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Movement Ecology and Conservation of the Migratory Bats Lasiurus cinereus and Lasionycteris noctivagans

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Movement Ecology and Conservation of the Migratory Bats *Lasiurus cinereus* and *Lasionycteris noctivagans*

by

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Abstract

Little is known about bat migration, but recently, fatalities of migratory bats at wind energy facilities across North America have offered the opportunity to gain insight into migratory-bat behaviour. Using a combination of stable-isotope and genetic analyses, I studied patterns of movement and relatedness of two species of migratory tree-roosting bats frequently killed by wind turbines: hoary bats (*Lasiurus cinereus*) and silver-haired bats (*Lasionycteris noctivagans*). Using stable isotopes of nitrogen ($\delta^{15}N$), carbon ($\delta^{13}C$), and hydrogen ($\delta^{2}H$) of the fur of bat carcasses collected at a wind energy facility in south-western Alberta, I determined that the bats killed were migratory and originated from latitudes 100s-1000's of kilometers to the north, with silver-haired bat originating from further north than hoary bats. The relationships between stable isotopes and arrival date suggests that the timing of migration may be governed by local habitat cues rather than latitudinal cues. I used a highly polymorphic portion (HVII) of the mitochondrial DNA control region and developed multilocus microsatellite markers to investigate the population genetics of hoary bats and silver-haired bats recovered at wind energy facilities in three Canadian provinces. Pairwise Fst values suggest subtle population-genetic structure among sites in both species, but greater structure in silver-haired bats. Two different Bayesian clustering analyses, STRUCTURE and TESS, suggested that there are at least two genetic clusters of hoary bats and six genetic clusters of silver-haired bats across the three sites. Lastly, I used microsatellite markers to examine whether bats learn migratory routes and behaviours from other closely related individuals. I tested whether the time between when individual bats were killed at a wind energy facility was influenced by their degree of relatedness and found that bats do not appear to be learning migratory routes or behaviours from closely related individuals. This suggests that bats may rely on endogenous genetic programs for migration and that migration may be a heritable trait.

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Dedication

This thesis is dedicated to my amazing partner, Brandon, whose support is truly staggering.

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CHAPTER 1: GENERAL INTRODUCTION

Although my research and this thesis, has a strong conservation focus, the underlying theme throughout is animal migration. Animal migration has long fascinated people, partly because migration occurs in a wide variety of animals: mammals, birds, sharks, reptiles, amphibians, ray-finned fishes, insects, and freshwater and marine invertebrates (Dingle 1996, Dingle and Drake 2007). To have evolved so frequently, the potential benefits of migration (e.g. increased access to resources) must outweigh the potential costs (e.g. increased mortality during migration; Dingle 1980, Alerstam et al. 2003, Fleming and Eby 2003). While we know that the movements of migrating animals are somehow fundamentally different from their other movements, such as foraging, dispersal, and ranging, defining migration is challenging (Baker 1978, Dingle 1996, Dingle and Drake 2007). Therefore, there are many, often taxon-specific, definitions of animal migration.

Early definitions, and many current definitions, tend to focus on the seasonal to-and-fro movements of birds (Dingle 1996). Attempts at a multi-taxon definition range from Baker's (1978) rather vague but inclusive - "the act of moving from one spatial unit to another"; to Dingle's (1980) attempt at including a behavioural component with – " [m]igration is specialized behaviour especially evolved for the displacement of the individual in space"; to Kennedy's (1985) more specific - "[m]igratory behavior is persistent and straightened out movement effected by the animal's own locomotory exertions or by its active embarkation upon a vehicle. It depends on some temporary inhibition of station keeping responses but promotes their eventual disinhibition and recurrence". Given the immense diversity of animals that migrate, a true multi-taxon definition of migration may be impossible. However, a working definition of migration should contain some key elements: longer than normal (i.e. daily) movement between habitats to

continually secure optimal environmental conditions; straightened out, persistent, and directional movement while en route; and significant behavioural and physiological adjustments before, during and after migration (Thomsan 1926 cited in Dingle 1980, Emlen and Emlen 1966, Dingle 1980, 1996, Fleming and Eby 2003, Dingle and Drake 2007).

Migration is governed by the interaction of external (e.g. social and environmental cues) and internal (e.g. genetic) factors (Dingle 1980, Berthold and Terrill 1991, Dingle 1996). The major components of animal migration are timing, orientation, and navigation. Animals must first decide when to migrate, and then decide where to go and how to get there. They may migrate in family groups and learn migratory behaviours and routes from group members, or rely on an endogenous genetic program. Migratory animals use a variety of visual and non-visual cues for orientation and navigation, such as the sun, the stars, polarized light, the Earth's geomagnetic field, and landmarks to orient themselves along migratory routes (Able 1980, Lohmann and Lohmann 1996, Alerstam et al. 2003, Holland 2007). These various cues interact with genetic and social cues during migration (Able 1980, Berthold and Terrill 1991, Bingman and Cheng 2005).

Migration can take many forms (e.g. obligative or facultative, round-trip or one-way, seasonal or irruptive; Dingle and Drake 2007) and occurs over many different spatial scales (Dingle 1996, Fleming and Eby 2003, Dingle and Drake 2007). In bats, migration is classified by Fleming and Eby (2003) as sedentary (moving <50km between summer and winter roosts), regional (moving approximately 100-500km between summer and winter roosts) and long-distance (moving >500km and potentially >1000km between summer and winter roosts). In bats in general, regional or short-distance migration is much more common than long-distance migrations. Of the approximately 176 genera of bats, only 15 contain species known to be long-

distance migrants (Popa-Lisseanu and Voigt 2009). The low number of bats that migrate, relative to birds, may be a consequence of the use of hibernation as an alternative over-wintering strategy by many temperate bat species, or a constraint of bats' tropical origins (Jones et al. 2005a, Bisson et al. 2009).

Lack of data plagues the study of bat migration and much of what we know is circumstantial or anecdotal. Long-distance migration by bats was suspected in the late 1800's when the winter disappearance of many species of temperate bats was noted (Allen 1939) and descriptive and anecdotal evidence of migration began accumulating (Miller 1897, Howell 1908). Many of the North American bats suspected of migrating were solitary, foliage-roosting species such as eastern red bats (*Lasiurus borealis*; Shump and Shump 1982a) and hoary bats (*L. cinereus*; Shump and Shump 1982b), or tree-cavity roosting silver-haired bats (*Lasionycteris noctivagans*; Kunz 1982). Until recently (Cryan 2003, Cryan et al. 2004, Cryan et al. 2014, Fraser and Longstaffe 2014), evidence for migration in hoary bats, eastern red bats, and silver-haired bats was circumstantial (Shump and Shump 1982a, Cryan 2003) and came primarily from records of bats appearing on islands (Hitchcock 1943, Hayman 1959, Tenaza 1966, Maunder 1988, Hill and Yalden 1990), landing on ships at sea (Thomas 1921, Norton 1930, Carter 1950, Mackiewicz and Backus 1956), migrating diurnally (Howell 1908, Hall 1946), colliding with buildings and other structures (Saunders 1930, Terres 1956, Van Gelder 1956, Taylor and Anderson 1973 , Crawford and Baker 1981, Timm 1989), and seasonally peaking in local abundance (Zinn and Baker 1979, Barclay 1984). In the first continental–scale studies of bat migration, Findley and Jones (1964) used museum records and available literature in an attempt to map the seasonal distribution of hoary bats throughout North America. Later work by Cryan (2003) re-evaluated and expanded Findley and Jones' study, presented further evidence of long-

distance migration in tree bats, and provided seasonal distributions for both eastern red bats and silver-haired bats. Long-distance migration (>1000 km) of individual hoary bats and silver-haired bats has since been confirmed by stable hydrogen isotope analysis of fur (Cryan et al. 2004, Cryan et al. 2014, Fraser and Longstaffe 2014)

The study of bat migration is challenging. Not only are migratory bats nocturnal, but they are all relatively fast, high altitude flyers (Norberg and Rayner 1987), thus they are difficult to capture on-the-wing. Due in part to their roosting behaviour, they are relatively dispersed across the landscape, thus they are also difficult to capture in the roost. As such, early studies of the seasonal movements of bats focused on banding and homing experiments, predominantly using colonial and/or cave-dwelling species as models (Allen 1921, Howell and Little 1924, Griffin 1934, 1936, Cockrum 1956, Davis 1966, Griffin 1970). Tree-roosting migrants, the North American species in particular, have been grossly understudied. However, a number of recent studies using radio-telemetry (McGuire et al. 2012), acoustic monitoring (Baerwald and Barclay 2009), stable isotope analysis (Cryan et al. 2014, Fraser and Longstaffe 2014), genetics (Russell et al. 2015, Vonhof and Russell 2015), and other lab-based approaches (McGuire and Ratcliffe 2011, Cryan et al. 2012a, McGuire et al. 2013) have been rapidly increasing our understanding of the biology of these species.

Gaining a better understanding of the basic biology of bat migration has become increasingly important because of bat fatalities at wind energy facilities because many of these fatalities involve bats during autumn migration. Bat fatalities have been reported at wind energy facilities across Europe (Zagmajster et al. 2007, Rydell et al. 2010, Camina 2012, Georgiakakis et al. 2012), Canada (Barclay et al. 2007, Arnett and Baerwald 2013), and the United States (Arnett et al. 2008, Arnett and Baerwald 2013), and at facilities in South Africa (Doty and

Martin 2013), Australia (Hall and Richards 1972, Hull and Cawthen 2013) and Brazil (Barros et al. 2015), but likely occur at other facilities worldwide. The numbers of fatalities are alarming from a population perspective. An estimated 840,000 – 1.7 million bats were killed by wind turbines across Canada and the United States from 2000-2011 (Arnett and Baerwald 2013), with current estimates of the annual number of fatalities falling between 400,000 and 800,000 (Arnett and Baerwald 2013, Hayes 2013, Smallwood 2013). The majority (~80%) of these fatalities are of hoary bats, eastern red bats, and silver-haired bats during autumn migration (Arnett and Baerwald 2013). I chose to focus my research on hoary bats and silver-haired bats because they make-up 54% and 37% of bat fatalities at wind energy installations in Alberta, respectively. Although not particularly closely related (Hoofer and Bussche 2003), both species belong to a group of bats colloquially referred to as migratory tree-bats, which, as the name suggests, are bats that migrate and roost in trees. I provide more detailed and relevant biological information about each species in the pertinent chapters.

Given that migratory animals are at greater risk of extinction than sedentary animals (Pimm et al. 1988, Pagel and Payne 1996, Meffe and Carroll 1997) and that migration in general may be becoming an endangered phenomenon (Brower and Malcolm 1991, Wilcove 2008, Wilcove and Wikelski 2008), it is becoming increasingly important to understand the migrations of animals in order to conserve them. To reduce the impact of wind energy installations on bats, we must first understand some basic biology of bat migration. With this in mind, the two main goals of my research were to: a) gain a better understanding of the migratory patterns and behaviours of migratory bats, and b) gain a better understanding of the impacts of wind energy installations on migratory bats.

In Chapter 2, I use stable isotope analysis of $\delta^{2}H$, $\delta^{13}C$, and $\delta^{15}N$ to examine the summer distributions, migratory patterns, and geographic origins of hoary bats and silver-haired bats killed at a wind energy facility in southwestern Alberta. Bats killed in summer and autumn at wind turbines in Germany originated from Estonia, Russia, and Scandinavia, thus revealing that wind turbines kill bats not only from sedentary local populations, but also from migratory populations, and thus negative impacts of fatalities extend far beyond political borders (Voigt et al. 2012). As in the German study, I expected that the bats killed at a single facility in southwestern Alberta come from a large catchment area. If this is the case, in combination with evidence from previous studies using acoustic and fatality data (Baerwald and Barclay 2009) it suggests that bats from broad geographic areas are using discrete migration routes.

In Chapter 3, I evaluate the degree of population genetic structure among potential migration routes of bats across Canada using a region of maternally inherited mitochondrial DNA (HVII) and 18-19 bi-parentally inherited microsatellite loci. I also used these data to assess the contemporary effective population sizes of both silver-haired and hoary bats and discuss the consequences of bat fatalities at wind energy facilities on genetic structure and population stability and the need to include genetic information in management decisions.

In Chapter 4, I assess the use of social transmission of migratory routes and behaviours in bats. I expected that, as in other long-lived animals with extended parental care (Sutherland 1998), bats learn migratory behaviours and routes through socially transmitted information rather than relying on endogenous genetic programs. I discuss my findings in terms of the costs and benefits of social transmission of information.

Finally, in chapter 5, I synthesise what I have learned about the basic biology of bat migration and the potential consequences of fatalities at wind energy facilities and use these

findings to make a series of recommendations for reducing the harm of wind turbines to bat populations.

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CHAPTER 2: ORIGINS AND MIGRATORY PATTERNS OF BATS KILLED BY WIND TURBINES IN SOUTHERN ALBERTA: EVIDENCE FROM STABLE ISOTOPES[1]

Introduction

Given the challenges of studying small, aerial, nocturnal migrants, the migration biology of bats is poorly understood (Fleming and Eby 2003, Cryan and Diehl 2009). Determining the origins and migratory patterns of bats has important conservation implications. Around the globe, large numbers of bats are being killed at wind energy facilities (Rydell et al. 2010, Arnett and Baerwald 2013). Most fatalities are of migratory bats during autumn migration and, although little is known regarding the use of migratory routes by bats, fatalities thus appear to occur along potential migration routes (Baerwald and Barclay 2009, Rydell et al. 2010, Arnett and Baerwald 2013). In North America, approximately 80% of fatalities are of migratory tree-roosting bats: hoary bats (*Lasiurus cinereus*), eastern red bats (*L. borealis*), and silver-haired bats (*Lasionycteris noctivagans*; Arnett and Baerwald 2013). In southern Alberta, the estimated fatality rate of bats ranges from 1.3 - 31.4 bats/turbine/year depending on the site (Baerwald and Barclay 2009) resulting in thousands of hoary bat and silver-haired bat fatalities in Alberta annually. If bats are primarily killed along migration routes, as has been hypothesized (e.g. Baerwald and Barclay 2009), and multiple wind facilities are placed along a route, the cumulative impact of these fatalities may be quite large (Arnett and Baerwald 2013).

[1] A modified version of this chapter was published in *Ecosphere* as: Baerwald, E. F., W. P. Patterson, and R. M. R. Barclay. 2014. Origins and migratory patterns of bats killed by wind turbines in southern Alberta: evidence from stable isotopes. Ecosphere **5**:118. I collected all hair samples, analyzed the data, and wrote the manuscript. W. P. Patterson performed the laboratory component of the stable isotope analysis and wrote the corresponding methods. R. M. R. Barclay assisted with the data analysis, writing, and editing.

Not only is there scant knowledge regarding the migration biology of migratory bats, little is known about their distributions and ranges. For example, two biodiversity databases, the Global Biodiversity Information Facility (GBIF) and the Mammal Networked Information System (MaNIS), list few records if any, of hoary bats or silver-haired bats in Canada. Additionally, range maps of hoary or silver-haired bats differ markedly among sources (e.g. Bat Conservation International 2012, Encyclopedia of Life 2012, The Redpath Museum 2012). Without information as basic as the distribution and movements of affected species, it is difficult to determine the cumulative effects of turbine-related fatalities. This is especially concerning given that migratory bats have ecological traits that increase their risk of extinction. In general, animals that migrate tend to be more vulnerable to extinction than those that do not (Pimm et al. 1988). Unlike sedentary bats, migratory bats require appropriate habitat in several, spatially disjunct locations: breeding/summering sites, hibernation/overwintering sites, stopover sites, and migratory corridors that link them (Hutson et al. 2001, Fleming and Eby 2003). Even though fatalities of bats may be a concern from a population perspective, especially given the slow life-history of bats (Barclay and Harder 2003), their carcasses provide the opportunity and impetus to study the migration of these species, and inform future conservation initiatives.

Migration has traditionally been studied by using exogenous markers, such as bands and radio-transmitters, and although these methods have been used to study various aspects of migratory-bat biology (e.g. Hutterer et al. 2005, Klug et al. 2012), they have been of limited use in tracking long-distance movements of tree-roosting bats in North America (Cryan and Diehl 2009). However, predictable relationships between an endogenous marker, the stable isotope values of animal tissue, and the environment, have enabled investigation of the origins and migratory behaviours of various animals (reviewed in Hobson 1999b, Rubenstein and Hobson

2004, Hobson and Wassenaar 2008). δ^2H decreases with increasing latitude and elevation (Poage and Chamberlain 2001, Bowen and Revenaugh 2003, Sellick et al. 2009). $\delta^{13}C$ values may also decrease with increasing latitude and elevation as vegetation shifts from predominantly C_4 to C_3 plants (Körner et al. 1988, Hobson et al. 2003, Suits et al. 2005). Likewise, $\delta^{15}N$ decreases with increasing latitude and the associated cooler, drier conditions (Handley et al. 1999, Hobson 1999a, Amundson et al. 2003). Animals incorporate the chemical composition (e.g. stable isotope values) of their diet into their tissues. Animal keratins, such as fur, are metabolically inert once grown and the stable isotope values of these tissues therefore reflect the environmental conditions (i.e. isoscape) where they were grown. Thus, the chemical composition of fur can be used to link an animal to the place where the fur was produced. The confidence in this estimate is greatly increased if we know the dietary ecology of an animal and the diet-to-tissue discrimination factor.

Fortunately, we know the dietary ecology of hoary bats and silver-haired bats summering in Canada (Barclay 1985, Reimer et al. 2010) and that diet-to-tissue discrimination in bats is similar to that for other vertebrates (Voigt et al. 2003, Voigt and Matt 2004, Mirón et al. 2006, Popa-Lisseanu et al. 2007). Stable-isotope analysis of bat tissue has been used in numerous dietary studies (Fleming et al. 1993, Voigt and Kelm 2006, Popa-Lisseanu et al. 2007, Painter et al. 2009, Cryan et al. 2012b), and also to investigate the origins and migratory movements of bats. For example, δ^2H values of hoary bat fur (δ^2H_f) accurately reflects δ^2H values of the precipitation (δ^2H_p) at the latitude at which it was grown (Cryan et al. 2004, Cryan et al. 2014). Combining this relationship with capture and museum records demonstrated that some hoary bats migrate long-distances (>2000 km; Cryan et al. 2004, 2014). Further studies have attempted to evaluate the use of stable-isotope analysis to assign origins to individuals of various bat

species across Europe (Popa-Lisseanu et al. 2012), the eastern United States (Britzke et al. 2009, Sullivan et al. 2012), and Canada (Fraser et al. 2012). All of these studies found that, for numerous species, bat fur accurately reflects δ^2H_p where the fur was grown, and in some, that $\delta^{13}C_f$ values are correlated with δ^2H values in both hair and precipitation, and with latitude. Not all results have been consistent across species or age/sex classes, and thus analyzing each group separately is important (Britzke et al. 2009). Given the difficulty in studying bat migration, the ability to use endogenous markers, such as stable isotopes, offers a feasible alternative to more traditional but inefficient methods (i.e. banding or radio-tracking).

To learn more about the basic biology of migratory bats, and to apply this information to the issue of bat fatalities at wind turbines, I used stable isotope analysis ($\delta^{13}C$, $\delta^{15}N$, and δ^2H) of fur ($\delta^{15}N_f$, $\delta^{13}C_f$, δ^2H_f) from turbine fatalities to test hypotheses regarding the distribution and migration of hoary and silver-haired bats. Given that hoary and silver-haired bats differ in size, morphology, and roosting and foraging behaviour, and thus likely differ in their optimal migration strategies (Barclay 1985, Hedenstrom 2008, 2009, Baerwald and Barclay 2011), my underlying hypothesis was that these differences influence their distribution and migration, which would be reflected in the isotopic signature of their fur. I thus predicted that the stable isotope values present in the fur of hoary and silver-haired bats differ. Based on published range maps, inferred migratory behaviour (Barclay 1984, Cryan 2003, Baerwald and Barclay 2009), and the seasonal timing of fatalities (Baerwald and Barclay 2011), I specifically hypothesized that, a) bats killed at turbines in south-western Alberta are migrants (i.e. not local) and that b) hoary bats originate from farther north and from a broader range than silver-haired bats do. If bat fatalities at turbines represent migrants, I predicted that both the variance and the mean isotope values of their fur are greater than the isotope values in the fur of resident bats (i.e. fur collected

from bats on their summering grounds) from the same latitude as the wind energy facilities. If hoary bats originate from farther north and from a broader area than silver-haired bats do, then the isotope values in hoary bat fur should be lower and have greater variance than the isotope values of silver-haired bat fur.

Bats commonly exhibit sexual segregation on their summering grounds as well as sex-biased migratory behaviour, with females tending to migrate more often and over longer distances than males do (Cryan 2003, Fleming and Eby 2003, Ibanez et al. 2009). I hypothesized that migration of both hoary and silver-haired bats differs between the sexes, much like in other bat species. I thus predicted that the isotope values in the fur of adult females are lower than those of adult males, reflecting the longer, more northern migration made by females. Based on capture and fatality records (Baerwald and Barclay 2011), I further predicted that the degree of sexual segregation, thus the difference in isotope values between males and females, is greater in hoary bats. Finally, to explore the temporal patterns of bat migration, I examined the relationship between isotope values and date. A correlation may indicate a pattern in the migratory movements of bats (e.g. differential or leapfrog migration; Mazerolle and Hobson 2007, Paxton et al. 2007, Langin et al. 2009).

Materials and Methods

Study species

Hoary bats occur throughout much of the Americas, from northern Canada to southern Argentina and Chile (Shump and Shump 1982b, Cryan 2003; Figure 2.1). Although the movement patterns and seasonal distributions of hoary bats in North America are poorly understood, in summer they occur throughout the aspen parkland and boreal forest of Canada. In

autumn, they leave and overwinter in Mexico and the southern United States (Findley and Jones 1964, Cryan 2003, Cryan et al. 2004; Figure 2.1). Hoary bats arrive in southern Canada in May to early June and autumn migration begins with males in late July and continues with females and juveniles from early August to mid-September (Barclay 1984, Baerwald and Barclay 2011). There appears to be some degree of sexual segregation during summer, with females potentially migrating greater distances than males (Findley and Jones 1964, Cryan 2003, Cryan et al. 2004). Although their distribution is unknown, the relatively high proportion of adult male hoary bat fatalities at wind energy installations in Alberta (Baerwald and Barclay 2011) suggests that more adult males move into the province than previously suspected. In Alberta, 54% of bat fatalities at wind energy installations are hoary bats (E. F. Baerwald, unpublished data).

