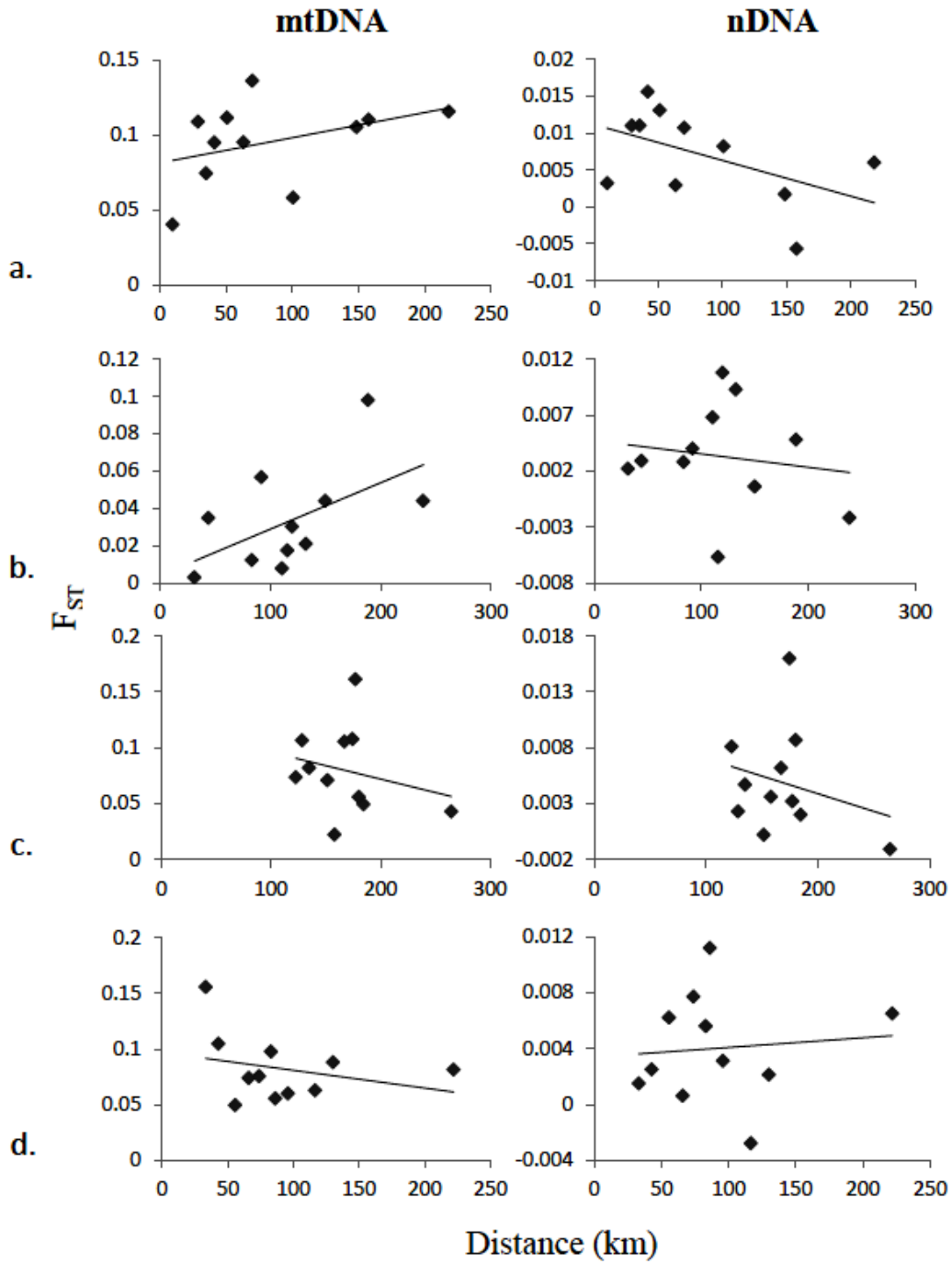
Figure 3: Correlation between pairwise F_{ST} and pairwise geographic distances. Pairwise values were calculated between a single summering site (a. Dollar Lake, b. Fundy National Park, c. Kejimkujik National Park, d. Nuttby) and 11 swarming sites using both mitochondrial and nuclear markers.
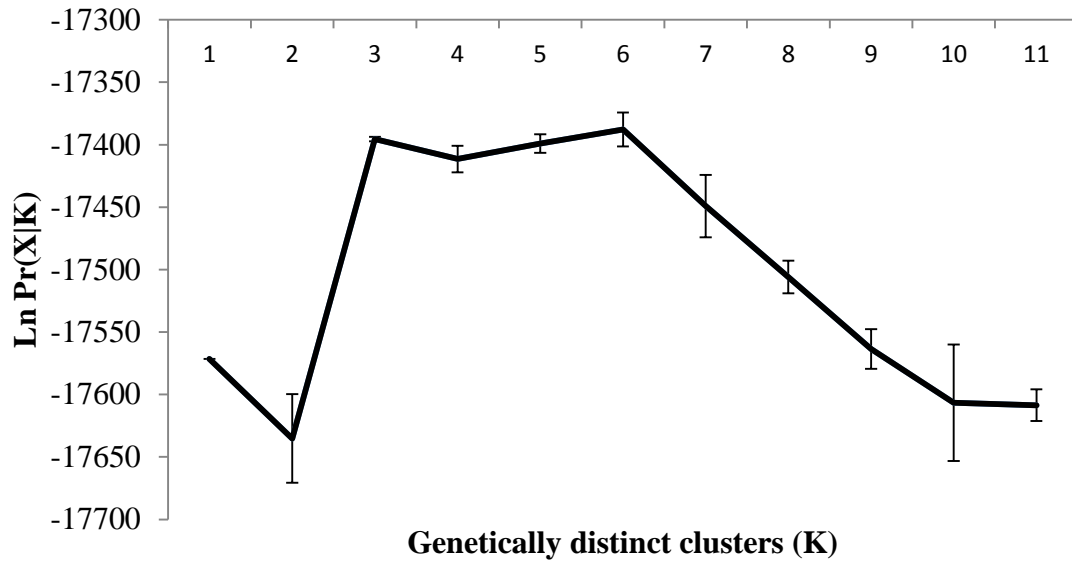
Figure 4: Probability of genetically distinct clusters (K) using STRUCTURE conducted on genotypic allele frequency data for northern long eared bats from Atlantic Canada.
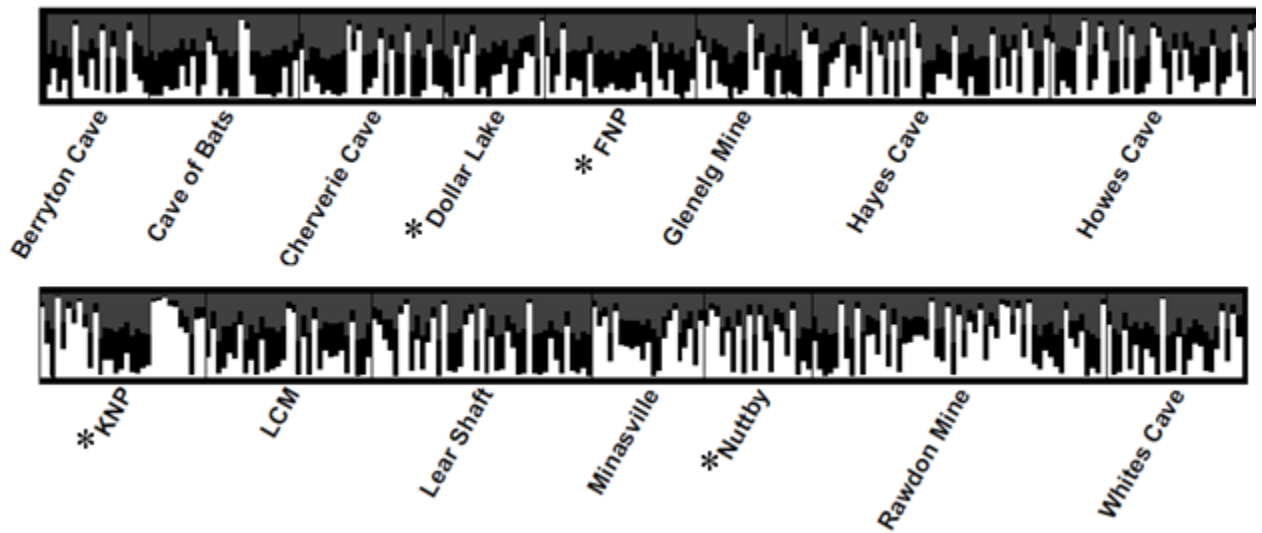
Figure 5: Membership probability of an individual to one of three distinct clusters determined using STRUCTURE. Individuals are categorized by location at which an individual was sampled.