Silver-haired bats range from south-eastern Alaska through southern Canada, south to central California and northern Mexico, and east to Georgia (Yates et al. 1976, Hall 1981; Figure 2.1). Wintering grounds seem to be in the Pacific Northwest, south-western states, and middle latitudes of the eastern United States (Izor 1979, Cryan 2003). In spring, silver-haired bats from the east migrate north and east, while silver-haired bats in the west migrate northward (Cryan 2003). In summer, silver-haired bats are common in aspen parkland, but are also found throughout the southern three-quarters of Canada's boreal forest (Figure 2.1). Silver-haired bats arrive in southern Canada in May to early June and autumn migration is from early August until mid to late September (Barclay 1984, Baerwald and Barclay 2011). As in hoary bats, there appears to be some degree of sexual segregation during summer, with females potentially migrating farther than males (Cryan 2003), although capture records indicate the presence of adult males in northern Alberta (Grindal et al. 2011) and fatalities at wind energy facilities suggest a relatively even sex ratio in southern Alberta during autumn migration (Baerwald and

Barclay 2011). In Alberta, 37% of bat fatalities at wind energy installations are silver-haired bats (E. F. Baerwald, unpublished data).

Although no formal studies have been conducted on the moulting patterns of either hoary or silver-haired bats (but see review by Fraser et al. 2013), observational and isotopic data suggest that both species moult on their summering grounds just prior to migration, thus between late June and early August (E. F. Baerwald and B. J. Klug, pers. observation; Cryan et al. 2004). This timing of moult is similar to that observed in other bat species (Cryan et al. 2012b, Fraser et al. 2012, Fraser et al. 2013). Thus, fur collected along the migratory route is fur grown on the summering grounds during the current year's moult.

Sampling

I collected hair samples from bat carcasses found under wind turbines in south-western Alberta, Canada (49° 35' 04" N, 113° 47' 48" W), from 15 July to 30 September 2006 and 2007. For each carcass, I recorded species, age (Anthony 1988) and sex (when possible), and the degree of decomposition. I collected hair from between the scapulae of bats killed the previous night. I placed samples into plastic micro-centrifuge tubes and stored them in a freezer until analysis.

The fur of resident silver-haired bats was collected by E. Fraser from the Cypress Hills, SK (approx. 49° 37' N, 109° 58' W) between 20 July – 05 August 2008 and 2009. I collected hair from resident hoary bats captured at a maternity site, Delta Marsh, Manitoba, Canada (50°11′02.44″ N, 98°22′55.15″ W) in July 1991 and from 08 June - 03 August 2010. Bats tend to be highly philopatric (Perry 2011) and reproductive female hoary bats return annually to Delta Marsh (Koehler and Barclay 2000). I thus assumed that any hair collected pre-moult was hair

grown at the same site the previous year. All methods were approved by the University of Calgary Life and Environmental Sciences Animal Care Committee, and all field captures were conducted under Manitoba Conservation Wildlife Scientific permit WB09615.

Stable carbon and nitrogen isotope analysis

Stable carbon and nitrogen analyses were conducted at the Saskatchewan Isotope Laboratory (SIL) in the Department of Geological Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. After washing hair samples with a mixture of chloroform:methanol (2:1) for 2 h, rinsing with distilled water, and oven drying overnight at 60^{o}C, they were weighed in tin capsules and loaded into the sample carousel. Stable isotope values were determined by combustion using a Thermo Finnigan Flash 1112 Elemental Analyzer via a Conflo III coupled to a Thermo Finnigan Delta Plus XL mass spectrometer. Samples were pyrolized under helium in an oxidation furnace packed with chromium (VI) oxide and silvered cobaltic/cobaltous oxide (to remove any halogens) at 1000°C. The resulting gas was passed through a reduction furnace packed with elemental copper at 680°C to reduce all nitrogen-bearing compounds to pure gaseous nitrogen. The resulting gases were passed through a water trap to eliminate moisture. A GC column at 50°C separated the carbon dioxide and nitrogen gases for analysis in the mass spectrometer. Data were blank-corrected, and then corrected for carbon isotope values for ^{17}O contribution using the Craig correction (Craig 1957). Carbon isotope ratios are reported in per mil (‰) notation relative to the VPDB scale. Nitrogen isotope ratios are reported in per mil notation relative to AIR. Carbon data were calibrated against the international standards L-SVEC (δ^{13}C = -46.6‰ VPDB) and IAEA-CH6 (δ^{13}C = -10.4‰ VPDB). IAEA-CH7, an intermediate international standard, gave the following result during

calibration of the in-house standards: $\delta^{13}C$ = -32.1 ± 0.04‰ VPDB (n = 11). The accepted value of $\delta^{13}C$ is -32.1 ± 0.10‰ VPDB. Nitrogen was calibrated against the international standards USGS-25 ($\delta^{15}N$ = -30.4‰ AIR) and IAEA-305A ($\delta^{15}N$ = 39.8‰ AIR). IAEA-NO3, an intermediate international standard, gave the following result during calibration of the in-house standards: $\delta^{15}N$ = 4.0 ± 0.08‰ AIR (n = 8). The accepted value of $\delta^{15}N$ is 4.7 ± 0.2‰ AIR. Accuracy of data was monitored via routine analyses of in-house standards that are calibrated against the IAEA standards above. The accuracy of $\delta^{13}C$ and $\delta^{15}N$ measurements are 0.1‰ and 0.2‰, respectively (n = 18, 2s).

Stable hydrogen isotope analysis

Deuterium analysis was also conducted at the SIL. Hair samples were washed in a mixture of chloroform:methanol (2:1) for 2 h, rinsed with distilled water, and oven dried overnight at 60°C. To avoid mass linearity correction, mass of the samples was matched to the mass of the standards to get comparable signal intensity. Fur samples and standards were weighed in silver capsules then equilibrated at room temperature for at least 96 h prior to $\delta^{2}H$ determination (based on Bowen et al.'s (2005) finding that complete replacement of the exchangeable fraction of H in hair can occur in as little as 3–4 days). Samples were analyzed using a Thermo Finnigan TC/EA coupled to a Conflo III and a Delta-Plus XL mass spectrometer. Samples were dropped into a glassy carbon furnace and pyrolyzed at 1350°C to form hydrogen and/or carbon monoxide gases. These gases were carried in a helium stream to a GC column held at 100°C to separate the gases before being diluted in the Conflo III and passed to the mass spectrometer for analysis. Isotope ratios were blank corrected and normalized against Kudu Horn Standard (Africa) (KHS) and Caribou Hoof Standard (North America) (CBS) provided by the

National Hydrology Institute, Saskatoon, Saskatchewan. Values are δ^2H = -197± 1.8 for CBS and -54.4 ± 0.6 (n=10) for KHS. Results are reported as per mil (‰) notation relative to VSMOW, VSLAP scale. A third standard, Spectrum Keratin (source unknown), was used as a check standard for drift correction with an accepted value of -121.6 ± 2 for δ^2H. The standard deviation for δ^2H was 1.8‰.

Data analysis

Based on potential inherent differences in isotopic ratios by age and sex (Britzke et al. 2009, Cryan et al. 2012), I divided fur samples from turbine fatalities into one of four categories; adult male, adult female, sub-adult male, and sub-adult female (Table 2.1). To explore the relationships among the three isotopes found in the fur, I used a correlation matrix. I analyzed variation in carbon, nitrogen, and deuterium isotope values among species, age, and sex classes using Analysis of Variance (ANOVA) tests. Due to the potential for year-to-year variation in the isotope values present in fur (Haché et al. 2012), I included year in all models. I included all interactions, and used backward stepwise model-selection to remove non-significant terms. I used Tukey's post-hoc tests to evaluate differences among groups.

To test my hypothesis that the majority of bats killed were migrants (i.e. not local), I compared the variance in isotope values from turbine-killed bats to the variance in values from the fur of resident silver-haired bats from the Cypress Hills, SK (data from Fraser 2011), and resident hoary bats from Delta Marsh, MB. I used F-tests and either standard t-tests or unequal variance t-tests, whichever was appropriate based on the variance.

To investigate the summer distribution and catchment area of bats killed at turbines, I used published relationships between δ^2H_f and δ^2H_p and an online GIS-based mapping tool,

IsoMAP (Bowen et al. 2012, Bowen et al. 2014). IsoMAP is a likelihood-based assignment method based on the isoscape of δ^2H_p. It incorporates both the analytical error surrounding δ^2H_f and the errors inherent in the δ^2H_p isoscape. I created a precipitation model similar to the Bowen et al. (2005) model used by Cryan et al. (2014) to assign a likelihood of geographic origin. To geographically assign individuals using IsoMAP, δ^2H_f values must first be converted to their predicted δ^2H_p using previously published relationships between the two variables. For hoary bats, I used the relationship published by Cryan et al. (2014). However, there is no published relationship for silver-haired bats, so I examined both the Cryan et al. (2014) relationship and the relationship published by Popa-Lisseanu et al. (2012). The latter relationship is based on multiple species of European bats, presumably making it more universal than other, more species-specific equations (e.g. Britzke et al. 2009, Fraser et al. 2012). Even though this equation is based on European species of bats, the general relationship between latitude and δ^2H_p is global, albeit with differences among continents. Additionally, there is no reason to think that bat species in Europe incorporate deuterium into their keratin differently than bats in North America do. I transposed the data so that the δ^2H_p was the response variable rather than the explanatory variable and then used the resulting regression equation to determine the predicted δ^2H_p (now y) given our δ^2H_f values (now x). The Cryan et al. (2014) data resulted in the regression equation $y = 0.7469x+2.635$, and the Popa-Lisseanu et al. (2012) data resulted in $y = 0.6722x-2.6195$. I used a two-sample t-test to compare the mean predicted δ^2H_p values derived by the two different regression equations. Then, to determine the accuracy of these regression equations for silver-haired bats and the IsoMAP models in general, I used isotope values in fur from silver-haired bats of known geographical origins (Cypress Hills, SK), and compared the results of both the geostatistical (likekrig) and regression (likereg) models. For further analysis, I chose the

regression equation (i.e. Cryan et al. 2014 or Popa-Lissaneau et al. 2012) and IsoMAP model that most accurately predicted geographic origin.

To better understand migration patterns of bats, I examined the relationship between isotope ratios and arrival date (i.e. night of death) via linear regressions between each isotope ($\delta^{13}C_f$, $\delta^{15}N_f$, or $\delta^{2}H_f$) and date. Based on the results of earlier analyses, I analyzed each species and year separately.

I used Shapiro-Wilk W tests to test for normality. I considered variables to be non-normal if $p<0.05$, but if the W statistic was sufficiently close to one (i.e. ≥ 0.90), then I considered the data near-normal and did not transform them (Sen and Srivastava 1990) because ANOVAs with large sample sizes are robust in dealing with violations of normality (Zar 1999). I conducted statistical analyses with JMP 10.0.0 (SAS Institute, Cary, North Carolina) and present means ± standard deviation (SD).

Results

I analyzed fur from 295 bats killed at turbines in south-western Alberta (Table 2.1). None of the isotope values from turbine fatalities were normally distributed, but all three had W statistic values close to one ($\delta^{13}C_f$, $W = 0.98$, $p = 0.001$; $\delta^{15}N_f$, $W = 0.98$, $p < 0.001$; $\delta^{2}H_f$, $W= 0.97$, $p < 0.0001$), and I thus considered them near-normal.

$\delta^{13}C_f$ and $\delta^{2}H_f$ values were positively correlated (Pearson's $r =0.47$, $p < 0.001$), but $\delta^{15}N_f$ values were not correlated with either $\delta^{13}C_f$ or $\delta^{2}H_f$ values ($\delta^{13}C_f$ Pearson's $r = 0.06$, $p = 0.30$; $\delta^{2}H_f$ Pearson's $r = -0.07$, $p = 0.23$). In the ANOVA that assessed the variation in $\delta^{13}C_f$ values among species, years, and age/sex classes, the model explained a significant proportion of the variation (ANOVA, $F_{8,286} = 14.90$, $p < 0.001$; Table 2.2). $\delta^{13}C_f$ values differed among species

and age/sex class, but not between years (Table 2.2). The significant interaction between species and age/sex class indicates that $\delta^{13}C_f$ values are influenced by species, but that this relationship was influenced by the age and sex class (Table 2.2). Adult male and female hoary bats had similar values of $\delta^{13}C_f$, but these were higher than both sexes of sub-adult hoary bats and all classes of silver-haired bats (Figure 2.2).

In the ANOVA that assessed the variation in $\delta^{15}N_f$ values among species, years, and age/sex classes, the model explained a significant proportion of the variation (ANOVA, $F_{8,286} = 3.91$, $p < 0.001$; Table 2.3). There was no significant difference between years, but there was a significant interaction between species and age/sex class, indicating that $\delta^{15}N_f$ values are influenced by species, and that this relationship was influenced by the age and sex class (Table 2.3; Figure 2.3).

In the ANOVA that assessed the variation in $\delta^{2}H_f$ values among species, years, and age/sex classes, the model explained a significant proportion of the variation (ANOVA, $F_{8,286} = 9.26$, $p < 0.0001$). The mean $\delta^{2}H_f$ value of silver-haired bats was lower than that of hoary bats (Table 2.4). The significant interaction between year and age/sex class indicates that $\delta^{2}H_f$ differed by age and sex class, but that this difference was influenced by year (Table 2.4; Figure 2.4).

With the exception of $\delta^{13}C$, isotope values of fur from bats killed at wind turbines (putative migrants) were more variable than values in the fur of bats captured in the Cypress Hills or at Delta Marsh (residents; Table 2.5). In addition, mean isotope values differed significantly between putative migrants and residents. With the exception of $\delta^{13}C_f$ in hoary bats, putative migrants had lower $\delta^{13}C_f$, $\delta^{15}N_f$, and $\delta^{2}H_f$ values than residents (Table 2.5).

There was no difference in the mean δ^2H_p predicted by the equations derived from the Cryan et al. (2014) data and the Popa-Lisseanu et al. (2012) data (Cryan et al. (2014) mean = -92.92‰, Popa-Lisseanu et al. (2012) mean = -88.61‰, t = 1.97, p = 0.06). The IsoMAP regression model, in conjunction with either equation, accurately predicted the origins of silver-haired bats resident in the Cypress Hills, SK (Figure 2.5). Thus, for simplicity, I used the equation derived from the Cryan et al. (2014) data and the regression model (likereg) in IsoMAP to predict the origins of all turbine-killed bats. The IsoMAP analysis indicated that bats killed at wind turbines in south-western Alberta potentially came from a large catchment area and that silver-haired bats originated from farther north or from higher elevations than hoary bats (Figures 2.6 and 2.7).

There was a negative correlation between $\delta^{15}N_f$ and fatality date for silver-haired bats in both 2006 and 2007 (2006, $r^2 = 0.43$, $p < 0.0001$; 2007, $r^2 = 0.18$, $p = 0.002$), and with the date of hoary bat fatality in 2007 ($r^2 = 0.08$, $p = 0.01$). There was a weak but significant negative correlation between $\delta^{13}C_f$ values and fatality date for silver-haired bats in 2006 ($r^2 = 0.07$, $p = 0.04$), but no other significant correlations between $\delta^{13}C_f$ or δ^2H_f values and date.

Discussion

Based on published range maps and inferred migratory behaviour (Cryan 2003, Baerwald and Barclay 2009), I hypothesized that bats killed in south-western Alberta were migrants and that hoary bats originated from farther north and from a broader area than silver-haired bats. The differences in isotope values between putative migrants and residents, the lower mean $\delta^{13}C_f$ and δ^2H_f values, and the greater variances in isotope values in turbine fatalities relative to resident bats, are consistent with the hypothesis that the bats killed were migrants.

As I also predicted, the isotope values in the fur of silver-haired bats and hoary bats killed at wind turbines differed, although not uniformly across all isotopes, years, or age/sex classes. Contrary to my prediction, however, silver-haired bats had lower $\delta^{13}C_f$ and $\delta^{2}H_f$ values than hoary bats did. These differences could result from differences in diet (Barclay 1985, Reimer et al. 2010) or foraging habitat (i.e. different association with standing water and aquatic foodwebs; Britzke et al. 2009, Sellick et al. 2009) or the combination of the two. The diets of hoary bats and silver-haired bats on the summering grounds are slightly different, as are the diets of sub-adult hoary bats (at least for the first two weeks post-volancy; Rolseth et al. 1994). Although both species are opportunistic aerial-hawkers that eat similar types of insects, silver-haired bats and sub-adult hoary bats tend to eat smaller prey items than adult hoary bats (Barclay 1985, Rolseth et al. 1994). However, the stomach-content analysis of 54 of the bats used in this study revealed few dietary differences among species, age and sex classes (Reimer et al. 2010). Although this type of dietary analysis only represents the diet of an individual over a single night during migration, it suggests that the dietary differences among age/sex classes are unlikely to cause the different patterns present in the stable isotope signatures. In addition, the correlation I found between $\delta^{13}C_f$ and $\delta^{2}H_f$ values suggests that the inter-specific differences in these isotopes also reflect differences in the latitude of origin of individuals.

On average, silver-haired bats killed in south-western Alberta had lower stable isotope values than hoary bats and thus appear to have originated from higher elevations and/or farther north, and from more boreal-like ecosystems than hoary bats, which appear to have originated from more aspen parkland-like ecosystems. This interpretation is reinforced by the IsoMAP analysis. That the observed ranges were different than the expected ranges suggests that the published range maps are inaccurate and/or that hoary bats originating from farther north or

higher elevations use a different migration route and thus are not killed at the wind energy site in south-western Alberta.

Care should be taken in the interpretation of the IsoMAP analysis. I am not suggesting that stable isotope analysis be used to redefine species ranges or that bats originate from every possible location, as this method is based on global patterns and not constrained by the biology of the animal, although it can be (e.g. Sullivan et al. 2012). For example, my IsoMAP analysis shows that, based on stable isotopes, silver-haired bats killed in southern Alberta could have originated from eastern Canada. This is highly unlikely given what we know about the species' biology and bat migration in general. It is more likely that the silver-haired bats killed in Alberta originated from locations nearer to the wind facility, perhaps from the boreal regions of Alberta, the Northwest Territories, and Saskatchewan or from the higher elevations of the Rocky Mountains. Regardless, the data show that migratory bats killed in south-western Alberta have the potential to come from large catchment areas, as recently found for bat species in Germany (Voigt et al. 2012).

I hypothesized that migratory bats exhibit sexual segregation during the summer and predicted that the isotope values of adult males would differ (likely by being higher) from adult females (i.e. females reside farther north). I further predicted that the degree of sexual segregation would be greater in hoary bats than in silver-haired bats. The lack of significant differences in mean isotope values between males and females of either species does not support my predictions. The lack of evidence for sexual segregation is surprising, especially for hoary bats. Sexual segregation and differential migratory behaviour in bats is common (Cryan 2003, Fleming and Eby 2003, Ibanez et al. 2009), as evidenced by the fact that male hoary bats arrive at our wind-energy study site before females do (Baerwald and Barclay 2011) and by the limited

number (or complete lack) of male hoary bats at maternity sites, such as the Cypress Hills or Delta Marsh, during the summer (Barclay 1984; C. Willis and J. Poissant, pers. comm.). It may be that males and females are segregated in such a way that the isotope values in their fur do not reflect the difference (e.g. elevationally and/or longitudinally). Indeed, given my site's proximity to the Rocky Mountains and that males are often found at higher elevations than females (Cryan et al. 2000, Cryan 2003), it seems likely that sexual segregation may be occurring by elevation.

I examined the correlations between isotope value and date for evidence of differential migration (e.g. Mazerolle and Hobson 2007, Paxton et al. 2007, Langin et al. 2009). The weak or non-existent correlations between $\delta^{13}C_f$ or $\delta^{2}H_f$ and fatality date (i.e. arrival date) do not provide conclusive evidence for differential migration (see also Cryan et al. 2014). However, $\delta^{15}N_f$ was negatively correlated with fatality date of silver-haired bats in both years and hoary bats in 2007. Given the lack of correlation between $\delta^{15}N_f$ and $\delta^{13}C_f$ or $\delta^{2}H_f$, it is unlikely that the relationship between $\delta^{15}N$ and date indicates a latitudinal pattern of migratory movement, but rather, indicates dietary differences. $\delta^{15}N$ values increase by ~3‰ with each trophic level or by variable amounts in animals that are water or nutritionally stressed (Hobson et al. 1993, Kelly 2000, Voigt and Matt 2004, Smith et al. 2010). Thus, it may be that bats arriving earlier in the season were either foraging at a higher trophic level, or were more water or nutritionally stressed at the time their fur was grown, than bats arriving later in the season. The relationship between $\delta^{15}N_f$ values and date suggests differences in habitat quality or body condition that influence the timing of migration either at an individual or landscape level. For example, early migrants could be individuals in relatively poor condition (from high or low quality habitat) or bats from a region of relatively low quality habitat.

My data show that migratory bats killed in south-western Alberta may come from forests hundreds of kilometers away and potentially from large catchment areas, as was recently reported in the European study by Voigt et al. (2012) and a North American study by Cryan et al. (2014). Thus, the impacts of these fatalities may have broad-reaching ecological consequences. Although some wind energy facilities have successfully reduced the number of bat fatalities (Baerwald et al. 2009, Arnett et al. 2010), fatalities of bats remain a conservation issue, especially given the important ecosystem services provided by bats. The combined losses of bats from white-nose syndrome (Frick et al. 2010) and wind energy development could cost the North American agricultural sector billions of dollars/year (Boyles et al. 2011). However, the ecological ramifications of bat fatalities extend beyond agriculture. Bats limit insects and their herbivory (Kalka et al. 2008, Williams-Guillén et al. 2008, Böhm et al. 2011), which is especially important when dealing with forest pests such as western spruce budworm (Wilson and Barclay 2006). Thus, the loss of migratory bats that summer throughout the boreal forest and aspen parkland, and then migrate through agricultural areas, has the potential to impact a wide variety of ecosystems over a large geographical range.

Table 2-1 Sample sizes of turbine-killed hoary bats (*Lasiurus cinereus*) and silver-haired bats (*Lasionycteris noctivagans*) used for fur samples.