* denotes summering collection sites
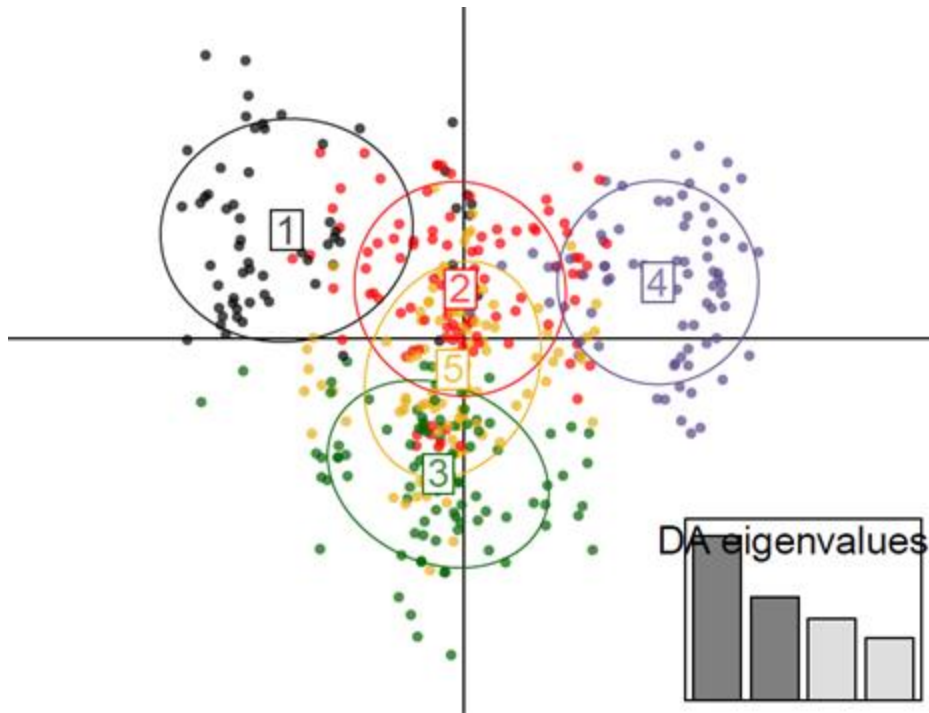
Figure 6: Five genetically distinct clusters as determined using Discriminate Analysis of Principal Components.

Table 1: Number of mitochondrial sequences and genotypic profiles obtained from male and female northern long-eared bats collected at four summering and 11 swarming sites from 2000-2012.

Site Name	Year	mtDNA			nDNA		
		Female	Male	**Total**	Female	Male	**Total**
Fundy National Park	2000	2	7	9	2	6	8
	2001	3	16	19	3	17	20
Dollar Lake	2005	4	0	4	3	0	3
	2006	3	0	3	5	0	5
	2007	10	1	11	10	1	11
Kejimkujik National Park	2001	14	3	17	16	3	19
	2003	7	0	7	7	0	7
	2004	5	0	5	5	0	5
Nuttby	2011	2	1	3	2	1	3
	2012	11	7	18	11	6	17
TOTAL SUMMERING		**60**	**35**	**96**	**63**	**34**	**98**
Berryton Cave	2010	10	7	17	11	8	19
Cave of Bats	2009	9	16	25	12	16	28
Cheverie Cave	2009	11	4	15	9	7	16
	2010	5	3	8	5	4	9
	2011	1	1	2	1	1	2
Glenelg Mine	2010	4	6	10	4	8	12
	2011	2	3	5	2	3	5
Hayes Cave	2009	22	21	43	25	24	49
Howes Cave	2010	7	11	18	7	11	18
	2011	2	18	20	2	18	20
Lake Charlotte Mine	2009	5	14	19	5	14	19
	2010	1	4	5	1	4	5
	2011	1	6	7	1	6	7
Lear Shaft	2009	14	12	26	16	18	34
	2010	0	5	5	1	6	7
Minasville Cave	2010	10	9	19	10	11	21
Rawdon Mine	2009	9	20	29	9	22	31
	2010	17	7	24	17	7	24
Whites Cave	2010	8	14	22	10	15	25
TOTAL SWARMING		**138**	**181**	**319**	**153**	**203**	**351**

Table 2: Microsatellite multiplex reaction information used to genotype 449 tissue samples collected from northern long-eared bats. See Burns et al. 2012 for more information.

Multiplex	Locus Name	Annealing Temp (°C)	Primer [] (μM)	Fluorescent Label
	Mluc4		0.15	VIC
1	Mluc5	60	0.10	6FAM
	Mluc8		0.1	PET
	Mluc1		0.16	6FAM
2		60		
	Mluc21		0.40	6FAM
	Mluc11		0.10	6FAM
3		55		
	Mluc25		0.15	PET
	Mluc7		0.10	NED
4	Mluc30	60	0.15	6FAM
	Mluc34		0.20	PET

Table 3: Mitochondrial haplotype data characteristics for each samples site; including number of sequences obtained (N), number of haplotypes (N), gene diversity (H) with standard deviation (S.D.) and nucleotide diversity (π) with standard deviation (S.D.).

Site Name	N	h	H	S.D.	π	S.D.
SUMMERING						
Dollar Lake	18	4	0.673	0.069	0.022	0.012
Fundy National Park	28	13	0.881	0.048	0.028	0.015
Kejimkujik National Park	29	9	0.84	0.041	0.035	0.018
Nuttby	21	12	0.914	0.041	0.031	0.017
TOTAL (SUMMERING)	**96**	**30**	**0.92**	**0.015**	**0.031**	**0.016**
SWARMING						
Berryton Cave	17	11	0.904	0.057	0.03	0.016
Cave of Bats	25	13	0.917	0.036	0.031	0.017
Cheverie Cave	25	17	0.963	0.021	0.037	0.02
Glenelg Mine	15	10	0.943	0.04	0.034	0.019
Hayes Cave	43	19	0.869	0.041	0.024	0.013
Howes Cave	38	18	0.916	0.028	0.029	0.015
Lake Charlotte Mine	31	11	0.875	0.036	0.027	0.015
Lear Shaft1	31	13	0.766	0.08	0.021	0.011
Minasville Cave	19	17	0.983	0.026	0.038	0.02
Rawdon Mine	53	20	0.932	0.016	0.036	0.019
Whites Cave	22	12	0.944	0.025	0.036	0.019
TOTAL (SWARMING)	**319**	**85**	**0.94**	**0.009**	**0.033**	**0.017**
TOTAL (ALL SITES)	**415**	**98**	**0.939**	**0.008**	**0.032**	**0.017**

Table 4: AMOVA results for mtDNA and nDNA global F_{ST} calculated for site types separately and combined.