	2006		2007	
Age/Sex	Hoary bat	Silver-haired bat	Hoary bat	Silver-haired bat
Adult male	37	19	26	7
Adult female	25	25	9	13
Sub-adult male	17	9	26	15
Sub-adult female	16	13	20	18

Table 2-2 Results of the ANOVA used to test the variation of $\delta^{13}C$ values in fur collected from hoary bats (*Lasiurus cinereus*) and silver-haired bats (*Lasionycteris noctivagans*) killed at wind turbines in south-western Alberta from 15 July to 30 September 2006 and 2007. Categories with different superscript letters are significantly different (Tukey's post-hoc test)

Parameter	Categories	LSmeans	SD	df	Type III Sum of Squares	F-stat	P
Year	2006	-24.5	1.0	1	1.9	1.79	0.18
	2007	-24.6	1.0				
Species	Silver-haired bat	-25.0	1.0	1	51.5	48.34	<0.0001
	Hoary bat	-24.1	1.1				
Age/sex class	Adult female[A,B]	-24.4	1.1	3	10.2	3.18	0.02
	Adult male[A]	-24.3	1.0				
	Sub-adult female[A,B]	-24.7	1.1				
	Sub-adult male[B]	-24.8	1.1				
Species x age/sex				3	27.9	8.72	<0.0001

Table 2-3 Results of the ANOVA used to test variation of $\delta^{15}N$ values in fur collected from hoary bats (*Lasiurus cinereus*) and silver-haired bats (*Lasionycteris noctivagans*) killed at wind turbines in south-western Alberta from 15 July to 30 September 2006 and 2007. Categories with different superscript letters are significantly different (Tukey's post-hoc test).

Parameter	Categories	LSmeans	SD	df	Type III Sum of Squares	F-stat	P
Year	2006	8.5	1.3	1	0.48	0.31	0.58
	2007	8.5	1.4				
Species	Silver-haired bat	8.6	1.3	1	4.5	2.91	0.09
	Hoary bat	8.4	1.3				
Age/sex class	Adult female^{A,B}	8.6	1.3	3	25.86	5.58	0.001
	Adult male^{B}	8.1	1.3				
	Sub-adult female^{A}	8.9	1.3				
	Sub-adult male^{A,B}	8.4	1.3				
Species x age/sex				3	18.56	4.01	0.008

Table 2-4 Results of the ANOVA used to test the variation of δ^2H values in fur collected from hoary bats (*Lasiurus cinereus*) and silver-haired bats (*Lasionycteris noctivagans*) killed at wind turbines in south-western Alberta from 15 July to 30 September 2006 and 2007.

Parameter	Categories	LSmeans	SD	df	Type III Sum of Squares	F-stat	P
Year	2006	-118.9	27	1	8191.55	12.69	<0.001
	2007	-129.9	26.4				
Species	Silver-haired bat	-133.8	26.1	1	24043.29	37.55	<0.0001
	Hoary bat	-115.0	26.5				
Age/sex class	Adult female	-121.3	27.6	3	4677.04	3.72	0.07
	Adult male	-119.5	27.1				
	Sub-adult female	-128.5	25.6				
	Sub-adult male	-128.3	26.4				
Year x age/sex				3	5862.73	4.25	0.03

Table 2-5 Comparison of isotope values between putative migrants killed at wind turbines in south-western Alberta and resident silver-haired bats (*Lasionycteris noctivagans*) captured in the Cypress Hills, SK or resident hoary bats (*Lasiurus cinereus*) captured in Delta Marsh, MB.

Species	Site	Mean isotope value ± SD	F-stat	F-test p	t-stat	t-test p
Silver-haired bat	Cypress Hills	$\delta^{13}C_f$ = -23.8 ± 0.7	1.80	0.09	6.11	<0.001
	Wind turbines	$\delta^{13}C_f$ = -25.0 ± 1.0				
Hoary bat	Delta Marsh	$\delta^{13}C_f$ = -25.0 ± 1.1	1.32	0.41	3.89	<0.001
	Wind turbines	$\delta^{13}C_f$ = -24.0 ± 1.2				
Silver-haired bat	Cypress Hills	$\delta^{15}N_f$ = 9.7 ± 0.6	7.55	<0.001	5.91	<0.001
	Wind turbines	$\delta^{15}N_f$ = 8.7 ± 1.5				
Hoary bat	Delta Marsh	$\delta^{15}N_f$ = 9.3 ± 0.8	1.97	0.04	5.95	<0.001
	Wind turbines	$\delta^{15}N_f$ = 8.3 ± 1.1				
Silver-haired bat	Cypress Hills	$\delta^{2}H_f$ = -101 ± 13.1	4.09	<0.001	9.7	<0.001
	Wind turbines	$\delta^{2}H_f$ = -134 ± 26.5				
Hoary bat	Delta Marsh	$\delta^{2}H_f$ = -107 ± 9.3	8.22	<0.001	3.28	0.001
	Wind turbines	$\delta^{2}H_f$ = -115 ± 26.5				

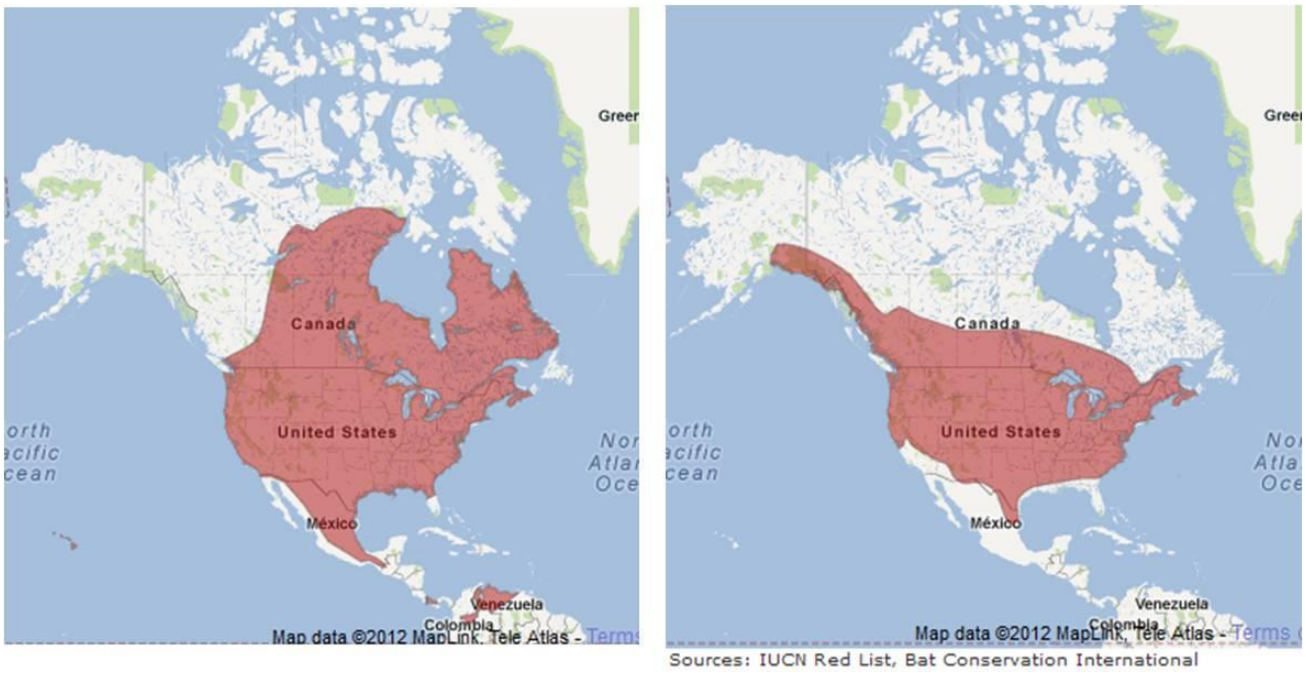

Figure 0-1 North American range maps of the hoary bat (*Lasiurus cinereus*, left), and the silver-haired bat (*Lasionycteris noctivagans*, right). I chose these range maps over other possibilities because they are used by the IUCN in its evaluation of a species' status. Maps from Bat Conservation International (2012).

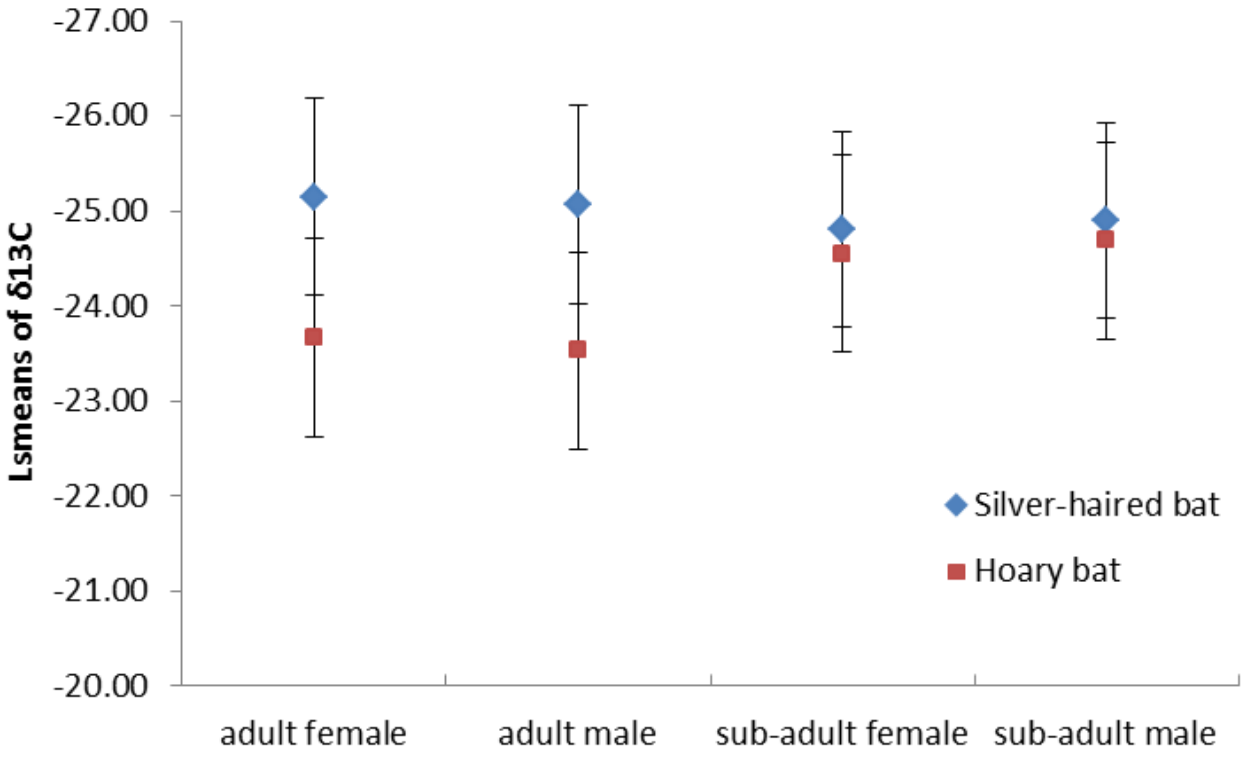

Figure 0-2 LSmeans plot (± SD) of the interaction between age and sex category and species from the ANOVA that assessed the variation in $\delta^{13}C$ values among species, years and age/sex classes. The interaction was significant (ANOVA, $F_{3,286} = 8.72$, $p < 0.0001$).

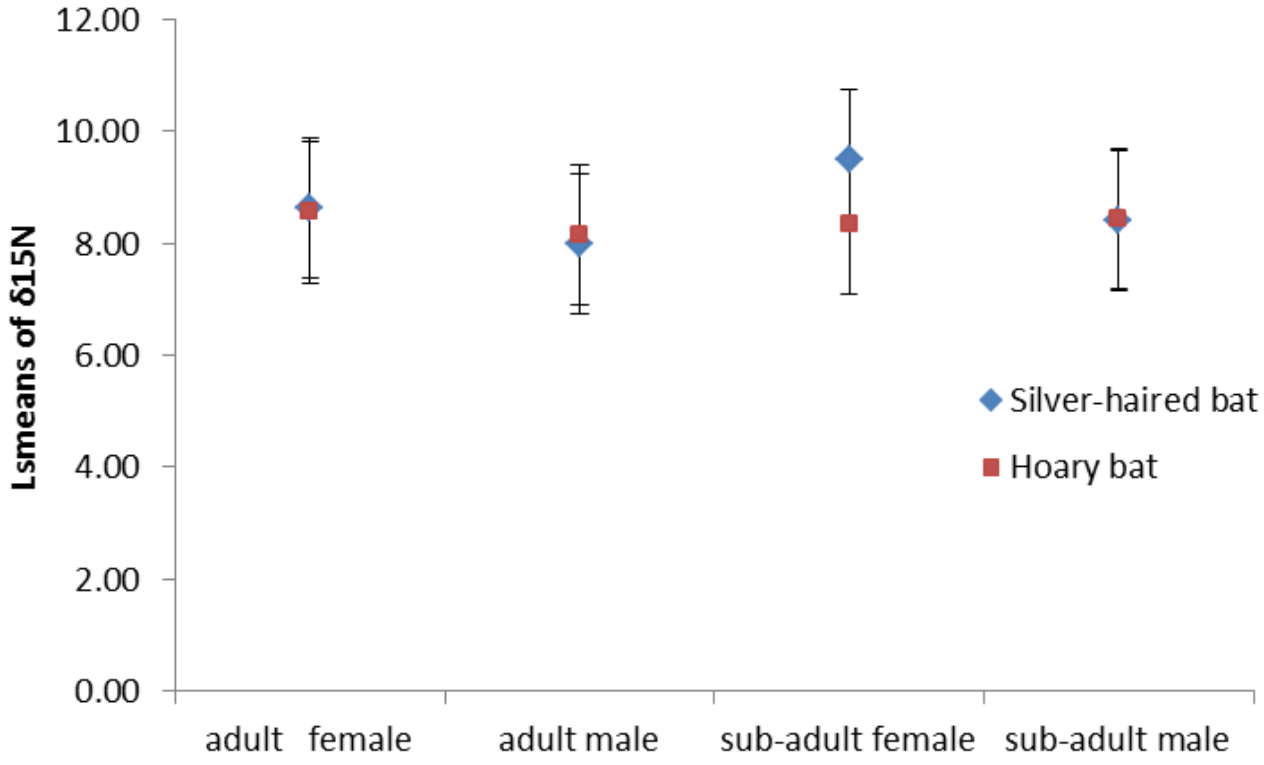

Figure 0-3 LSmeans plot (± SD) of the interaction between age and sex category and species from the ANOVA that assessed the variation in $\delta^{15}N$ values among species, years and age/sex classes. The interaction was significant (ANOVA, $F_{3,286} = 18.56$, $p = 0.008$).

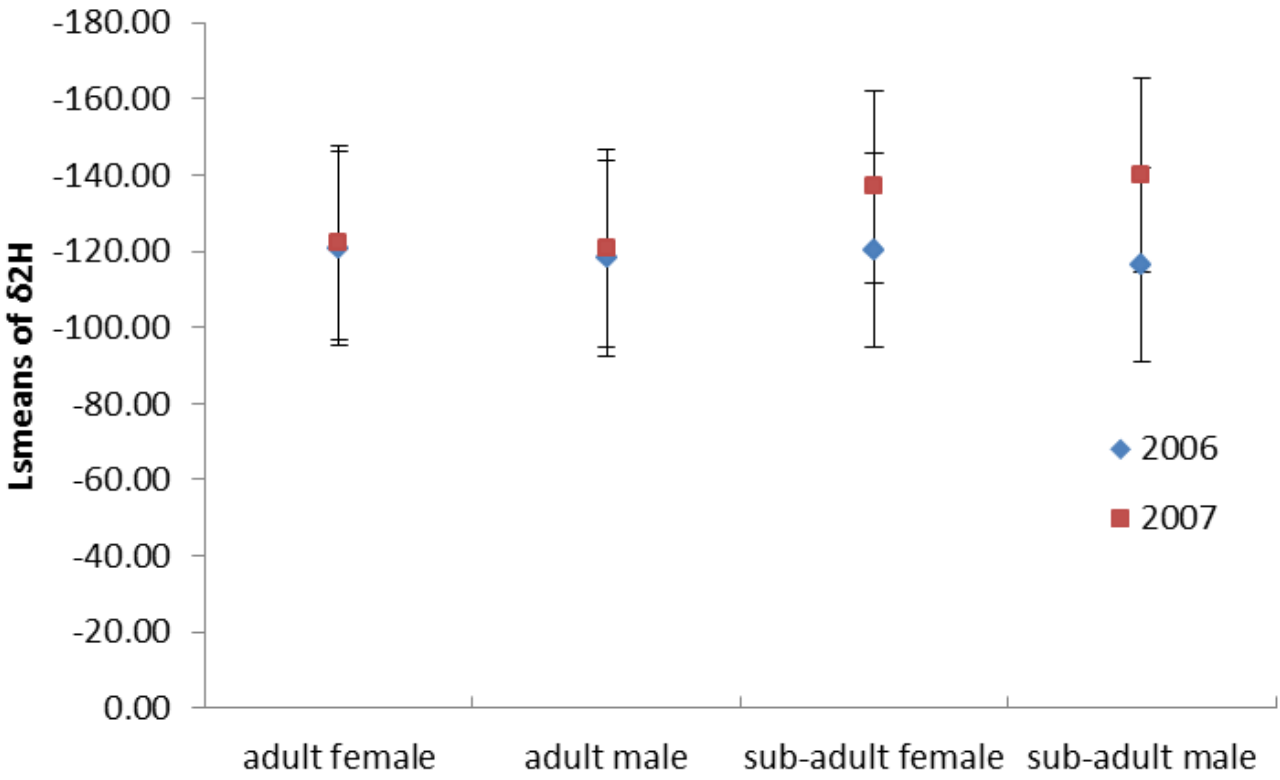

Figure 0-4 LSmeans plot (± SD) of the interaction between year and sex category and species from the ANOVA that assessed the variation in δ^2H values among species, years and age/sex classes. The interaction was significant (ANOVA, $F_{3,286} = 5862.73$, $p = 0.03$).

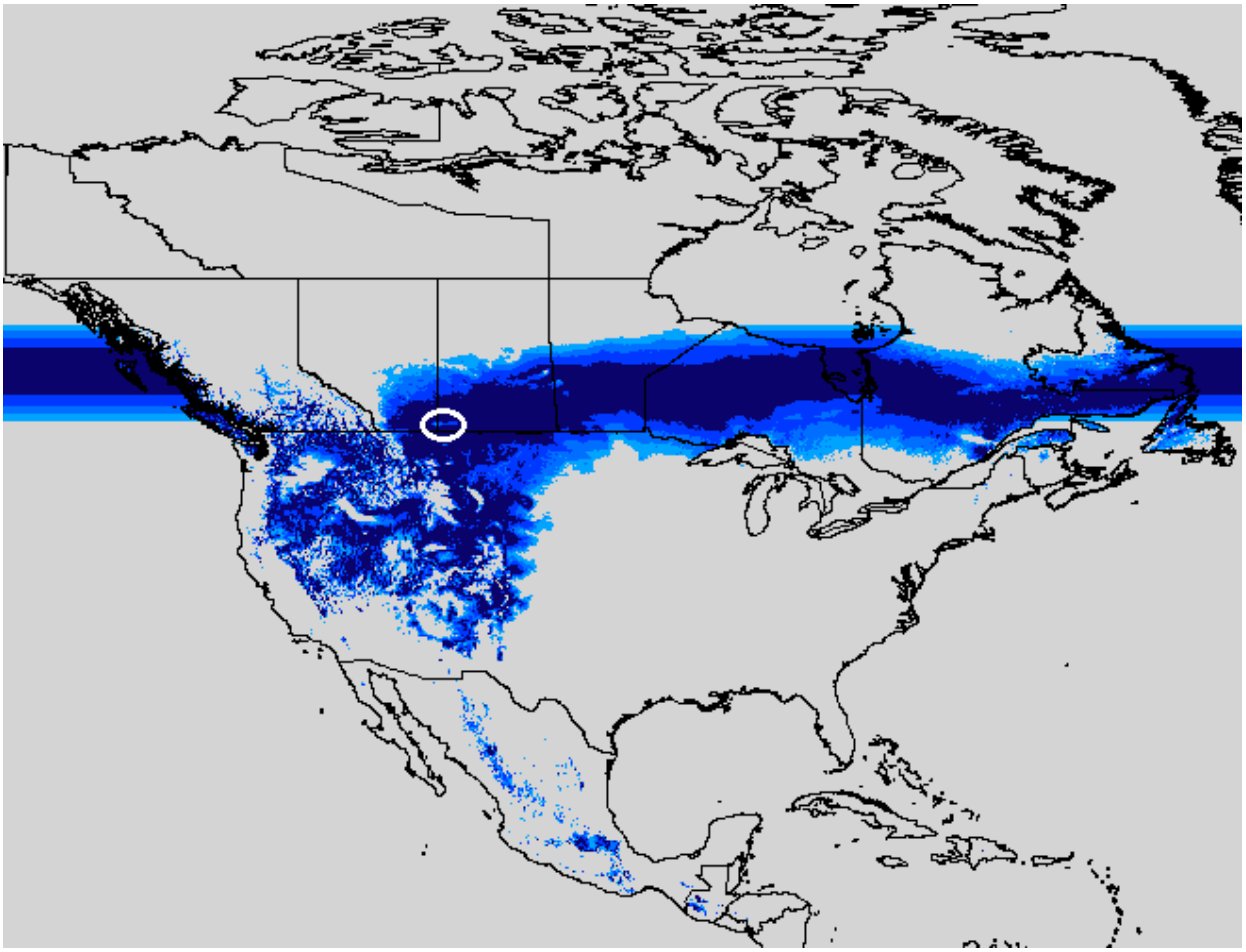

Figure 0-5 The results of the IsoMAP assignment of fur collected from silver-haired bats (*Lasionycertis noctivagans*) that were putative residents of Cypress Hills, SK (approximate location indicated by the white circle). Shading darkens with increasing likelihood of origin. Full interactive maps can be viewed online at http://isomap.rcac.purdue.edu:8080/gridsphere/gridsphere?cid=DisplayAssignPortlet (job id 29121)