Site Type	mtDNA				nDNA	
	% Variation		F_{ST}	p-value	F_{ST}	p-value
	Among	Within				
Summering	11.65	88.35	0.117	<0.0001	0.007	<0.0001
Swarming	4.31	95.69	0.043	<0.0001	0.001	0.02
All Sites	6.17	93.83	0.062	<0.0001	0.003	<0.0001

Table 5: Microsatellite data characteristics; including the number of alleles observed (A), observed and expected heterozygosity (H_o/H_E), significance of Hardy-Weinberg (H-W) tests, and null allele frequencies.

Locus Name	A	H_o	H_E	HW	Null alleles
Mluc1	12	0.762	0.791	NS	0.017
Mluc4	7	0.437	0.540	p<0.001	0.098
Mluc5	10	0.607	0.682	NS	0.058
Mluc7	53	0.532	0.947	ND*	0.281
Mluc8	22	0.532	0.780	p<0.001	0.193
Mluc11	25	0.709	0.843	p<0.001	0.086
Mluc21	17	0.712	0.776	NS	0.038
Mluc25	22	0.696	0.910	p<0.001	0.133
Mluc30	62	0.689	0.968	ND*	0.168
Mluc34	35	0.930	0.961	ND*	0.015

* denotes cases where the number of individuals was too few to conduct the test.

Table 6: Pairwise F_{ST} values calculated using mitochondrial data for 11 swarming sites (1-11) and 4 summering sits (12-15) (Site numbers correspond with Figure 1). Pairwise F_{ST} values are located above diagonal and relative *p-values* are below the diagonal. Bolded values indicate statistically significant values after Bonferroni correction.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1		0.044	0.050	0.035	0.019	0.018	0.094	0.025	-0.003	0.028	0.009	0.110	0.003	0.108	0.088
2	0.327		0.034	-0.007	0.034	0.036	0.035	0.079	0.018	0.029	0.018	0.040	0.044	0.022	0.074
3	0.146	0.192		0.031	0.063	0.047	0.066	0.117	0.002	**0.026**	0.027	0.136	0.057	0.073	0.055
4	0.170	0.457	0.142		0.036	0.032	0.041	0.084	0.016	0.029	0.021	0.058	0.044	0.043	0.060
5	0.420	0.150	0.222	0.104		0.029	0.090	0.019	0.011	**0.036**	0.043	0.074	0.021	0.105	0.105
6	0.333	0.040	0.004	0.053	0.039		**0.088**	0.049	0.008	0.013	0.037	0.116	0.012	0.107	0.081
7	0.006	0.047	0.038	0.040	0.001	0.000		**0.139**	0.059	**0.072**	0.055	0.109	0.098	0.049	0.098
8	0.386	0.021	0.048	0.023	0.282	0.029	0.000		0.056	**0.070**	0.079	0.095	0.017	0.161	0.156
9	0.162	0.251	0.749	0.479	0.307	0.014	0.067	0.076		-0.002	0.004	0.112	0.008	0.071	0.050
10	0.019	0.012	0.000	0.107	0.000	0.027	0.000	0.000	0.026		0.030	0.095	0.030	0.082	0.076
11	0.550	0.932	0.243	0.516	0.294	0.071	0.031	0.084	0.433	0.024		0.105	0.035	0.056	0.063
12	0.690	0.558	0.140	0.241	0.126	0.085	0.010	0.061	0.162	0.031	0.794		0.107	0.090	0.201
13	0.267	0.047	0.249	0.059	0.380	0.018	0.002	0.265	0.300	0.001	0.127	0.079		0.113	0.092
14	0.040	0.373	0.040	0.294	0.003	0.000	0.107	0.000	0.085	0.000	0.281	0.179	0.001		0.115
15	0.231	0.376	0.391	0.177	0.165	0.007	0.121	0.040	0.270	0.001	0.521	0.273	0.120	0.162	