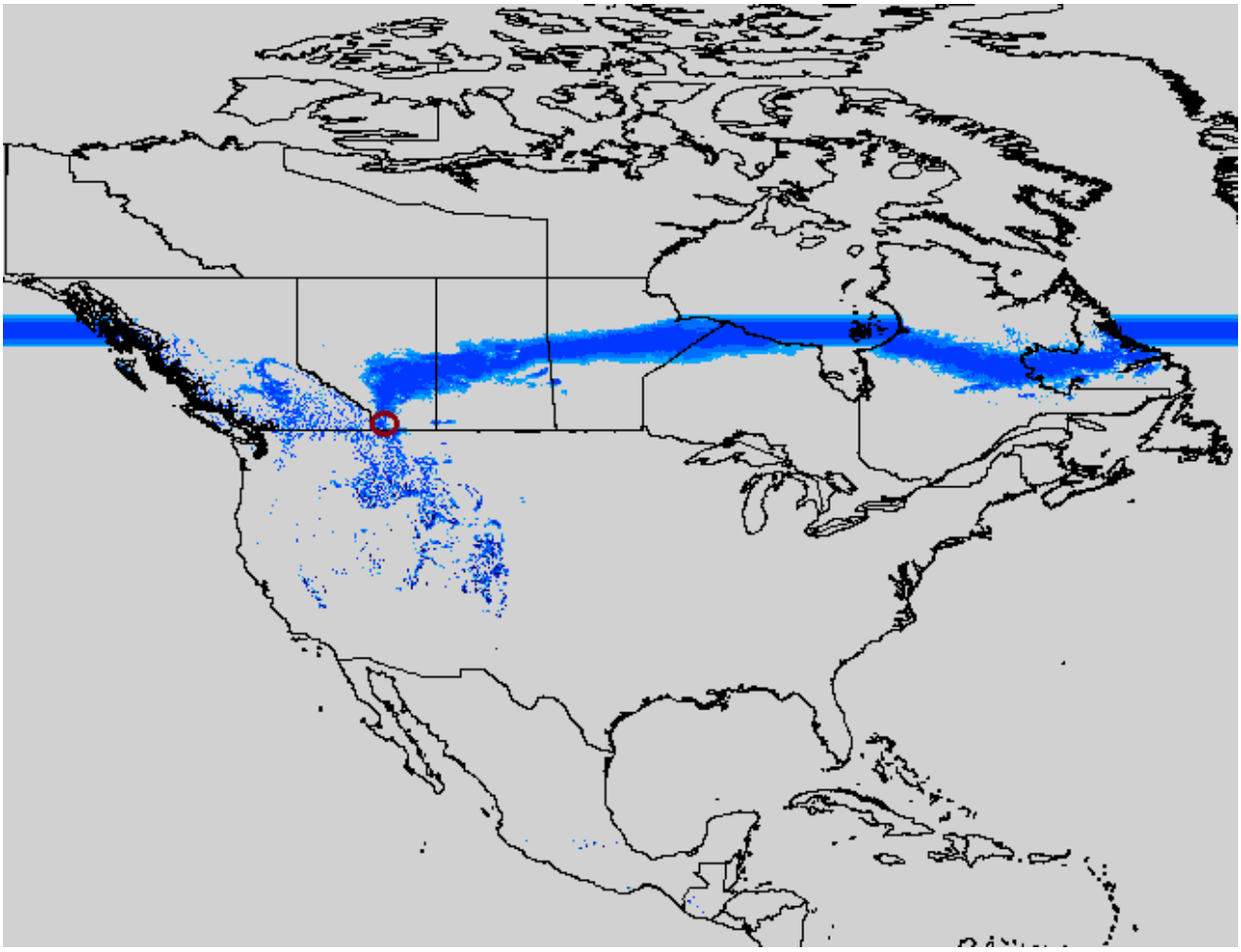

Figure 0-6 IsoMAP analysis of the geographic origins of hoary bats (*Lasiurus cinereus*) killed at wind turbines in south-western Alberta, Canada (approximate location indicated by the red circle). This likelihood-based assignment method is based on δ^2H values in precipitation. Shading darkens with increasing likelihood of origin. Full interactive maps can be viewed online at http://isomap.rcac.purdue.edu:8080/gridsphere/gridsphere?cid=DisplayAssignPortlet (job id 29120)

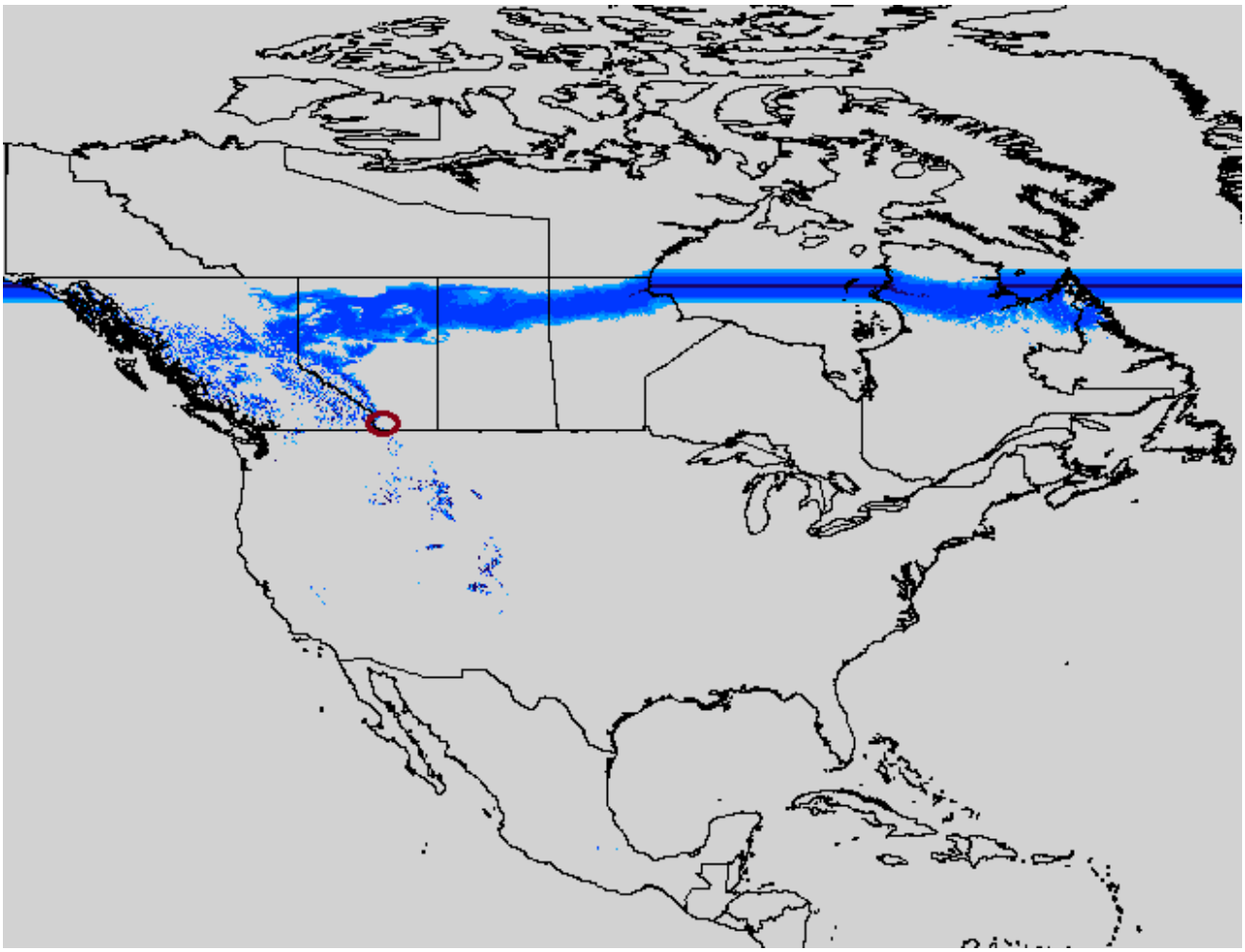

Figure 0-7 IsoMAP analysis of the geographic origins of silver-haired bats (*Lasionycteris noctivagans*) killed at wind turbines in south-western Alberta, Canada (approximate location indicated by the red circle). This likelihood-based assignment method is based on δ^2H values in precipitation. Shading darkens with increasing likelihood of origin. Full interactive maps can be viewed online at http://isomap.rcac.purdue.edu:8080/gridsphere/gridsphere?cid=DisplayAssignPortlet (job id 29126)

CHAPTER 3: POPULATION GENETICS OF TWO SPECIES OF MIGRATORY TREE-BATS AFFECTED BY WIND TURBINES

Introduction

Habitat loss and degradation, over-exploitation, climate change, and increasing resource demands of a growing human population have pushed many species to extinction and made innumerable extinctions over the next century inevitable (Sala et al. 2000, Butchart et al. 2010, Pereira et al. 2010, Ceballos et al. 2015). Extinction risk is not equal among organisms. For example, animals that migrate tend to be more vulnerable to extinction than those that do not (Pimm et al. 1988, Pagel and Payne 1996, Meffe and Carroll 1997). Unlike sedentary animals, migratory animals require appropriate habitat in several, spatially disjunct locations: breeding/summering sites, hibernation/overwintering sites, stopover sites, and migratory corridors that link them (Hutson et al. 2001, Fleming and Eby 2003). A high risk of extinction is also associated with isolated populations (Hughes et al. 1997, Ceballos and Ehrlich 2002) and those with small geographic range size (Purvis et al. 2000, Jones et al. 2003, Cardillo et al. 2006, Cardillo et al. 2008). Migratory animals are considered to have large ranges, but if migration funnels animals into discrete routes or geographic bottlenecks, then this may increase the risk of extinction in migrants because only a small area needs to be negatively affected to result in large population declines. Migratory bats have a number of the aforementioned ecological traits that increase risk of extinction, in addition to having slow life histories (Barclay and Harder 2003).

In recent years, there has been growing concern over the stability of migratory-bat populations due to the impact of fatalities at wind energy facilities (Kunz et al. 2007, Arnett and Baerwald 2013). Across North America, hundreds of thousands of bats are killed annually by

wind turbines; an estimated 655,579 to 1,318,743 bats were killed from 2000-2011 (Arnett and Baerwald 2013). Of these fatalities approximately 80% are of migratory tree-bats: hoary bats (*Lasiurus cinereus*; 38%), eastern red bats (*L. borealis*; 22%), and silver-haired bats (*Lasionycteris noctivagans*; 18.4%; Arnett and Baerwald 2013). In Alberta, 54% of bat fatalities at wind energy installations are hoary bats and 37% are silver-haired bats (Baerwald et al. 2014). Wind energy facilities placed along migratory routes increase the mortality risk of an already risky behaviour and could potentially endanger specific sub-populations or matrilines using particularly risky migratory routes (i.e. those routes with high concentrations of wind energy facilities), or select for more sedentary and therefore less risky behaviour.

Managing imperilled species over entire ranges may not be feasible, particularly in the case of animals that migrate long-distances. An alternative population genetic approach has been used extensively in identifying conservation priorities and management units (e.g. Gorbics and Bodkin 2001, Haig et al. 2001, Salgueiro et al. 2004, Brown et al. 2011). In these approaches, the primary conservation goal is to preserve genetic diversity (Vane-Wright et al. 1991, Faith 1992), because genetic diversity is the raw material for evolution (Bowen and Roman 2005) and is important for the long-term persistence of species and populations (Reed and Frankham 2003, Spielman et al. 2004, Frankham 2005). Knowledge of genetic variability and structure is also important for the estimation of effective population size, which is useful in the management and conservation of species at risk of extinction (Lande and Barrowclough 1987, Nunney 2000).

The effective size of a population (N_e) is the size of an idealized population (Fisher 1930, Wright 1931) that has the same genetic drift properties as an actual population (Conner and Hartl 2004). In essence, N_e is the number of individuals in a population who contribute to the genetic diversity of the next generation. This number tends to be much lower than the census size (N_c) of

a population because N_e is influenced by numerous demographic, ecological, and life-history traits, such as past bottlenecks, mating systems, and sex ratios. Populations with low N_e lose genetic diversity more quickly than populations with larger N_e (Conner and Hartl 2004) and this makes them more sensitive to population declines. Although ancient (the past thousands or millions of generations) and historical (the past tens to thousands of generations) N_e can be estimated (Wang 2005), for conservation purposes, estimates of contemporary (the past one to few generations) N_e are the most relevant. However, contemporary estimates of N_e may not be feasible if N_e is large, because precision of the estimate tends to decrease as N_e increases (Wang 2005). Although contemporary N_e of eastern red bats appears to be large, with average estimates ranging from 74,500 (Vonhof and Russell 2015) to 2.78 million (Pylant 2014), it does not appear to be so in hoary bats. The contemporary N_e of hoary bats in the central Appalachians was estimated to be 1,611 (95% Confidence Interval: 424 – 4,697; Pylant 2014). Understanding the relationship between contemporary N_e and genetic structuring of populations is important for conservation strategies (Franklin and Frankham 1998, Waples 2002).

Migratory animals with great dispersal ability tend to have lower amounts of population structure relative to non-migratory animals with less dispersal ability (Bohonak 1999, Bailey et al. 2007, Bradbury et al. 2008) and this pattern holds true for bats (Chen et al. 2010, Moussy et al. 2012, Burns and Broders 2014). However, this pattern may be complicated by numerous factors, such as barriers that impede dispersal, sex-biased dispersal and/or gene flow, and behaviours such as roost philopatry and social systems that have close, long-term relationships among group members (Avise 1994, Petit et al. 2001, Rivers et al. 2005).

Animals that form close social groups (e.g. manatees and dugongs (Order Sirenia); Deutsch et al. 2003, Sheppard et al. 2006) and exhibit extended parental care (e.g. swans (Order

Anseriformes) and storks (Order Ciconiiformes); Rees 1989, Chernetsov et al. 2004) often transmit information about migration socially, either from conspecifics or directly from mother to offspring (Akesson and Hedenstrom 2007). Given the social structure, social transmission of behaviour (Ratcliffe and ter Hofstede 2005, Page and Ryan 2006), and extended mother-pup associations (Brigham and Brigham 1989, Rossiter et al. 2002, Geipel et al. 2013) seen in some bats, migration routes and behaviours may be socially transmitted. If migration routes are socially transmitted, then migratory routes may be matrilineal and inherited, as seen in whales and elephants (McComb et al. 2001, Archie et al. 2006), and this would be reflected in the genetic structure of maternally inherited mitochondrial DNA (mtDNA).

Even if migratory routes are not transmitted socially, but are determined by endogenous genetic programs, philopatry may further dampen the effects of gene flow and serve to create genetic structure (Lande and Barrowclough 1987, Esler 2000, O'Corry-Crowe et al. 2002). Bats tend to be highly philopatric, with females returning to maternity roosts year-after-year (e.g. Gannon et al. 2000, Barclay and Brigham 2001), but site fidelity often varies with roost type: building, cave, and tree-cavity roosting species tend to have greater fidelity than foliage roosting species (Lewis 1995). Although roost fidelity may be lower in foliage-roosting species because of the ephemeral nature of their roosts, it may be that foliage-roosting species are loyal to a maternity area rather than a specific maternity roost. If population genetic structure exists among migration routes and maternity sites, we could use this structure to guide conservation efforts for migratory bats, such as hoary bats and silver-haired bats killed by wind turbines.

Hoary bats roost solitarily in tree foliage throughout much of North and South America (Shump and Shump 1982b, Cryan 2003). Although we still do not understand the movement patterns or seasonal distributions of hoary bats, it seems they winter in Mexico and the southern

United States, then migrate north and east in the spring (Findley and Jones 1964, Cryan 2003, Cryan et al. 2004, Cryan et al. 2014). In summer, hoary bats are found throughout the prairies, aspen parkland, and the southern boreal forests of Canada (Chapter 2, Buehler and Piersma 2008, Ulanovsky and Moss 2008). Reproductive females exhibit some degree of year-to-year fidelity to summer sites (Koehler and Barclay 2000).

There may be some sexual segregation of hoary bats during summer, with females potentially migrating further than males (Findley and Jones 1964, Cryan 2003, Cryan et al. 2004), but fatality and stable-isotope data show no strong evidence for this (Chapter 2, Baerwald and Barclay 2011). Although their distribution is unknown, the high proportion of adult-male hoary bat fatalities at wind energy installations in Alberta (Baerwald and Barclay 2011) suggests that there are more adult male hoary bats in Alberta than once thought (Ulanovsky and Moss 2008). Long-distance migration (>2000 km) has been confirmed by stable-hydrogen isotope analysis of fur (Cryan et al. 2004).

Silver-haired bats range throughout most of the United States and the forested areas of Canada (Kunz 1982). Known wintering grounds are in the Pacific Northwest, south-western states, and middle latitudes of the eastern United States (Izor 1979, Cryan 2003). The limited data on their migratory patterns suggest that in spring, silver-haired bats from the east migrate north and east, and western silver-haired bats migrate northward (Cryan 2003). Despite having a wing morphology associated with intermediate dispersal ability, similar to that of little brown Myotis (*Myotis lucifugus*) but less than that of hoary bats (Norberg and Rayner 1987), long-distance migration of individual silver-haired bats (>1000 km) in eastern Canada and the US has recently been confirmed by stable hydrogen isotope analysis of fur (Fraser and Longstaffe 2014).

In summer, silver-haired bats roost singly or in small colonies (Kunz 1982). Maternity colonies are located in tree cavities, not in bark crevices or other roosting places used by males and non-reproductive females (Mattson et al. 1996, Betts 1998). There is some degree of sexual segregation during summer, with females potentially migrating farther than males (Cryan 2003). However, capture records indicate the presence of adult males in northern Alberta (Hutterer et al. 2005), fatalities at wind energy facilities suggest a relatively even sex ratio in southern Alberta during fall migration (Baerwald and Barclay 2011), and stable-isotope analysis of the fur of those fatalities showed no evidence of sexual segregation (Chapter 2).

I hypothesized that silver-haired bats and hoary bats, particularly females, are philopatric and return to maternity sites annually, and that they use matrilineal migration routes. Thus, I predicted that there is genetic structure among migration routes. I assumed that gene flow is facilitated by male dispersal, as seen in other bat species (e.g. Petit and Mayer 1999, Kerth et al. 2000), so I also predicted that there is greater structure in the maternally inherited mtDNA than in bi-parentally inherited microsatellites. Given that the degree of genetic structuring in bats is influenced by dispersal ability, social structure, and roost fidelity (Carstens et al. 2004, Moussy et al. 2012, Rossiter et al. 2012), and given the aforementioned biological and ecological differences, I further predicted that the degree of genetic structure differs between the species such that silver-haired bats have a greater amount of genetic structure than hoary bats do. I was also interested in how contemporary N_e differed between the species, and from previous estimates of historical and contemporary N_e of eastern red bats and hoary bats, respectively (Pylant 2014, Vonhof and Russell 2015).

To test my predictions, I used mtDNA and microsatellites to evaluate the genetic structure of silver-haired bats and hoary bats across Canada. Because detailed information about

bat migration routes is lacking, I could not directly compare migration routes, but rather looked for evidence of genetic structure among bats across a broad geographical area, from three provinces: Alberta, Manitoba, and Ontario.

Methods

Sampling

I obtained tissue samples from a total of three locations across Canada (Figure 3.1). Tissue samples were collected, using dissecting scissors, from the dactylopatagium of bat carcasses found under wind turbines. Tissue collection occurred opportunistically during autumn migration (July-September) between 2006 and 2010, but timing differed slightly among sites (Tables 3.1 and 3.2). Tissue was stored in 70-90% ethanol and kept in a -20°C freezer until DNA extraction.

mtDNA extraction, amplification, and sequencing

I extracted genomic DNA from 2mm biopsy punches using the Dilution Protocol of the Phire Animal Tissue Direct PCR Kit (Thermo Scientific). I PCR-amplified a highly polymorphic portion of the mitochondrial DNA control region, the hypervariable II region (HVII), using the primers L16517 (Fumagalli et al. 1996) and sH651 (Castella et al. 2001). I carried out PCRs in a final volume of 20 µL, containing 10 µL of 2X Phire Animal Tissue PCR buffer, 0.5 µL of BSA, 1 µL of each primer, 0.4 µL of Phire Hot Start II DNA polymerase, and 1 µL of DNA from the dilution-protocol extraction. I amplified the DNA with the following protocol: 98 °C for 5 min; 34 cycles of 98°C for 5 s, 57 °C for 20 s, 72 °C for 45 s; 72 °C for 10 min.

I sequenced the PCR products using the BigDye Cycle Sequencing Kit (Applied Biosystems) in a 10 μL reaction containing 4 μL of 1:4 diluted Big Dye, 1 μL of the forward primer (L16517), 4 μL of PCR product and 1 μL of doubly distilled water. The pre-sequencing reaction protocol was: 96 °C for 1 min; 26 cycles of 96°C for 10 s, 50 °C for 5 s, 60 °C for 4 min. I analysed products on an ABI Prism 3500 Genetic Analyzer (Applied Biosystems).

To ensure high quality results throughout the remaining pipeline, I discarded all sequences with an overall quality less than 50. I edited chromatograms with BIOEDIT 7.2.5 (Hall 1999) and then aligned the sequences using CLUSTALW (Larkin et al. 2007) within BIOEDIT. I cropped the 128 hoary bat sequences to 396 base pairs and the 130 aligned silver-haired bat sequences to 378 base pairs using the information-based view in BIOEDIT to determine the informative region. I used DNASP, version 5.10.1 (Librado and Rozas 2009) to calculate haplotype diversity (h) (Nei and Tajima 1981a) and nucleotide diversity (π) (Nei 1987), and to collapse individual sequences into haplotypes for further analyses.

Microsatellite extraction, amplification, and genotyping

Primer development and microsatellite analysis was conducted at the University of Georgia's Savannah River Ecology Laboratory (see Appendix A for details on primer development). DNA was extracted using Qiagen DNEasy Blood and Tissue kit. PCR amplifications were performed in a 12.5 μL volume (10 mM Tris pH 8.4, 50 mM KCl, 25.0 μg/ml BSA, 0.4 μM unlabeled primer, 0.04 μM tag labeled primer, 0.36 μM universal dye-labeled primer, 3.0 mM $MgCl_2$, 0.8 mM dNTPs, 0.5 units AmpliTaq Gold® Polymerase (Applied Biosystems), and 20 ng DNA template) using an Applied Biosystems GeneAmp 9700. Touchdown thermal cycling programs (Don et al. 1991) encompassing a 10°C span of annealing

temperatures, ranging between 65 and 55°C (TD65), were used for all loci. Touchdown cycling parameters consisted of an initial denaturation step of 5 min at 95°C followed by 20 cycles of 95°C for 30 s, highest annealing temperature (decreased 0.5°C per cycle) for 30 s, and 72 °C for 30 s; and 20 cycles of 95 °C for 30 s, lowest annealing temperature for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min. PCR products were run on an ABI-3130xl sequencer and sized with Naurox size standard prepared as described in DeWoody et al. (2004), except that unlabeled primers started with GTTT. Results were analyzed using GENEMAPPER version 3.7 (Applied Biosystems).

I calculated the number of alleles (N_a), observed (H_o) and expected heterozygosity (H_e), deviations from Hardy-Weinberg equilibrium (HWE), and linkage disequilibrium (LD) using ARLEQUIN 3.5.1.3 (Excoffier and Lischer 2010). I used MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) to search for loci with the following genotyping errors: dropout of large alleles, stuttering error, and null alleles.

Analysis of genetic structure

I refer to samples by the province they were collected in (i.e. Alberta, Manitoba, and Ontario; Tables 3.1 and 3.2, Figure 3.1). To examine genetic structure, I used ARLEQUIN to calculate pairwise Fst values among sampling locations for both the mtDNA and microsatellite data. For the microsatellites, I also examined pairwise comparisons of Fst using FREENA (Chapuis and Estoup 2007) because FREENA corrects Fst estimates for the positive bias caused by the presence of null alleles (Chapuis and Estoup 2007).

To further test for population subdivision, I used two different Bayesian clustering methods, STRUCTURE 2.3.4 (Pritchard et al 2000, Falush et al 2003) and TESS 2.3.1 (Chen et al. 2007). STRUCTURE assigns individuals into clusters (K) in a way that minimizes deviations from HWE and LD within each cluster, without using spatial data. The program uses a Markov Chain Monte Carlo (MCMC) procedure to estimate the posterior probability that the data fit the hypothesis of K clusters. I assumed an admixture ancestry model with correlated allele frequencies and ran STRUCTURE for K = one to 10, with 4 replicates for each value of K. For each replicate, I ran 100,000 burn-in steps followed by 900,000 MCMC steps. I determined the optimal number of clusters using the method of Evanno et al. (2005) as implemented by STRUCTURE HARVESTER (Earl 2012).

TESS also allows for admixture, but incorporates spatial data (i.e. where samples were taken) to inform clustering. Before running the analyses, I used the geographical coordinates of each sampling location to generate spatial coordinates for each individual using the Generate Spatial Coordinates option within TESS and allowed a range of 1° for both the x and y coordinates. I assumed admixture (BMY admixture model; Durand et al. 2009) and ran TESS for K = two to 10 clusters, with 4 replicates for each value of K. For each replicate, I ran 10,000 burn-in steps followed by 100,000 total sweeps. The optimal number of clusters was determined by identifying the K at which DIC values began to plateau.

Bottleneck analysis

I used two approaches to examine whether either species of bat shows the genetic signature of a population bottleneck. First, I used the program BOTTLENECK (Piry et al. 1999), which compares the distribution of heterozygosity expected from the observed number of alleles

given the sample size for each locus and population (Cornuet and Luikart 1996). I excluded loci containing null alleles and ran 5000 iterations. For the two-phased mutation model (TPM), I used a variance of 12 and 90% SMM (stepwise mutation model). To assess significance, I used two-tailed Wilcoxon signed-rank tests, which are fairly robust with a small (i.e. <20) number of loci (Piry et al. 1999), and used a Bonferroni correction.

I also used ARLEQUIN to calculate modified M-ratios (Garza and Williamson 2001, Excoffier and Lischer 2010). Garza and Williamson's M-ratio test compares the number of alleles with the allelic size range and assumes that in a population undergoing a bottleneck, the number of alleles is reduced faster than the allelic size range (Garza and Williamson 2001). The M-ratio in ARLEQUIN is modified to avoid dividing by zero in monomorphic populations (Excoffier and Lischer 2010). M-ratios below a critical value of 0.68 generally indicate a recent population bottleneck (Garza and Williamson 2001), but I also generated project and species-specific critical M-ratios using CRITICAL_M (Garza and Williamson 2001). I varied θ from 0.1 to 10 and used default values of 0.1 for p_g and 3.5 steps for Δ_g (Busch et al. 2007).

Estimates of contemporary effective population sizes

I used the microsatellite data to estimate contemporary N_e using three methods: temporal (Waples 1989, Jorde and Ryman 1995, 2007), LD single-sample (Waples and Do 2008), and sib-ship assignment (Wang 2009). I used NEESTIMATOR V2 (Do et al. 2014) for the temporal and LD analyses, and COLONY (Jones and Wang 2010) for the sib-ship analysis. All of these methods handle null alleles well if the null alleles are distributed evenly among samples (Zeller et al. 2008, Jones et al. 2010, Sved et al. 2013). However, both null and low frequency alleles can bias the estimates of N_e (Waples and Do 2010, Sved et al. 2013), so I excluded alleles

occurring in frequencies less than 1% (Waples and Do 2010) and loci that contained null alleles at frequencies >20% (Laci25 and 38, Lano4, 26, 31, 41, and 44). I restricted analyses to the Alberta group because it was the only group with a sufficiently large sample of individuals of known ages and sexes that was collected over two generations (2006 and 2007), which is required for the temporal and sib-ship analyses.

The temporal method assumes that the primary process affecting changes in allele frequencies is genetic drift and considers the rate of change in allele frequencies between generations: the magnitude of change between generations will increase and N_e decreases (Nei and Tajima 1981b, Waples 1989). Overlapping generations, as occur in bats, lead to bias in standard temporal methods (Nei and Tajima 1981b, Pollak 1983), so I used an unbiased measure of genetic drift and N_e for populations with non-discrete generations (Jorde and Ryman 1995, 2007).

The LD single-sample method to estimate N_e is based on the premise that as N_e decreases and genetic drift increases, the smaller number of mating pairs will result in non-random association of alleles, i.e. linkage disequilibrium will increase (Hill 1981). The LD method assumes discrete generations, but bats have overlapping generations, so I included multiple cohorts (the 2006 and 2007 cohorts) in my analysis, thus ensuring my results could be interpreted as an estimate of N_e (Schwartz et al. 1998). I assumed random mating and report estimates of N_e ± 95% confidence intervals determined with jackknife re-sampling (Waples and Do 2008).

The Sib-ship method is based on the principle that a small N_e will result in a high proportion of siblings and the smaller the N_e, the higher the probability that two individuals drawn at random from the same cohort of a population are siblings (Wang 2009). I used genotyping error rates calculated by COLONY, assumed a random-mating population with a

polygamous mating system, and report N_e ± 95% confidence intervals determined by maximum likelihood.

Results

Sequence characteristics and diversity

I found 72 polymorphic (segregating) sites in the hoary bat samples and 28 polymorphic sites in the silver-haired bat samples. Sequences of hoary bats had a G+C content of 43.5% and sequences of silver-haired bats had a G+C content of 35.5%. I identified 78 unique haplotypes from 103 hoary bat individuals and 49 unique haplotypes from 105 silver-haired bat individuals. Overall haplotype diversity (Hd) in hoary bats was 0.963 ±0.015 (Hd ± standard deviation) and ranged from 0.966 to 1.0 (Table 3.1). Overall nucleotide diversity (π) in hoary bats was 0.07 ±0.008 and ranged from 0.075 to 0.182 (Table 3.1). Haplotype diversity in silver-haired bats was 0.937 ±0.014 and ranged from 0.946 to 1.0 (Table 3.2). Overall, nucleotide diversity in silver-haired bats was 0.014 ±0.001 and ranged from 0.016 to 0.046 (Table 3.2). In both species, most haplotypes were singletons (hoary bat = 91.0%; silver-haired bat = 79.6%).

Microsatellite diversity

157 hoary bats across three sample locations were genotyped at 19 microsatellite loci, but one locus (Laci28) was monomorphic in both the Manitoba and Ontario groups (Table 3.3). The number of alleles per locus ranged from three to 66 (Table 3.3). All groups showed similar levels of average observed heterozygosity, but it was slightly lower in the Alberta group (AB = 0.73 ± 0.23, MB = 0.80 ± 0.13, ONT = 0.80 ± 0.15). Nine loci in the Alberta group showed significant deviations from HWE after a Bonferroni correction for multiple comparisons; all nine of these

loci contained null alleles (Table 3.3). Deviations from HWE are likely due to a combination of null alleles and the Wahlund effect, which is reduction in Ho caused by subpopulation structuring within the group being tested, as might be expected along a migratory route and as suggested by my results (see Results). Sixty-five of 513 comparisons (12.67%) showed significant linkage disequilibrium, but there was no evidence for consistent LD between any two loci, thus indicating that loci were independent.

120 silver-haired bats across three sample locations were genotyped at 18 microsatellite loci (Table 3.4). The number of alleles per locus ranged from four to 20 (Table 3.4). All groups showed similar levels of average observed heterozygosity, but it was slightly higher in the Manitoba group (AB = 0.67 ± 0.17, MB = 0.71 ± 0.21, ONT = 0.68 ± 0.19). Seven loci showed significant deviations from HWE (after a Bonferroni correction) in some but not all locations; all of these loci contained null alleles (Table 3.4). Again, deviations from HWE are likely due to a combination of null alleles and the Wahlund effect. Sixty-five of 459 comparisons (14.16%) showed significant linkage disequilibrium, but there was no evidence for consistent LD between any two loci, thus indicating that loci were independent.

Analysis of mtDNA structure

In hoary bats, the pairwise Fsts revealed no significant genetic distance between any of the sampling locations (Table 3.5). In silver-haired bats, the pairwise Fsts revealed significant genetic distance between the Alberta and Ontario sites (Table 3.6).

Analysis of microsatellite structure

For hoary bats, the pairwise Fsts in both ARLEQUIN and FREENA revealed significant genetic distances between the Alberta group and both the Manitoba and Ontario group, but no difference between the Manitoba and Ontario groups (Table 3.7). The STRUCTURE analysis suggested there were two putative populations (Figure 3.2) across the three sampling locations, whereas the TESS analysis suggested six putative populations (Figure 3.3).

For silver-haired bats, the pairwise Fsts in both ARLEQUIN and FREENA revealed significant genetic distance between the Alberta group and the Manitoba group, but no other differences (Table 3.8). Both the STRUCTURE and the TESS analyses suggested there were six putative populations across the three sampling locations (Figures 3.4 and 3.5).

Bottleneck analysis

The BOTTLENECK analysis for hoary bats revealed no evidence of a historical population bottleneck in any group (two-tailed Wilcoxon tests; AB $p = 0.12$, MB $p = 1.00$, ONT $p = 0.35$). However, all modified M-ratios were substantially lower than the critical value of 0.68 (mean ± SD; AB = 0.31 ± 0.11, MB = 0.15 ± 0.03, ONT = 0.19 ± 0.06, overall 0.19 ± 0.11). Modified M-ratios were also much lower than all critical values of M generated between $\theta = 0.1$ ($M_{crit} = 0.87$) and $\theta = 10$ ($M_{crit} = 0.76$).

The BOTTLENECK analysis for silver-haired bats also revealed no evidence of a historical population bottleneck in any group (two-tailed Wilcoxon tests; AB $p = 0.12$, MB $p = 0.97$, ONT $p = 0.39$). However, all modified M-ratios were again substantially lower than the critical value of 0.68 (mean ± SD; AB = 0.22 ± 0.08, MB = 0.14 ± 0.06, ONT = 0.16 ± 0.06,

overall 0.19 ± 0.05). Modified M-ratios were also much lower than all critical values of M generated between $\theta = 0.1$ ($M_{crit} = 0.94$) and $\theta = 10$ ($M_{crit} = 0.76$).

Estimates of contemporary effective population sizes

Estimates of the contemporary effective population size of hoary bats were highly variable and imprecise, with N_e values ranging from 84 to infinity (Table 3.9). Estimates of the contemporary effective population size of silver-haired bats were also highly variable and imprecise, with N_e values ranging from 17 to infinity (Table 3.10).

Discussion

As I predicted, both silver-haired bats and hoary bats exhibit genetic structure among the provinces sampled. This is in direct contrast to other studies on the genetic structure of migratory tree-bats, specifically, hoary bats (Pylant 2014, Amy Russell pers.comm) and eastern red bats (Korstian 2012, Vonhof and Russell 2015). Given that migratory tree-bats are highly vagile and thus gene flow is potentially possible over large distances, the detection of genetic structure may not be possible at the relatively small spatial scales used in some other studies (e.g. within the central Appalachians; Pylant 2014). It may also be that populations are more defined in the northern parts of their range, but become more mixed as migration routes come together further south.

Marker use may also affect the amount of genetic structure detected. Although mtDNA as a whole evolves much faster than most single-copy nuclear DNA (Avise 1994), different regions of mtDNA evolve at different rates and thus some regions may be more useful than others in detecting intraspecific population-genetic structure; regions with high mutation rates (e.g. the

hypervariable II region of the Control Region) are best suited for intraspecific studies, whereas regions with a slower mutation rate (e.g. cytochrome *b* (cyt*b*) and cytochrome oxidase I (COI)) are best suited for studies at the species, genus, or family level (Avise 1994). Thus, my use of the HVII region, rather than cyt*b* (Pylant 2014) or COI (Korstian 2012), may have further contributed to my ability to detect genetic structure in migratory tree-bats.

Because of the ecology and morphology of hoary bats and silver-haired bats, I expected low-levels of genetic variation. Wide-ranging migratory animals with high-dispersal abilities tend to exhibit limited population structure (Ball et al. 1988, Russell et al. 2005, Burns and Broders 2014). However, the structure I found (as represented by Fst values) is within the range of values found in both the little brown Myotis (*Myotis lucifugus*; Burns et al. 2014), a regional migrant, and the big brown bat (*Eptesicus fuscus*; Vonhof et al. 2008, Turmelle et al. 2011), a sedentary species. I had further predicted that I would see a greater amount of genetic structure in the maternally inherited mtDNA than in the biparentally inherited microsatellites. However, based on the number of significant pairwise differences, this prediction was only supported for silver-haired bats. This pattern could be explained by sex-biased dispersal of males, and route or roost philopatry by females (Petit and Mayer 1999, Petit et al. 2001, Kerth et al. 2002). When there is genetic structure among populations of a migratory animal, it is often due to philopatry, as seen in sandhill cranes (*Grus canadensis*; Jones et al. 2005b), sea turtles (Bowen and Karl 2007), and noctule bats (*Nyctalus noctula*; Petit et al. 1999). Thus, the pattern of greater genetic structure in the microsatellites of hoary bats may suggest dispersal by both sexes, greater dispersal by females, or philopatry to migratory routes or summering grounds by males.

I predicted that there would be a greater degree of structure in silver-haired bats relative to hoary bats and found the pairwise Φst values and the number of clusters suggested by

STRUCTURE were greater in silver-haired bats. This difference may be because of the lesser dispersal ability of silver-haired bats relative to hoary bats, differences in their social systems, or due to other ecological factors. It is likely that the colonial nature of silver-haired bat maternity roosts compared to the solitary nature of hoary bat maternity roosts contributed significantly to the differences in genetic structure. The genetic structure of maternity colonies is highly variable among and within species (Kerth 2008), with some maternity colonies of some species containing a mix of both unrelated and closely related individuals (e.g. big brown bats; Metheny et al. 2008b) and some maternity colonies consisting of predominantly closely related individuals (e.g. Bechstein's bats (*Myotis bechsteinii*); Kerth et al. 2000). The formation of new colonies of big brown bats is initiated by a group of closely related females splitting from the original colony (Metheny et al. 2008a). This would serve to increase structure among colonies.

When there is genetic structure among maternity colonies, the timing of sample collection becomes important as it may influence the amount of genetic structure detected. If migratory tree-bats funnel into migration routes from a wide area, as suggested by previous studies using stable isotopes (Baerwald et al. 2014, Cryan et al. 2014), then I would expect genetic structure created by female philopatry to maternity sites to be diluted along the route. Thus, sampling during autumn migration likely results in less detectable structure than sampling at summering/maternity sites, but if bats are mating during autumn migration, the degree of genetic structuring among routes becomes increasingly important.

Genetic diversity, as measured by haplotype diversity (h) and mean observed heterozygosity (H_o), was lower in the hoary bats and silver-haired bats included in this study than in eastern red bats (hoary bats, $H_o = 0.78$, $h = 0.96$; silver-haired bats $H_o = 0.69$, $h = 0.94$; eastern red bats, $H_o = 0.82$, $h = 0.99$; Vonhof and Russell 2015). Additionally, the hoary bat

population in the central Appalachians appears to have a low N_e, with an estimated contemporary nuclear N_e of 1,611 (95% Confidence Interval: 424 – 4,697; Pylant 2014), which is surprisingly small, especially when compared to estimates of N_e of eastern red bats, which are in the millions (Pylant 2014, Vonhof and Russell 2015) or Mexican free-trailed bats (which is ~10-12 million; Russell et al. 2011). However, my measures of the contemporary effective population size of both species were highly variable and imprecise.

Effective population size can be affected by many factors which tend to fall into two categories: biases caused by human error (i.e. sampling, calculation etc.) and the biology of the organism (e.g. life-history, demographic history, mating system, etc.; Conner and Hartl 2004, Luikart et al. 2010, Robinson and Moyer 2013). Most of the methods used to estimate contemporary N_e lack precision when N_e is large (i.e. >500; Luikart et al. 2010, Waples and Do 2010, Robinson and Moyer 2013), although it has been suggested that precision of the temporal method is reasonable when many individuals (50-100) and loci (15-30 microsatellites) are sampled (Waples 1989, Jorde and Ryman 1995) and that the LD method is reasonably precise with N_e up to 500, even with overlapping generations (Robinson and Moyer 2013).

One of the defining characteristics of an idealized population and thus a major assumption of the methods used to estimate N_e is that of discrete generations. However, like many animals, bats have overlapping generations and this can affect the estimate of N_e. These biases are highly variable and can be either downward or upward depending on numerous factors, such as life-history characteristics (Luikart et al. 2010, Robinson and Moyer 2013). For example, species with slow life-histories have shown a strong (50%) upward bias (i.e. overestimation; Waples and Yokota 2007) and for those with a strong reproductive skew, estimates have been biased downward by up to 34% (i.e. underestimated; Robinson and Moyer

2013). Thus, the lack of precision, and ultimately usefulness, of my estimates of contemporary effective population sizes is likely due to a combination of large (>500) effective population sizes and the life-history characteristics of bats. However, if effective population sizes are small, as suggested by Pylant (2004), this may be due to a variety of factors.

Fluctuating population sizes (i.e. bottlenecks), unequal sex ratios, and variation in reproductive success will all decrease N_e relative to census size (N_c). Although the BOTTLENECK tests for both species did not provide evidence for a bottleneck, the Garza-Williamson M-ratio tests did, with all M-ratios falling substantially below the critical value of 0.68 and all generated critical values. The Cornuet and Luikart (1996) method implemented by BOTTLENECK is more sensitive and better at detecting more recent and less severe bottlenecks than the Garza and Williamson (2001) M-ratio test, which is better at detecting older, more severe declines (Williamson-Natesan 2005). Thus, it appears that both hoary bats and silver-haired bats have experienced a bottleneck, but not recently (i.e. within the last tens of generations). Regardless of the timing of the bottleneck, the population decline would have served to decrease N_e.

We know little about sex ratios and variation in reproductive success in bats, especially in the migratory-tree bats. However, fatality data from the Alberta site suggest that sex ratios are relatively even, at least among the bats that were killed (Baerwald and Barclay 2011). One of the hypotheses regarding bat fatalities at wind turbines is that male bats, particularly hoary bats, congregate around turbines to wait for females, possibly engaging in a kind of lekking behaviour (Cryan 2008). If male bats are lekking, then this could lead to a skewed reproductive success and further decrease N_e relative to N_c.

Given that genetic structure exists among populations of hoary bats and silver-haired bats, and that fatalities at wind energy installations are not distributed equally among these populations (Arnett and Baerwald 2013), genetic diversity could be lost more rapidly in the populations experiencing the highest levels of fatality, especially if their N_e is low. This loss of genetic variation may increase negative genetic effects such as depressed evolutionary potential and weakened reproductive success, and ultimately, increase the extinction risk of these populations (Amos and Balmford 2001, Spielman et al. 2004).

Additionally, if aspects of bat migration are heritable, as seen across numerous taxa (Pulido and Berthold 2003, Liedvogel et al. 2011), then these aspects of bat migration may evolve. For example, migratory tendency and personality are often correlated: "bold" individuals are more likely to migrate and travel longer distances than "shy" individuals (Mettke-Hofmann et al. 2005, Nilsson et al. 2010, Chapman et al. 2011). If bats are attracted to turbines and killed while investigating them, as has been hypothesised (Kunz et al. 2007, Cryan and Barclay 2009), and if "bold" individuals are those most likely to approach the turbines and be killed, and if "boldness" is heritable, then turbines may select against boldness, and ultimately migratory behaviour, in bats. Because animal personality can have major ecological and evolutionary effects, such as the ability of species or populations to respond to environmental change (Dall et al. 2004, Sih et al. 2004), the loss of genetic diversity in migratory bats may have unforeseen consequences beyond the reduction of the overall population size.

If preserving genetic diversity is a priority, then we can use genetic structure to inform conservation decisions, such as, at what scale we should manage the species. It may not be feasible to manage either entire populations or individual colonies. Rather than trying to manage the entire species, we could focus our conservation efforts on further exploration of the genetic

structure among populations and then use this structure to define management units. Management units, in the conservation-genetic sense, are defined as "populations of conspecific individuals among which the degree of connectivity is sufficiently low so that each population should be monitored and managed separately" (Palsbøll et al. 2007, page 11; see also Moritz 1994, Taylor and Dizon 1999). If we find further evidence of matrilineal migration routes and limited movement of females among routes, then the management units may be the migration routes used by bats. If individuals from multiple colonies/areas are funnelling into these routes, then managing at the migration route level may be an effective way to manage bats that are distributed over a large area.

Table 3-1 Sampling details, sample sizes, and molecular diversity indices of hoary bats (*Lasiurus cinereus*) used in mtDNA analyses.

Site	Sampling dates	Sample size	Number of haplotypes	Haplotype diversity (Hd) ±SD	Nucleotide diversity (π) ±SD
Alberta Summerview Wind Energy Facility	July-Sept. 2006 & 2007	92	71	0.966±0.01	0.075±0.08
Manitoba St. Leon Wind Energy Facility	August 2009	5	5	1±0.13	0.182±0.06
Ontario Wolfe Island Wind Energy Facility	May-Sept. 2010	6	6	1±0.10	0.121±0.03

Table 3-2 Sampling details, sample sizes, and molecular diversity indices of silver-haired bats (*Lasionycteris noctivagans*) used in mtDNA analyses.

Site	Sampling dates	Sample size	Number of haplotypes	Haplotype diversity (Hd) ±SD	Nucleotide diversity (π) ±SD
Alberta Summerview Wind Energy Facility	July-Sept. 2006 & 2007	81	44	0.946±0.02	0.016±0.002
Manitoba St. Leon Wind Energy Facility	August 2009	11	11	1±0.039	0.036±0.004
Ontario Wolfe Island Wind Energy Facility	May-Sept. 2010	13	13	1±0.030	0.046±0.006

Table 3-3 Molecular diversity indices of 19 hoary bat (*Lasiurus cinereus*) microsatellite loci. Hardy-Weinberg p-values in bold are significant after a Bonferroni correction for multiple comparisons. Frequency of null alleles is based on Van Oosterhout et al (2004) and values in bold are loci that contain null alleles. The locus Laci28 was monomorphic in both the Manitoba and Ontario groups, so was excluded from several of the calculations.