Table 7: Pairwise F_{ST} values calculated using microsatellite data for 11 swarming sites (1-11) and 4 summering sits (12-15) (Site numbers correspond with Figure 1). Pairwise F_{ST} values are located above diagonal and relative *p-values* are below the diagonal. Bolded values indicate statistically significant values after Bonferroni correction.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1		-0.003	0.000	0.009	0.001	0.001	0.008	-0.001	-0.001	**0.009**	0.006	-0.006	0.002	**0.016**	0.002
2	0.038		-0.004	-0.005	-0.002	-0.003	-0.003	-0.003	-0.004	**0.002**	0.003	0.003	0.001	**0.004**	0.001
3	0.050	0.617		0.004	0.000	-0.002	0.006	-0.002	0.004	-0.001	0.013	0.011	0.004	0.008	**0.011**
4	0.003	0.016	0.016		0.004	0.000	-0.002	-0.002	-0.005	**0.007**	0.005	0.008	-0.002	-0.001	0.003
5	0.004	0.025	0.106	0.005		**0.001**	**0.001**	0.004	0.001	**0.002**	0.006	0.011	**0.009**	**0.006**	0.003
6	0.001	0.016	0.166	0.037	0.000		**0.003**	-0.002	-0.004	0.000	**0.003**	0.006	**0.003**	**0.002**	**0.007**
7	0.003	0.016	0.003	0.083	0.000	0.000		-0.002	**0.002**	**0.001**	0.001	0.011	**0.005**	**0.002**	**0.006**
8	0.010	0.036	0.294	0.090	0.001	0.030	0.054		0.000	**0.004**	-0.004	0.003	-0.006	**0.003**	0.002
9	0.093	0.031	0.016	0.037	0.004	0.009	0.001	0.012		**-0.001**	0.010	0.013	**0.007**	0.000	**0.006**
10	0.000	0.000	0.004	0.000	0.000	0.003	0.000	0.000	0.000		**0.010**	**0.016**	**0.011**	**0.005**	0.008
11	0.038	0.023	0.002	0.020	0.003	0.000	0.046	0.208	0.005	0.000		0.002	**0.003**	**0.009**	-0.003
12	0.501	0.007	0.015	0.007	0.002	0.008	0.003	0.002	0.006	0.000	0.129		0.003	**0.013**	0.006
13	0.006	0.006	0.005	0.012	0.000	0.000	0.000	0.092	0.000	0.000	0.000	0.003		**0.003**	**0.007**
14	0.000	0.000	0.002	0.046	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000		**0.012**
15	0.016	0.008	0.000	0.006	0.002	0.000	0.000	0.002	0.001	0.000	0.027	0.028	0.000	0.000	

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Chapter 3:

Subtle population structuring found within northern long-eared bats (*Myotis septentrionalis*) inhabiting mainland Atlantic Canada: Conclusion

Population structure across and within sites occupied during a particular 'season' is a vital component of the overall dynamics of migration, as it is the symptom of how individuals move between sites and interact with other migrants. Subsequently, population structuring of seasonal sites has consequences for the ecology, evolution, and management of migratory species. In chapter two I identified the population structuring occurring within and between two seasonal sites, used by the northern long eared bat (*Myotis septentrionalis*); forested regions, which are occupied throughout the summer and the entrances of hibernacula which are occupied during the autumn. From chapter two, it was first found that female's exhibit high degrees of philopatry towards summering sites, while males are less philopatric. Second, males are likely the primary dispersers within this species as very low levels of genetic structuring were revealed among swarming sites using microsatellite loci. Third, gene diversity compared between swarming and summering sites suggest that swarming sites are comprised of individuals from multiple summering sites. Additionally, using clustering assessment techniques, 5 clusters appear to occur across mainland Atlantic Canada, with three clusters showing a high degree of genetic similarity. However, individual assignment to one of the 5 clusters revealed that neither site location nor spatial distance is driving this structuring.

It is unclear as to what is driving this population structuring. As described in chapter 2, behavioural or historical processes may explain the genetic clusters observed. It is possible that these clusters are remnants of historical refugia, which subsequently merged post glaciation. As the last glaciation event only occurred 12,000 years ago (Demboski and Cook 2001), the bats inhabiting Atlantic Canada may not have had

sufficient time to redistribute their genetic material across all clusters. Alternatively individuals from different lineages may exhibit differences in behaviour that affect their likelihood to breed with individuals from other lineages. For example, this could be manifested by differences in phenology, including the timing of mating events. Elucidating whether any behavioural or ecological differences exist in individuals from these five clusters would provide important insight into the processes maintaining structuring in this species.

Isolation by distance has rarely been observed in bat species (Burland et al. 1999), and as I found, the northern long-eared bat is not affected by geographic distance up to 350 km. However, this ability to move across large spatial scales may have consequences for the transmission of parasites and harmful pathogens. As a greater amount of structuring was observed using mitochondrial markers, this study suggests that females show some degree of site fidelity towards swarming sites. Additionally as mentioned above, males have little fidelity towards swarming sites and appear to freely move. If males are the primary dispersers, they are likely the main vectors increasing transmission between sites. This finding is very important for explaining the rapid spread of the pathogenic fungus *Pseudogymnoascus destructans*, more commonly known as White-nose Syndrome, across North America (Blehert et al. 2009). This fungus has devastated bat populations with millions of bat mortalities (Blehert et al. 2009; Reynolds and Barton 2014). Since 2006, WNS has spread across North America and as of 2014 has been confirmed in 26 states and 5 provinces, including New Brunswick and Nova Scotia, and has been identified on seven species of hibernating bats: the northern long eared bat (*M.*

septentrionalis), the little brown bat (*Myotis lucifugus)*, the Indiana bat (*Myotis sodalis*), the eastern pipistrelle (*Perimyotis subflavus*), the eastern small-footed myotis (*Myotis leibii*), the grey bat (*M. grisescens*) and the big brown bat (*E. fuscus* to date; U.S. Fish and Wildlife Services; Reeder et al. 2012). As different bat species often occupy the same hibernacula, understanding interspecific movement patterns can aid in predicting the spread and epidemiology of emergent infectious diseases, which currently represent the principal threat to North American bat populations.

The apparent difference in male and female movement behaviours has consequences for their susceptibility for different human impacts. Male vagility may result in a heightened mortality rate as long distance dispersal may increase risk of predation and exposure to environmental stressors. Additionally, fatalities of bats have been globally documented at wind turbine facilities, raising concerns that this may be impacting bat populations (Arnett & Baerwald 2013). Mortality risk due to wind turbines has been found to be greater in adult male bats (Arnett et al. 2008), consistent with these findings on male-biased dispersal. This has potential consequences for both population and spatial dynamics. The majority of wind turbine fatalities occur during the fall migration from late summer to mid-autumn (Johnson 2005). If male mortality is especially high during migration towards mating events, and males maintain genetic connectivity across sites, gene flow may be reduced or inhibited by wind turbine facilities. Further investigation using a before-after, control-impact experimental design may provide insight on how wind turbines potentially alter gene flow between seasonal sites used by bat species.

Migratory species in general, and bats in particular, are at a heightened risk of decline due to their broad-scale movements and multiple habitat requirements. Management and conservation efforts require an adequate understanding of migratory processes, particularly in light of increased anthropogenic development. Using molecular markers can provide inferences on migratory connectivity and population structuring that would otherwise be likely overlooked by direct observations. Molecular markers can provide a great amount of information on the movement patterns for migratory species by identifying population structuring between and within seasonal sites. A combination of maternally and biparentally inherited markers can also reveal differences in movement behaviour between sexes. Analysis of population genetics has crucial implications for the management strategies for migratory species.

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