Alberta Summerview (n = 132)						
Loci	Number of alleles	Number of genotypes	Observed heterozygosity	Expected heterozygosity	HWE p-value	Frequency of null alleles
Laci1	65	130	0.762	0.972	**<0.001**	**0.107**
Laci15	11	130	0.831	0.826	0.362	-0.007
Laci20	9	131	0.779	0.771	0.649	-0.006
Laci22	19	131	0.901	0.865	0.114	-0.024
Laci25	10	132	0.273	0.786	**<0.001**	**0.313**
Laci26	28	130	0.938	0.910	0.626	-0.018
Laci28	3	132	0.015	0.015	1.000	**0.109**
Laci29	19	129	0.674	0.867	**<0.001**	**0.109**
Laci31	18	132	0.879	0.887	0.713	0.002
Laci33	23	132	0.856	0.884	0.555	0.014
Laci34	23	131	0.863	0.934	**<0.001**	**0.037**

Laci35	66	132	0.894	0.977	0.004	0.041
Laci36	21	132	0.894	0.882	0.636	-0.008
Laci38	39	131	0.534	0.946	**<0.001**	**0.217**
Laci39	27	132	0.856	0.872	0.170	0.005
Laci41	33	131	0.771	0.941	**<0.001**	**0.088**
Laci42	12	131	0.634	0.835	**<0.001**	**0.119**
Laci43	17	132	0.689	0.878	**<0.001**	**0.105**
Laci48	24	132	0.750	0.868	**<0.001**	**0.066**
Manitoba St. Leon (n = 10)						
Loci	Number of alleles	Number of genotypes	Observed heterozygosity	Expected heterozygosity	HWE p-value	Frequency of null alleles
Laci1	15	10	1.000	0.974	1.000	-0.041
Laci15	5	10	0.800	0.837	0.970	-0.002
Laci20	6	10	0.800	0.800	0.049	-0.057
Laci22	7	10	1.000	0.842	0.966	-0.137
Laci25	4	10	0.600	0.774	0.563	0.080
Laci26	8	10	0.800	0.858	0.581	0.012
Laci28	.	.	.	.	.	0.021
Laci29	8	10	0.700	0.884	0.114	0.070
Laci31	9	10	0.900	0.911	0.855	-0.022

Laci33	9	10	0.800	0.900	0.231	0.032
Laci34	7	10	0.800	0.868	0.561	0.014
Laci35	16	10	0.800	0.979	0.024	0.070
Laci36	8	10	0.900	0.847	0.900	-0.059
Laci38	7	10	0.800	0.868	0.557	0.014
Laci39	15	10	0.700	0.968	0.001	**0.122**
Laci41	12	10	1.000	0.937	1.000	-0.064
Laci42	9	10	0.500	0.889	0.003	**0.206**
Laci43	8	10	0.700	0.868	0.469	0.073
Laci48	10	10	0.800	0.900	0.539	0.038
Ontario Wolfe Island (n = 15)						
Loci	Number of alleles	Number of genotypes	Observed heterozygosity	Expected heterozygosity	HWE p-value	Frequency of null alleles
Laci1	20	15	0.867	0.966	0.139	0.037
Laci15	8	15	0.667	0.814	0.222	0.077
Laci20	5	15	0.867	0.793	0.097	-0.061
Laci22	8	15	0.867	0.857	0.926	-0.022
Laci25	5	15	0.400	0.706	0.006	**0.202**
Laci26	10	15	0.867	0.897	0.203	-0.003

Laci28	.	.	.	.	.	0.011
Laci29	10	15	0.600	0.908	0.009	**0.149**
Laci31	12	15	0.867	0.892	0.788	0.000
Laci33	11	15	1.000	0.910	0.271	-0.070
Laci34	9	15	0.933	0.834	0.999	-0.088
Laci35	16	15	0.867	0.936	0.210	0.021
Laci36	10	15	0.733	0.885	0.313	0.075
Laci38	9	15	0.933	0.834	0.999	-0.088
Laci39	16	15	0.867	0.936	0.214	0.021
Laci41	12	15	0.867	0.913	0.508	0.009
Laci42	7	15	0.533	0.846	0.006	**0.173**
Laci43	8	15	0.867	0.874	0.572	-0.019
Laci48	9	15	0.800	0.855	0.165	0.013

Table 3-4 Molecular diversity indices of 18 silver-haired bat (*Lasionycteris noctivagans*) microsatellite loci. Hardy-Weinberg p-values in bold are significant at $\alpha = 0.05$. Frequency of null alleles is based on Van Oosterhout et al (2004) and values in bold are loci that contain null alleles.

Alberta Summerview (n = 87)						
Loci	Number of alleles	Number of genotypes	Observed heterozygosity	Expected heterozygosity	HWE p-value	Frequency of null alleles
Lano1	7	87	0.644	0.709	0.531	0.05
Lano3	8	87	0.862	0.796	0.134	-0.05
Lano4	11	86	0.488	0.843	**<0.001**	**0.21**
Lano8	13	85	0.800	0.858	0.733	0.03
Lano10	17	87	0.655	0.867	**<0.001**	**0.12**
Lano13	14	87	0.828	0.880	0.411	0.03
Lano15	10	84	0.750	0.767	0.486	0.01
Lano22	18	86	0.907	0.869	0.990	-0.03
Lano26	12	87	0.345	0.829	**<0.001**	**0.29**
Lano28	6	86	0.674	0.671	0.784	-0.01
Lano29	12	87	0.816	0.862	0.059	0.03
Lano31	14	85	0.471	0.850	**<0.001**	**0.22**

Lano32	12	86	0.547	0.840	**<0.001**	**0.17**
Lano34	13	87	0.701	0.857	0.038	**0.09**
Lano35	20	87	0.782	0.910	0.035	0.07
Lano40	17	87	0.805	0.817	0.501	0.00
Lano41	10	83	0.386	0.813	**<0.001**	**0.25**
Lano44	19	86	0.535	0.917	**<0.001**	**0.21**
Manitoba St. Leon (n = 13)						
Loci	Number of alleles	Number of genotypes	Observed heterozygosity	Expected heterozygosity	HWE p-value	Frequency of null alleles
Lano1	4	13	0.769	0.750	0.958	-0.03
Lano3	6	13	0.923	0.793	0.945	-0.11
Lano4	10	13	0.538	0.867	0.007	**0.17**
Lano8	11	13	0.923	0.855	0.969	-0.07
Lano10	7	13	0.923	0.766	1.000	-0.15
Lano13	7	13	0.923	0.812	0.955	-0.10
Lano15	7	13	0.923	0.766	1.000	-0.15
Lano22	12	13	0.923	0.920	0.257	-0.03
Lano26	8	13	0.461	0.867	0.003	**0.22**
Lano28	5	13	0.692	0.593	0.505	-0.14
Lano29	8	13	0.923	0.880	0.225	-0.05

Lano31	6	13	0.461	0.790	0.021	**0.19**
Lano32	8	13	0.538	0.883	0.020	**0.18**
Lano34	7	13	0.692	0.864	0.170	0.08
Lano35	12	13	0.769	0.901	0.152	0.04
Lano40	7	13	0.769	0.803	0.719	0.00
Lano41	8	13	0.384	0.827	**<0.001**	**0.26**
Lano44	7	13	0.307	0.781	**0.001**	**0.27**
Ontario Wolfe Island (n = 20)						
Loci	Number of alleles	Number of genotypes	Observed heterozygosity	Expected heterozygosity	HWE p-value	Frequency of null alleles
Lano1	5	19	0.842	0.729	0.958	-0.10
Lano3	6	20	0.800	0.808	0.607	-0.01
Lano4	7	18	0.500	0.825	0.006	**0.19**
Lano8	13	19	0.842	0.896	0.041	0.02
Lano10	8	19	0.736	0.815	0.317	0.03
Lano13	9	18	0.833	0.876	0.072	0.02
Lano15	8	20	0.750	0.830	0.127	0.03
Lano22	14	19	0.894	0.889	0.559	-0.02
Lano26	9	20	0.300	0.841	**<0.001**	**0.31**

Lano28	5	19	0.736	0.672	0.540	-0.09
Lano29	7	19	0.631	0.806	0.321	0.10
Lano31	9	19	0.684	0.854	0.044	0.09
Lano32	9	18	0.388	0.850	**<0.001**	**0.26**
Lano34	9	19	0.789	0.880	0.314	0.04
Lano35	12	18	0.888	0.912	0.271	-0.01
Lano40	12	20	0.800	0.829	0.273	0.01
Lano41	8	18	0.388	0.806	**0.001**	**0.24**
Lano44	12	18	0.500	0.877	**<0.001**	**0.21**

Table 3-5 Pairwise Fst comparisons (using mtDNA) among sampling locations of hoary bat (*Lasiurus cinereus*). Values below the diagonal are Fst values and values above the diagonal are p-values. Values in bold are significant at $\alpha = 0.05$

	Alberta	Manitoba	Ontario
Alberta	.	0.649	0.559
Manitoba	-0.0164	.	0.450
Ontario	-0.0118	-0.0198	.

Table 3-6 Pairwise Fst comparisons (using mtDNA) among sampling locations of silver-haired bat (*Lasionycteris noctivagans*). Values below the diagonal are Fst values and values above the diagonal are p-values. Values in bold are significant at $\alpha = 0.05$.

	Alberta	Manitoba	Ontario
Alberta	.	0.342	**0.009**
Manitoba	0.0031	.	0.144
Ontario	**0.0416**	0.0175	.

Table 3-7 Pairwise Fst comparisons (using microsatellites) among sampling locations of hoary bats (*Lasiurus cinereus*). Values below the diagonal are Fst values from ARLEQUIN and values above the diagonal are Fst values that have been adjusted for null alleles using the ENA method of Chapuis and Estoup (2007) in FREENA. Values in bold are significant at $\alpha = 0.05$.

	Alberta Summerview	Manitoba St. Leon	Ontario Wolfe Island
Alberta Summerview	.	**0.007**	**0.011**
Manitoba St. Leon	**0.009**	.	0.003
Ontario Wolfe Island	**0.010**	0.006	.

Table 3-8 Pairwise Fst comparisons (using microsatellites) among sampling locations of silver-haired bats (*Lasionycteris noctivagans*). Values below the diagonal are Fst values from ARLEQUIN and values above the diagonal are Fst values that have been adjusted for null alleles using the ENA method of Chapuis and Estoup (2007) in FREENA. Values in bold are significant at $\alpha = 0.05$.

	Alberta Summerview	Manitoba St. Leon	Ontario Wolfe Island
Alberta Summerview	.	**0.002**	0.002
Manitoba St. Leon	**0.006**	.	-0.007
Ontario Wolfe Island	0.003	-0.000	.

Table 3-9 Estimates of contemporary effective population size (N_e) of hoary bats (*Lasiurus cinereus*) from Alberta (n = 132). LD and temporal estimates were calculated by NE ESTIMATOR V2 and the sib-ship estimate was calculated by COLONY. LD and temporal estimates are shown for a pcrit of 0.01 and 95% confidence intervals from jackknifing. Note the extreme variability and lack of precision.

Measure	N_e	lower 95%	upper 95%
LD	1499	721	infinite
Temporal (Jorde/Ryman model)	532	138	infinite
Sib-ship (assuming random mating)	112	84	152

Table 3-10 Estimates of contemporary effective population size (N_e) of silver-haired bats (*Lasionycteris noctivagans*) from Alberta (n = 87). LD and temporal estimates were calculated by NE ESTIMATOR V2 and the sib-ship estimate was calculated by COLONY. LD and temporal estimates are shown for a pcrit of 0.01 and 95% confidence intervals from jackknifing. Note the extreme variability and lack of precision

Measure	N_e	lower 95%	upper 95%
LD	570	201	infinite
Temporal (Jorde/Ryman model)	56	17	infinite
Sib-ship (assuming random mating	49	32	78

Figure 3-1 Sampling locations for hoary bats (*Lasiurus cinereus*) and silver-haired bats (*Lasionycteris noctivagans*). Image from Google Maps.

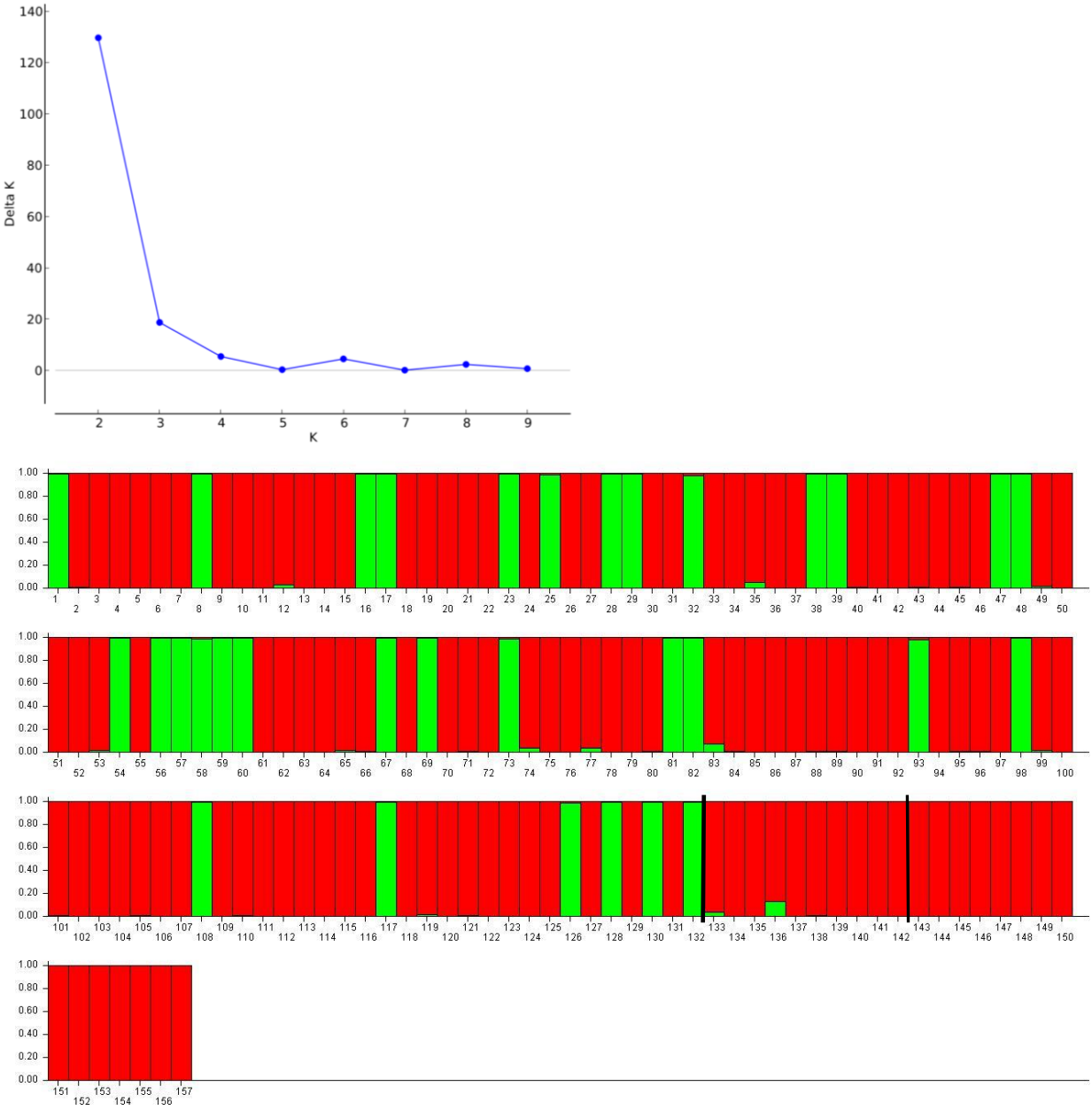

Figure 3-2 STRUCTURE results for 19 microsatellite loci in hoary bats (*Lasiurus cinereus*). Top panel: the most likely K number of distinct genetic clusters (denoted with the highest ΔK; Evanno et al. 2005). Bottom panel: barplot showing the probabilities of individual assignment to each genetic cluster, assuming two genetic clusters; black lines separate sampling locations (AB, MB, ONT).

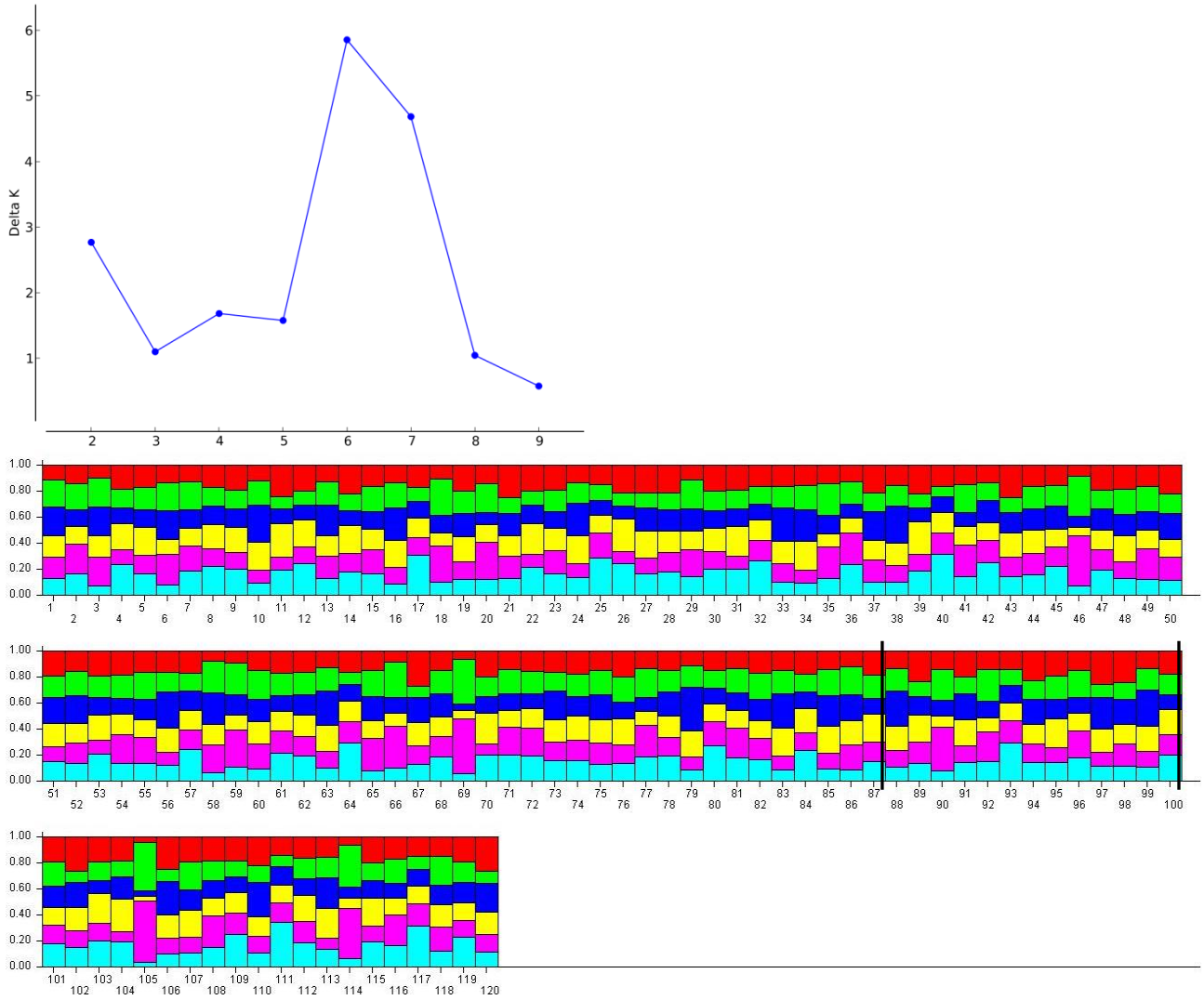

Figure 3-3 STRUCTURE results for 18 microsatellite loci in silver-haired bats (*Lasionycteris noctivagans*). Top panel: the most likely K number of distinct genetic clusters (denoted with the highest ΔK; Evanno et al. 2005). Bottom panel: barplot showing the probabilities of individual assignment to each genetic cluster, assuming six genetic clusters; black lines separate sampling locations (AB, MB, ONT).

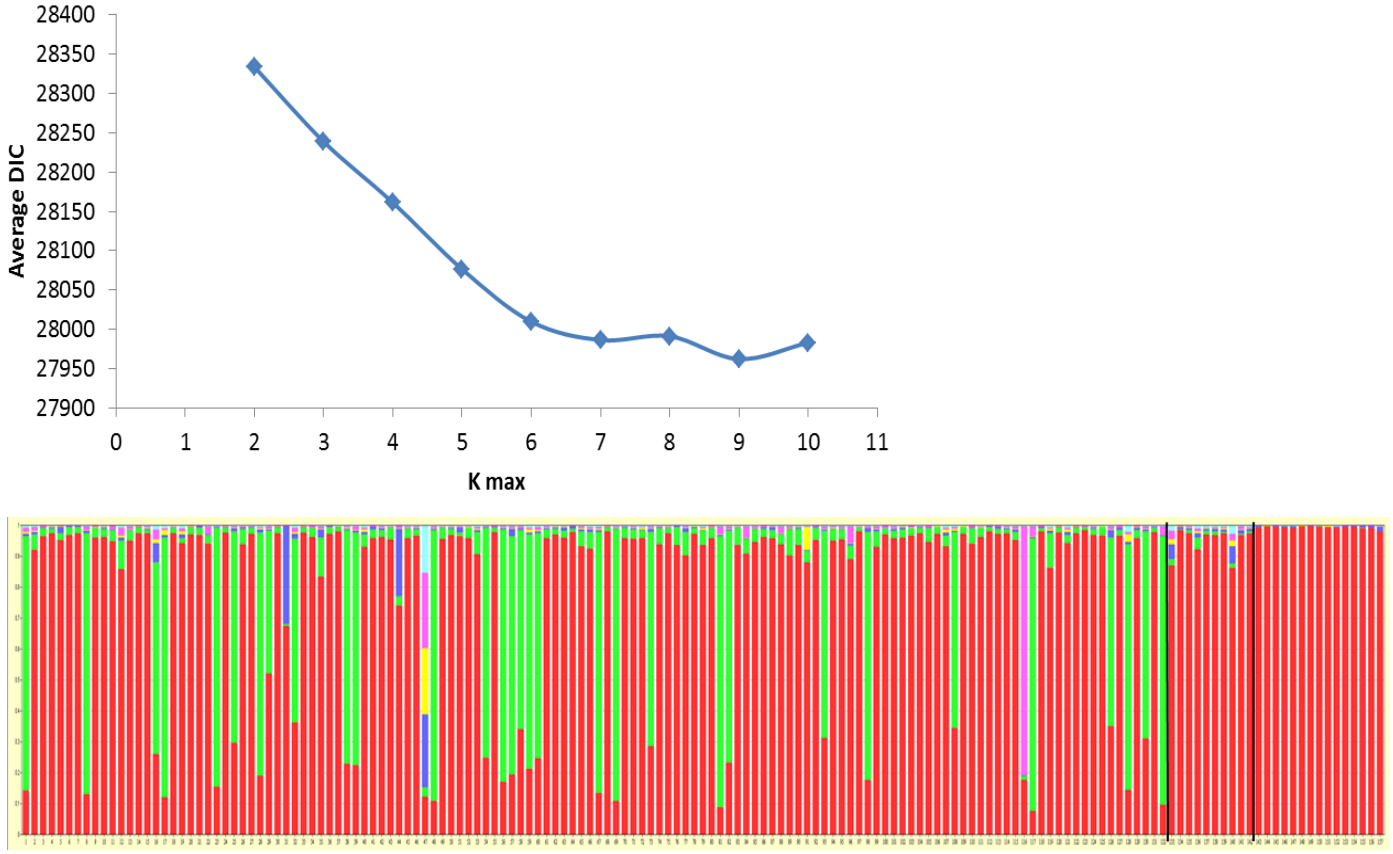

Figure 3-4 TESS results for 19 microsatellite loci in hoary bats (*Lasiurus cinereus*). Top panel: the most likely K number of distinct genetic clusters (denoted by the beginning of the plateau of DIC; Chen et al 2007). Bottom panel: barplot showing the probabilities of individual assignment to each genetic cluster, assuming six genetic clusters; black lines separate sampling locations (AB, MB, ONT).

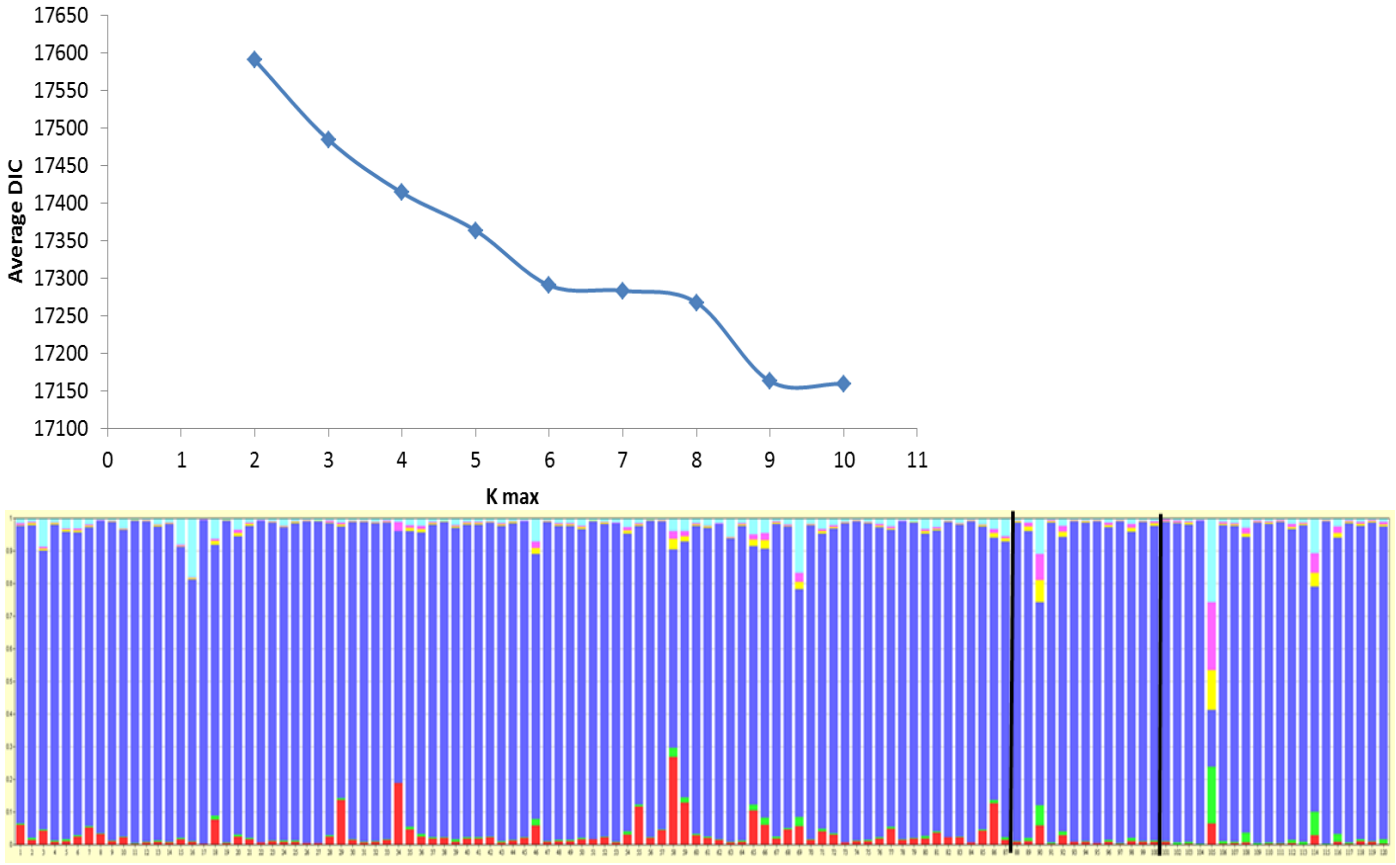

Figure 3-5 TESS results for 18 microsatellite loci in silver-haired bats (*Lasionycteris noctivagans*). Top panel: the most likely K number of distinct genetic clusters (denoted by the beginning of the plateau of DIC; Chen et al 2007). Bottom panel: barplot showing the probabilities of individual assignment to each genetic cluster, assuming six genetic clusters; black lines separate sampling locations (AB, MB, ONT).

CHAPTER 4: AN ASSESSMENT OF THE SOCIAL TRANSMISSION OF MIGRATORY BEHAVIOURS AMONG BATS

Introduction

How animals find their way during migration has long fascinated us and inspired centuries of research. Migratory animals rely on endogenous, genetically inherited programs, or socially transmitted information about routes and behaviours, or a combination of the two (Dodson 1988, Berthold 1991, Pulido 2007, Liedvogel et al. 2011). The degree to which each source of information is used depends on numerous factors, including the age, experience, personality, and sociality of an individual (Németh and Moore 2014). Thus, social transmission of migratory information may be more likely to occur in animals that live in groups that contain a mix of experienced leaders and naïve followers than in solitary animals or in a group containing only experienced or only naïve individuals.

Transmission of migratory behaviours is most likely to occur from experienced to inexperienced individuals. This may be because navigational abilities of experienced individuals are superior to those of inexperienced individuals, as seen in white-crowned sparrows (*Zonotrichia leucophrys*; Thorup et al. 2007) and white storks (*Ciconia ciconia*; Chernetsov et al. 2004). In fact, following an experienced leader may be necessary for successful migration, as seen in the ultralight-led migrations of whooping cranes (*Grus americana*), Canada geese (*Branta canadensis*), and trumpeter swans (*Cygnus buccinators*; Sladen et al. 2002, Ellis et al. 2003). Often, the leaders are parents guiding their offspring during their first migration. This is most commonly seen in mammals such as whales (e.g. gray whales (*Eschrichtius robustus*),

humpback whales (*Megaptera novaeangliae*), and southern right whales (*Eubalaena australis*); Martin et al. 1984, Corkeron and Connor 1999, Valenzuela et al. 2009) and ungulates (e.g. pronghorn (*Antilocapra americana*), mule deer (*Odocoileus hemionus*), and white-tailed deer (*Odocoileus virginianus*) McCullough 1985, Nelson 1998, Sawyer et al. 2005). However, the experienced individual may not always be a parent. In the great bustard (*Otis tarda*) for example, migratory behaviours of immature males are not learned from their parents, but from other adult males and migration in immature females is learned from their mothers in their first year, but from other adult females in their second year (Palacín et al. 2011).

Animals that learn components of migration from social transmission show increased flexibility and adaptability of migratory behaviour and a decreased use of suboptimal routes relative to those relying on genetic programs (Sutherland 1998). For example, in whooping cranes, social learning reduces suboptimal migration strategies; inexperienced birds that followed older birds had a 38% improvement in accurately arriving at their over-wintering location relative to inexperienced birds that flew only with other inexperienced birds (Mueller et al. 2013).

Life-history affects the degree to which social transmission of migratory information occurs. In long-lived animals with extended parental care, migration tends to be socially transmitted. In birds, for example, migration is socially transmitted in cranes, geese, swans, storks, and bustards, but under endogenous control in the majority of small songbirds (Berthold 2001, Pulido 2007). Long lifespans facilitate the transmission of historic traditions. For example, matriarchs of African elephant (*Loxodonta africana*) herds retain knowledge of their surroundings, and lead their herd to superior sites in times of resource scarcity, sometimes decades after they last visited them (McComb et al. 2001, Archie et al. 2006). This retention and

transmission of knowledge by the long-lived matriarch enables them to quickly adapt to changing environments and is a fundamental component of migration in elephants.

Social transmission of migratory information and the use of optimal migration routes may be particularly important for animals with slow life histories because they have low reproductive rates and migration is a risky behaviour, thus increasing the importance of successful migrations of juveniles. In black-throated blue warblers (*Setophaga caerulescens*), 85% of annual mortality occurs during their migration between New Hampshire and Jamaica, and mortality rates are 15 times higher during migration than during stationary seasons (Sillett and Holmes 2002). In three species of raptor that migrate between Northwestern Europe and Western Africa, mortality rates are six times greater during migration and half of their annual mortality occurs while migrating (Klaassen et al. 2014). In a mule deer population that was partially migratory, all annual mortality of migratory adult females occurred during migration, but among the non-migratory females, all mortality occurred over winter (Nicholson et al. 1997). Migration is particularly risky for first-time migrants (Strandberg et al. 2009). In both barnacle geese (*Branta leucopsis*) and snow geese (*Chen caerulescens*), over 95% of adults successfully complete autumn migration, but only ~65% of young-of-the-year survive their first autumn migration (Owen and Black 1989, Menu et al. 2005).

Bats have slow life-histories and long lives (Barclay and Harder 2003), and it is thus likely that social transmission of migratory behaviour is common in bats. However, we know little about migratory behaviours in bats. Bats are certainly capable of observational learning (Gaudet and Fenton 1984, Wright et al. 2011), teaching (i.e. deliberate demonstration of behaviours; Bunkley and Barber 2014), and social transmission of behaviour (Wilkinson 1992, Ratcliffe and ter Hofstede 2005, Page and Ryan 2006, Clarin et al. 2014). Mothers and offspring

are also capable of recognizing each other acoustically, even in large maternity colonies (Balcombe and McCracken 1992, Knörnschild et al. 2013, Jin et al. 2015), which suggests that bonds occur between mother and offspring. There is also evidence of extended mother-pup associations (Brigham and Brigham 1989, Rossiter et al. 2002). In the common big-eared bat (*Micronycteris microtis*), mothers provisioned pups with food for five months post-weaning (Geipel et al. 2013). Given that parental investment is pronounced in Chiroptera, I hypothesized that migratory bats transmit information about migratory routes and behaviours to juveniles, as seen in other animals with similar levels of parental investment.

Although highly gregarious species of bat likely migrate as a group, as seen in Mexican free-tailed bats (*Tadarida brasiliensis*; Cockrum 1969) and straw-coloured fruit bats (*Eidolon helvum*; Thomas 1983), it is not known how many species of bat flock while migrating. There are a handful of historical accounts of flocks of tree bats migrating during the day (Mearns 1898, Howell 1908, Allen 1939, Hall 1946). One of those flocks consisted of at least three sizes of bats, but it was noted that the flock did not behave like flocks in the classic sense (i.e. with coordinated movements), but rather, appeared to be a group of individuals moving through the same place at the same time (Howell 1908). Echolocation activity and fatalities of bats recorded at wind energy facilities also appear in waves (Baerwald and Barclay 2011), but it is unclear whether bats form flocks in the classic sense or whether multiple individuals simply take advantage of the same favourable environmental conditions to migrate (e.g. in low wind speeds; Baerwald and Barclay 2011). Regardless, flying in a group would allow for social transmission of migratory behaviours among both con- and hetero-specifics (Clarin et al. 2014).

Social transmission is most likely from mother to offspring, but perhaps also from other conspecifics. For a young bat to learn migration via social transmission, they would need to

follow an experienced individual, most likely one roosting nearby. Therefore, I predicted that bats travelling together on the same night originate from the same place. It is also likely that young bats follow their mothers or other close relatives, so I predicted that bats travelling together on the same night are more closely related to each other than bats travelling on different nights. This pattern should be particularly apparent in adult females and juveniles because they roost together in family groups, while males roost independently. Given that some species of migratory bat are solitary (e.g. hoary bats; *Lasiurus cinereus*) and some are colonial (e.g. silver-haired bats; *Lasionycteris noctivagans*), I hypothesized that the degree to which migration is socially transmitted differs among species, such that it is more prevalent in colonial than in solitary bats. If so, then I predicted that the degree of relatedness and the similarity in geographic origins of individuals travelling on the same night would be greater in colonial bats than in solitary bats. To test my predictions, I used recently developed multilocus microsatellite genotypes (Appendix A) and stable isotope values of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ (which can be used to determine the geographic origin of fur grown on the summering grounds; Chapter 2, Popa-Lisseanu et al. 2012, Cryan et al. 2014) to analyse the temporal relatedness and geographic origins of migrating hoary bats and silver-haired bats killed at a wind energy facility in southwestern Alberta over two consecutive autumn migrations.

Study species

Hoary bats roost solitarily in tree foliage throughout North and South America (Shump and Shump 1982b, Cryan 2003). Although we still do not understand the movement patterns or seasonal distributions of hoary bats, it seems they winter in Mexico and the southern United States, then migrate long distances north and east in the spring (Findley and Jones 1964, Cryan

2003, Cryan et al. 2014). In summer, hoary bats are found throughout the prairies, aspen parkland, and the southern boreal forests of Canada (Buehler and Piersma 2008, Ulanovsky and Moss 2008, Baerwald et al. 2014). Reproductive females exhibit some degree of year-to-year site fidelity (Koehler and Barclay 2000). There may be some degree of sexual segregation of hoary bats during summer, with females potentially migrating further than males (Findley and Jones 1964, Cryan 2003, Cryan et al. 2004), but fatality and stable isotope data show no strong evidence for this (Chapter 2, Baerwald and Barclay 2011). However, males roost separately from mother-pup family units (Koehler and Barclay 2000). Mating may occur in autumn during migration or on the wintering grounds, with one to four, but usually two pups born the following spring/early summer (Shump and Shump 1982b, Cryan et al. 2012a). Pups are volant at about 4 weeks of age, but not weaned until about 7 weeks of age (Koehler and Barclay 2000). At the onset of migration, the mean mass of each pup is less than the mean mass of adults, but mean forearm length (a proxy of body size) is similar (Koehler and Barclay 2000).

Known wintering grounds of silver-haired bats are in the Pacific Northwest, south-western states, and middle latitudes of the eastern United States (Izor 1979, Cryan 2003). The limited data on their migratory patterns suggest that in spring, silver-haired bats from the east migrate long distances north and east and western silver-haired bats migrate northward (Cryan 2003, Fraser and Longstaffe 2014). In summer, silver-haired bats are common throughout the forested areas of Canada where males roost singly and reproductive females form small colonies (Kunz 1982). Maternity colonies are located in tree cavities, not in bark crevices or other roosting places used by males and non-reproductive females (Mattson et al. 1996, Betts 1998). There is some degree of sexual segregation during summer, with females potentially migrating farther than males (Cryan 2003). However, fatalities at wind energy facilities suggest a relatively

even sex ratio in southern Alberta during autumn migration (Baerwald and Barclay 2011), and stable isotope analysis of the fur of those fatalities showed no evidence of sexual segregation (Chapter 2). Mating appears to occur in autumn during migration and one to two, but usually two, pups are born the following spring/early summer (Kunz 1982, Cryan et al. 2012a). Lactation appears to last about 5 weeks and volancy appears to occur at about 4 weeks (Kunz 1982; Baerwald and Klüg unpublished data).

Methods

Sample collection

I collected hair and skin samples from bat carcasses found under wind turbines in southwestern Alberta, Canada (49° 35'04" N, 113° 47'48" W). I searched for bat carcasses from 15 July to 30 September 2006 and 2007. I searched 10 randomly chosen turbines every day and the remaining 29 turbines once a week (see Baerwald and Barclay 2009 for details). For each carcass, I recorded species, age (Anthony 1988) and sex (when possible), and the degree of decomposition. In 2007, I verified age classifications during necropsies by noting the presence or absence of a thymus gland, which is present in sub-adults but absent in adults (Kallen 1977, Chakraborty and Chakravarty 1984). I collected skin tissue from the wingtips of freshly killed bat carcasses (i.e. those killed the previous night) using dissecting scissors. Tissue was stored in 70-90% ethanol and kept in a -20°C freezer until DNA extraction. I collected hair from between the scapulae of bats killed the previous night, placed samples into plastic micro-centrifuge tubes, and stored them in a freezer until analysis.

DNA extraction, amplification, and genotyping

Development of primers and microsatellite analysis were conducted at the University of Georgia's Savannah River Ecology Laboratory (see Appendix A for details on primer development). DNA was extracted using Qiagen DNEasy Blood and Tissue kit. PCR amplifications were performed in a 12.5 μL volume (10 mM Tris pH 8.4, 50 mM KCl, 25.0 μg/ml BSA, 0.4 μM unlabeled primer, 0.04μM tag labeled primer, 0.36μM universal dye-labeled primer, 3.0 mM $MgCl_2$, 0.8 mM dNTPs, 0.5 units AmpliTaq Gold® Polymerase (Applied Biosystems), and 20 ng DNA template) using an Applied Biosystems GeneAmp 9700. Touchdown thermal cycling programs (Don et al. 1991) encompassing a 10°C span of annealing temperatures ranging between 65 and 55°C (TD65) were used for all loci. Touchdown cycling parameters consisted of an initial denaturation step of 5 min at 95°C followed by 20 cycles of 95°C for 30 s, highest annealing temperature (decreased 0.5°C per cycle) for 30 s, and 72 °C for 30 s; and 20 cycles of 95 °C for 30 s, lowest annealing temperature for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min. PCR products were run on an ABI-3130xl sequencer and sized with Naurox size-standard prepared as described in DeWoody et al. (2004), except that unlabeled primers started with GTTT. Results were analyzed using GENEMAPPER version 3.7 (Applied Biosystems).

I calculated the number of alleles (N_a), observed (H_o) and expected heterozygosity (H_e), and linkage disequilibrium (LD) using ARLEQUIN 3.5.1.3 (Excoffier and Lischer 2010). I used MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) to search for loci with the following genotyping errors: dropout of large alleles, stuttering error, and null alleles. I did not remove null alleles from further analysis because the software programs I used are equipped to handle them.

Stable isotope analysis

Stable carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and deuterium ($\delta^{2}H$) analyses were conducted at the Saskatchewan Isotope Laboratory in the Department of Geological Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada (see Chapter 2 or Baerwald et al. 2014 for details).

Analysis of relatedness

I examined relatedness of individual bats in two ways. First, I used the full-likelihood method of COLONY 2.0.5.8 (Jones and Wang 2010) to distinguish dyads as Unrelated, Half-sibs, Full-sibs, or Parent-Offspring, from the genotypes of potential offspring and the potential mothers and fathers. I considered all young-of-the-year (from both 2006 and 2007) to be potential offspring and, given that bats have overlapping generations, I also considered adults from 2007 to be potential offspring of 2006 and 2007 adults, I considered all adult females and males to be potential mothers or fathers, respectively, each with a 0.5 probability of parenting one of the candidate offspring. To be classified as belonging to one of the relatedness categories, dyads had to have a ≥95% probability of belonging to that category. I used the genotyping error estimated by COLONY, assumed polygamy for both sexes, re-calculated allele frequencies, and used no sibship prior (i.e. I made no assumptions about the average paternal or maternal sibship size).

I also used COANCESTRY 1.0.1.5 (Wang 2011) to calculate pairwise relatedness of all possible dyads. COANCESTRY calculates seven measures of relatedness simultaneously, but only the two maximum likelihood methods (TrioML and DyadML) allow you to incorporate

genotyping errors, which, if not accounted for, can affect the precision of the relatedness estimates. I used the genotyping errors calculated by COLONY during the previous analysis and compared the results of TrioML and DyadML to determine which model to use. For both species, the two models were highly correlated ($r^2 > 0.90$), but the relatedness values were slightly higher in DyadML, so I used these values. A relatedness of 0.25 is indicative of half-siblings, but to minimize the likelihood of making a Type one error, I considered a dyad to be related if its relatedness was ≥0.20.

Statistical analyses

To investigate whether related individuals were killed on the same night more frequently than expected by chance, I compared the number of related and unrelated dyads killed on the same night to the number of related and unrelated dyads killed on different nights using chi-squared tests. I ran two different chi-squared tests, one using the relatedness categories of COLONY and one using the relatedness values of COANCESTRY. I restricted the analysis to within-year dyads (i.e. I did not include dyads that contained individuals from different years). I used an α value of 0.05.

I also examined whether the time between individuals within a dyad (in days) was influenced by their relatedness or the similarity in their geographic origins by using Generalized Linear Models (GLMs) in JMP 10 (SAS Institute, Cary NC). I used the relatedness values from the COANCESTRY analysis as described above as a predictor variable, but included all relatedness values (i.e. 0 – 1). As a proxy for geographic origins, I used the absolute difference in the stable isotope values of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ between each dyad that had stable isotope values available for both individuals (hoary bats, n = 91 individuals and 2063 dyads; silver-haired bats n

= 59 individuals and 1711 dyads) as the other predictor variables. I included the interactions between relatedness and each of the isotope variables, but removed the interactions from the model if they were not significant. For the GLM, I used the log-link function for Poisson distributions, accounted for overdispersion, and used an α value of 0.05.

Results

Genetic diversity

133 hoary bats were genotyped at 19 microsatellite loci. The number of alleles per locus ranged from three to 66 (Table 4.1). Average observed heterozygosity was 0.73 ± 0.23. Ten loci contained null alleles (Table 4.1). Forty-four of 171 comparisons (25.73%) showed evidence of significant linkage disequilibrium.

87 silver-haired bats were genotyped at 18 microsatellite loci. The number of alleles per locus ranged from six to 20 (Table 4.2). Average observed heterozygosity was 0.67 ± 0.17. Eight loci contained null alleles (Table 4.2). Thirty-seven of 153 comparisons (24.18%) showed evidence of significant linkage disequilibrium.

Statistical Analyses

For hoary bats, COLONY identified 134 related dyads, 84 of which occurred within the same year. The majority of related dyads (97%) were half-sibling dyads, but there were two full-sibling dyads, one mother-offspring dyad and one father-offspring dyad. Neither the full-sibling dyads nor the parent-offspring dyads involved individuals killed on the same day. COANCESTRY identified 84 related dyads, 52 of which occurred within the same year. Relatedness values across all possible dyads ranged from 0-0.31 (mean = 0.03 ± 0.05 standard

deviation; median = 0).There was no evidence that related dyads of hoary bats were killed on the same night more frequently than expected by chance, regardless of the method used to determine related dyads (Tables 4.2 and 4.3).

In the GLM that assessed the influence of relatedness and geographic origin on the days between individuals in dyads of hoary bats, the model explained a significant proportion of the variation (Pearson's $\chi^2/df_{2061} = 12804.42$, $p < 0.001$; Deviance/$df_{2061} = 13254.11$, $p < 0.001$; Overdisperson = 6.21). The number of days between the fatalities of dyad members increased as the difference in their $\delta^{13}C$ values increased and was influenced by the interaction between dyad relatedness and the difference in their δ^2H values (Table 4.3); the number of days between fatalities was influenced by the similarity of their δ^2H values, but when relatedness increased, this relationship decreased. Relatedness on its own did not influence the number of days between fatalities of dyad members (Table 4.3).

For silver-haired bats, COLONY identified 96 related dyads, 61 of which occurred within the same year. The majority of related dyads (98%) were half-sibling dyads, but there was one full-sibling dyad and one father-offspring dyad. Neither the full-sibling dyad nor the father-offspring dyad involved individuals killed on the same day. COANCESTRY identified 155 related dyads, 80 of which occurred within the same year. Relatedness values across all possible dyads ranged from 0-0.36 (mean = 0.0 ± 0.002 standard deviation; median = 0.01).The analysis using COLONY relatedness categories suggested that individuals killed on the same night were more related than expected, but the analysis using relatedness values from COANCESTRY did not (Tables 4.6 and 4.7).

In the GLM that assessed the influence of relatedness and geographic origin on the days between individuals in dyads of silver-haired bats, the model explained a significant proportion

of the variation (Pearson's $\chi^2/df_{842} = 7878.55$, $p < 0.001$; Deviance/$df_{842} = 8274$, $p < 0.001$; Overdisperson = 9.34). The number of days between the fatalities of dyad members increased as the difference in their $\delta^{15}N$ values increased but was not influenced by any other predictor variable (Table 4.8).

Discussion

Contrary to my predictions, I found no conclusive evidence that either hoary bats or silver-haired bats migrate in family groups. However, the single significant result from the analysis of silver-haired bat relatedness categories from COLONY partially supports my prediction that social transmission is more common in silver-haired bats than in hoary bats. Additionally, my hypothesis that bats migrating on the same night originate from a similar location was not fully supported. It appears that bats travelling on the same night originate from similar habitats, as indicated by the significant effect of $\delta^{13}C$ for hoary bats and $\delta^{15}N$ in silver-haired bats, but not necessarily from similar latitudes, as a significant effect of $\delta^{2}H$ would have indicated (Chapter 2). Instead, the negative interaction between relatedness of hoary bats and $\delta^{2}H$ indicates that the correlation of $\delta^{2}H$ with the number of days between dyad members is diminished as relatedness increases. This may suggest a subtle impact of relatedness on timing, but I cannot be certain given my dataset. My stable isotope results may well suggest that individuals travelling together on the same night are not doing so in a coordinated manner, but rather, responding to similar cues in similar habitats and moving accordingly.

There is evidence from other species of bats that mothers may embark on autumn movements before their young do (Strelkov 1969, Rodrigues and Palmeirim 2007, Steffens et al. 2007). This is surprising given the slow life-histories of bats and the high mortality commonly

associated with the first migration of juvenile animals (e.g. Strandberg et al. 2009). Although mortality rates of juvenile bats during their first migration are not known, migratory bats in the genera *Perimyotis*, *Nyctalus*, *Lasiurus,* and *Lasionycteris* commonly have twins, which is unusual among bats (Barclay and Harder 2003). This, and the fact that juvenile hoary bats and silver-haired bats of both sexes are ready to mate during their first autumn, which is also unusual among bats (Cryan et al. 2012a), suggests that mortality of first year individuals is relatively high. Survival estimates of migratory Leisler's bat (*Nyctalus leisleri*) are fairly high for adults, (annual survival estimates for adult females vary from 0.45-0.61 and from 0.55-0.91 for adult males; Schorcht et al. 2009, Giavi et al. 2014), but are lower for first year individuals, with annual survival estimates of 0.45 for juvenile females and 0.04 for juvenile males (Schorcht et al. 2009).

Because information obtained socially is often easier to obtain, but less reliable than information obtained asocially, there is usually equilibrium within a group in the number of individuals acquiring information asocially and those acquiring information socially (Laland 2004, Galef and Laland 2005). Information should be obtained socially when the costs of independently acquiring that information outweigh the benefits ("copy when asocial learning is costly"; Laland 2004) or when the independently acquired information results in too much uncertainty ("copy when uncertain"; Laland 2004; Boyd and Richerson 1985, 1988, Galef and Laland 2005). I assumed that both of these criteria were fulfilled and that the benefit of increasing the probability of a successful first migration of offspring would outweigh any costs a mother incurred from travelling with the pups. However, there are multiple costs and benefits that affect the decision to lead, and also to follow.

The costs and benefits of social transmission of migratory information can be divided into two categories, those associated with being in a group, and those associated with the teaching or learning of behaviours. Benefits of being in a group include increased predator detection and social thermoregulation, while costs include increased conspicuousness and competition for resources. Because these costs and benefits are related to being in a group they should be shared among group members (e.g. among leaders and followers), although not necessarily equally. It may be that for solitary-roosting hoary bats, the costs of travelling in a group outweigh any benefits and they become solitary again during migration, thus precluding social transmission of migratory behaviours.

If the social transmission of migratory information is purposeful (i.e. migratory behaviours are taught), then it is likely that the costs are higher for the leaders/demonstrators than for the followers/observers. In fact, one of the main characteristics of teaching behaviour is that the demonstrator incurs a cost, or at least not an immediate benefit, from altering their behaviour in the presence of a naïve observer (Caro and Hauser 1992). Costs may be measured in time, energy, and mortality (Alerstam et al. 2003). We know virtually nothing about the importance of migratory timing to bats, but if timing is important and travelling in a group with naïve individuals slows the experienced individuals down, then experienced individuals may forego social transmission. Diets of adult and juvenile bats frequently differ (Rolseth et al. 1994, Adams 1996, Hamilton and Barclay 1998), as seen in the bats used in the present study (Reimer et al. 2010). If the dietary differences result in longer foraging times in juveniles than adults, and this slows migratory movement, this delay may be overly costly for adults.

We also know little about the mating behaviour of hoary bats, but as in other temperate-zone bats, they likely mate in the autumn during migration (Shump and Shump 1982b, Cryan et

al. 2012a). Mating may occur along migrations routes, potentially with males intercepting females at lekking sites (Cryan 2008), although there is limited evidence to support this. Juveniles of both species may be ready to mate during their first migration, but there is no evidence they successfully do so (Cryan et al. 2012a). Regardless, if migrating with juveniles negatively affects mating opportunities for the mothers, perhaps by affecting timing, they may forego social transmission.

Bats may forego extended stopovers for refuelling, as seen in some silver-haired bats (McGuire et al. 2012), and employ a "fly-and- forage strategy", foraging periodically during migration flights rather than foraging solely at emergence in the evening, as seen in Nathusius′ pipistrelle (*Pipistrellus nathusii*; Šuba et al. 2012). If attention is required to maintain contact with group members and if this involves a trade-off between attention and foraging, the costs of attention may be too high, thus favouring solitary migration. It may be particularly challenging to maintain contact among small-bodied individuals travelling through the vast aerosphere, especially at night. Although bats may use echolocation (Jones and Siemers 2011, Bohn and Smotherman 2015) and passerines may use flight calls, to communicate with group members (Hamilton 1962, Farnsworth 2005), the range of these vocalizations may be insufficient to ensure group cohesion. Perhaps this constraint partially explains why migration in the majority of nocturnally migrating songbirds is under endogenous control rather than learned through social transmission (Berthold 2001, Pulido 2007).

If migratory behaviours in bats are the result of a genetic program rather than social transmission, how do individuals find their way? The literature on orientation and navigation systems of animals during migration is vast and rich, particularly for birds (e.g. Griffin 1952, Able 2001, Wiltschko and Wiltschko 2003, 2009). Bats are capable of perceiving stars (Childs

and Buchler 1981), and using post-sunset glow (Buchler and Childs 1982, Holland et al. 2010), the Earth's magnetic field (Holland et al. 2006, Holland et al. 2008, Holland et al. 2010), and geographical landmarks and linear features (Barclay 1984, Timm 1989, Ahlen 1997, Serra-Cobo et al. 2000, Racey and Entwistle 2003, Johnson et al. 2004, Lausen 2006) for orientation and the creation of large-scale navigational maps (Tsoar et al. 2011). Although bats are highly specialized for echolocation, it seems unlikely that they use echolocation to navigate long distances (Griffin 1970). Bats and other echolocating animals, such as porpoises (Verfuß et al. 2005), use echolocation for spatial orientation at small/local scales (Schnitzler et al. 2003) and not for long-distance movements.

I had hypothesized that the benefits of social transmission, such as increased flexibility and decreased use of sub-optimal routes (Sutherland 1998), would outweigh any costs associated with travelling in a group or teaching, but my findings suggest otherwise. This leads to many interesting questions and areas for further research. How do bats, particularly juvenile bats, find their way to their over-wintering grounds? Do bats travel in groups of unrelated individuals, and if so, how do they maintain cohesion? Is learning more likely in the nomadic migrations of nectarivores such as greater and lesser long-nosed bats (*Leptonycteris nivalis* and *L. curasoae*, respectively) that follow the flowering of plants? What is the mortality rate of juvenile bats during their first migration? How do these mortality rates differ with social structure, roosting ecology, and migration distance? In short, much more work is needed on the use and relative importance of social transmission and endogenous programs to bats before we can begin to understand their migration biology.

Table 4-1 Molecular diversity indices of 19 hoary bat (*Lasiurus cinereus*) microsatellite loci from 133 individuals. Frequency of null alleles is based on Van Oosterhout et al (2004) and values in bold are loci that contain null alleles.

Loci	Number of	Number of genotypes	Observed heterozygosity	Expected heterozygosity	Frequency of null

	alleles				alleles
Laci1	65	130	0.762	0.972	**0.107**
Laci15	11	130	0.831	0.826	-0.007
Laci20	9	131	0.779	0.771	-0.006
Laci22	19	131	0.901	0.865	-0.024
Laci25	10	132	0.273	0.786	**0.313**
Laci26	28	130	0.938	0.910	-0.018
Laci28	3	132	0.015	0.015	**0.109**
Laci29	19	129	0.674	0.867	**0.109**
Laci31	18	132	0.879	0.887	0.002
Laci33	23	132	0.856	0.884	0.014
Laci34	23	131	0.863	0.934	**0.037**
Laci35	66	132	0.894	0.977	0.041
Laci36	21	132	0.894	0.882	-0.008
Laci38	39	131	0.534	0.946	**0.217**
Laci39	27	132	0.856	0.872	0.005
Laci41	33	131	0.771	0.941	**0.088**
Laci42	12	131	0.634	0.835	**0.119**
Laci43	17	132	0.689	0.878	**0.105**
Laci48	24	132	0.750	0.868	**0.066**

Table 4-2 Molecular diversity indices of 18 silver-haired bat (*Lasionycteris noctivagans*) microsatellite loci. Frequency of null alleles is based on Van Oosterhout et al (2004) and values in bold are loci that contain null alleles.

Alberta Summerview					

(n = 87)					
Loci	Number of alleles	Number of genotypes	Observed heterozygosity	Expected heterozygosity	Frequency of null alleles
Lano1	7	87	0.644	0.709	0.05
Lano3	8	87	0.862	0.796	-0.05
Lano4	11	86	0.488	0.843	**0.21**
Lano8	13	85	0.800	0.858	0.03
Lano10	17	87	0.655	0.867	**0.12**
Lano13	14	87	0.828	0.880	0.03
Lano15	10	84	0.750	0.767	0.01
Lano22	18	86	0.907	0.869	-0.03
Lano26	12	87	0.345	0.829	**0.29**
Lano28	6	86	0.674	0.671	-0.01
Lano29	12	87	0.816	0.862	0.03
Lano31	14	85	0.471	0.850	**0.22**
Lano32	12	86	0.547	0.840	**0.17**
Lano34	13	87	0.701	0.857	**0.09**
Lano35	20	87	0.782	0.910	0.07
Lano40	17	87	0.805	0.817	0.00
Lano41	10	83	0.386	0.813	**0.25**
Lano44	19	86	0.535	0.917	**0.21**

Table 4-3 Contingency table based on relatedness categories of hoary bats (*Lasiurus cinereus*) as determined by COLONY ($\chi^2 = 0.07$, $p = 0.80$).

	Related dyads	Unrelated dyads
Dyads killed on the same night	8	457

Dyads killed on different nights	75	3,888

Table 4-4 Contingency table based on relatedness values of hoary bats (*Lasiurus cinereus*) as determined by COANCESTRY ($\chi^2 = 0.07$, $p = 0.80$).

	Related dyads	Unrelated dyads
Dyads killed on the same night	7	458
Dyads killed on different nights	45	3,918

Table 4-5 Results of the generalized linear model that assessed the influence of relatedness and geographic origin (as determined by stable isotope values) of hoary bats (*Lasiurus cinereus*) on the number of days between fatalities of dyad members. Values in bold are significant at $\alpha = 0.05$.

Parameter	Estimate	SE	χ^2	p-value

Intercept	2.157	0.049	1606.88	< **0.001**
ML relatedness	-0.202	0.399	0.26	0.61
Difference in $\delta^{13}C$	0.046	0.019	5.72	**0.02**
Difference in $\delta^{15}N$	-0.045	0.024	3.42	0.06
Difference in $\delta^{2}H$	-0.003	0.001	7.75	**0.001**
ML relatedness X difference in $\delta^{2}H$	-0.042	0.019	4.92	**0.03**

Table 4-6 Contingency table of relatedness categories of dyads of silver-haired bats (*Lasionycteris noctivagans*) as determined by COLONY ($\chi^2 = 3.89$, $p = 0.049$).

	Related dyads	Unrelated dyads
Dyads killed on the	9	147

same night		
Dyads killed on different nights	49	1644

Table 4-7 Contingency table of relatedness values of dyads of silver-haired bats (*Lasionycteris noctivagans*) as determined by COANCESTRY ($\chi^2 = 0.10$, $p = 0.76$).

	Related dyads	Unrelated dyads
Dyads killed on the same night	6	150
Dyads killed on different nights	74	1619

Table 4-8 Results of the generalized linear model that assessed the influence of relatedness and geographic origin (as determined by stable isotope values) of silver-haired bats (*Lasionycteris noctivagans*) on the number of days between fatalities of dyad members. Values in bold are significant at $\alpha = 0.05$.

Parameter	Estimate	SE	χ^2	p-value
Intercept	2.409	0.073	897.79	< **0.001**
ML relatedness	-0.641	0.504	1.66	0.20
Difference in $\delta^{13}C$	-0.019	0.035	0.29	0.59
Difference in $\delta^{15}N$	0.106	0.024	18.91	<**0.001**
Difference in $\delta^{2}H$	-0.002	0.002	1.65	0.20

GENERAL SYNTHESIS

My PhD research focused on the basic biology of bat migration and the implications for conservation of migratory bats. The issue of bat fatalities at wind energy facilities has made this research highly relevant and timely. I had two main goals for my research, to a) gain a better understanding of the migratory patterns and behaviours of bats, and b) gain a better understanding of the impacts of wind energy installations on migratory bats. Given that, I will first synthesize what I have learned about the basic biology of bat migration, and then discuss the consequences of bat fatalities at wind energy facilities. Finally, I will conclude with a series of recommendations to reduce bat fatalities at wind energy installations.

I found no evidence that migratory behaviours and routes are transmitted socially, at least not among kin (Chapter 4). However, I cannot rule out the possibility that bats learn components of migration from non-related conspecifics. If information about migration is not transmitted socially, then migration in bats may be governed by endogenous genetic programs. To test this, future studies should include behavioural components such as a comparison of the departure dates of mothers and offspring, and departure orientation after sensory-manipulation or translocation/displacement. Sensory-manipulation experiments could include the altering of magnetic receptors of bats via a Helmholtz coil (Holland et al. 2006, Holland et al. 2008, Holland et al. 2010) or altering their perception of post-sunset glow via polarized glass (e.g. Greif et al. 2014) prior to release. Translocation or displacement experiments test the navigation ability of animals after moving them to an unfamiliar area. They can cover relatively short distances (i.e. ≤100 km; e.g. Keiser et al. 2005, Luschi et al. 2007, Tsoar et al. 2011) or extremely long-distances (i.e. ≥1000 km; e.g. Thorup et al. 2007, van Toor et al. 2013).

Despite the fact that bats do not appear to learn migration routes from kin, my stable isotope and population genetic analyses (Chapters 2 and 3), in conjunction with previous studies using acoustic and fatality monitoring (Baerwald and Barclay 2009), suggest that bats migrate using discrete migration routes rather than migrating in broad fronts. Migratory routes may be associated with the availability and distribution of appropriate stopover and roosting sites, and geographical landmarks (e.g. rivers and foothills or mountains). My stable isotope data also suggest that the summer range of silver-haired bats extends farther north, into the boreal forest, than the range of hoary bats does, which appears to be predominantly in the aspen parkland (Chapter 2). To refine our understanding of the ranges and migratory routes used by bats, future studies using stable isotopes could include an analysis of the stable isotopes of strontium ($^{87}Sr/^{86}Sr$), which have recently been shown to reflect the longitude of where animal tissues have been grown (Sellick et al. 2009, Flockhart et al. 2015).

The stable isotope analysis also revealed that the timing of migration may be governed by habitat cues on the summer grounds (Chapters 2 and 4). In multiple analyses, time of arrival at the wind energy facility was correlated with the stable isotopes of carbon and/or nitrogen, both of which are indicators of habitat type and quality (Chapter 2). This suggests that bats depart for their southward migration based on local habitat cues, such as changing prey availability and/or quality, rather than departing based on latitudinal-based cues, such as changing day length.

Bats commonly exhibit sexual segregation on their summering grounds as well as sex-biased migratory behaviour (Cryan 2003, Fleming and Eby 2003, Ibanez et al. 2009), but the stable isotope values of males and females in my study did not differ. Thus there is no evidence for sexual segregation of either hoary bats or silver-haired bats (Chapter 2). The genetic data, however, provided evidence for male-biased dispersal of silver-haired bats (Chapter 3). I found

a greater degree of genetic structuring in the maternally inherited mtDNA than in the biparentally inherited microsatellite markers of silver-haired bats. This pattern can be explained by sex-biased dispersal of males, and route or roost philopatry by females (Petit and Mayer 1999, Petit et al. 2001, Kerth et al. 2002).

My population genetics analyses revealed structure in the population of silver-haired bats and hoary bats across Canada (Chapter 2). Multiple analyses of both mtDNA and microsatellites separated the silver-haired bats collected in Ontario from those collected in the other provinces. For hoary bats, both mtDNA and microsatellite analyses suggested that the bats collected in Alberta differed from those collected in Manitoba and Ontario. Further studies of the landscape and population genetics of both hoary bats and silver-haired bats should be conducted, ideally with more thorough coverage across their ranges and potential migration routes. Expanded studies could help delineate potential sub-populations and migration routes, which would be useful for understanding and mitigating the effects of wind energy on migratory bats. For example, knowledge of the movements of migratory bats in Saskatchewan is lacking and I had no DNA samples from the province, which would have contributed greatly to our understanding of migratory movements of bats.

My study has provided further evidence for the use of migration routes by bats and previous work has shown that fatalities of bats at wind turbines increase as bat activity (as measured by acoustics) increases (Baerwald and Barclay 2009, Baerwald and Barclay 2011), therefore it is highly likely that bat fatalities are highest along migration routes. Bats killed along these migration routes appear to come from large catchment areas (Chapter 2), thus fatalities at a single facility can have far-reaching consequences. Given this, it becomes increasingly important to consider cumulative effects of wind energy installations built along a migration route.

The genetic structuring I found (Chapter 3) has implications for conservation genetics. Neither genetic diversity nor bat fatalities are distributed evenly across the landscape (Chapter 3, Baerwald and Barclay 2009, Arnett and Baerwald 2013). If fatality rates are higher for one group, then the loss of genetic diversity will also be greater for that group and this could be particularly deleterious for groups with lower genetic diversity. If conserving genetic diversity is important, then this disparity should be considered in species' management plans. Loss of genetic diversity may have consequences for the migration of bats because migration may be governed by endogenous genetic programs (Chapter 4). If migratory routes are based on a genetically-based clock-and-compass model, as in many first-year passerines (Gwinner and Wiltschko 1978, 1980), then losing genetic diversity could decrease the diversity of those programs. Because culture changes more quickly than genes, animals that rely on genetic programs for migration are less adaptable than those that rely on social transmission (Sutherland 1998); bats may thus not be able to rapidly alter their migratory routes to avoid new threats that appear as the landscape changes.

Based on my findings, I recommend the following to reduce the impact of wind energy installations on migratory bats:

1) Given that bats appear to use discrete migration routes, and that fatality rates at wind energy facilities appear to be highest along migration routes, the delineation and conservation of migratory routes should be a priority in bat conservation plans.
2) Given that bats in North America are killed at wind energy facilities placed along migration routes, it is essential that cumulative effects of bat fatalities be considered. It is no longer acceptable to assess the risk of a wind energy site in isolation. The

number of facilities along a route and the cumulative fatality rates of bats at these facilities need to be considered.

3) Given that bats killed in one jurisdiction may originate in another, management efforts need to be coordinated among jurisdictions. For example, bats killed at wind energy facilities in Montana (e.g. at the Judith Gap Wind Farm in central Montana) may have originated in Saskatchewan and migrated through Alberta into Montana (as suggested in Baerwald and Barclay 2009). Thus, conservation of these bats would require coordination among two provinces and two countries.
4) Given that there appears to be population genetic structure of bats across Canada, this should be considered in management plans. It may be necessary to conduct further research to refine this structure and use this information to determine if multiple management units (see Chapter 3) should be created for migratory bats across Canada.
5) Given that information on the basic biology of migratory bats and bat migration can be used to inform conservation strategies, future research should combine basic research with applied studies.

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Appendix A: Development of novel polymorphic microsatellite markers for two bat species affected by wind turbines, hoary bats (*Lasiurus cinereus*) and silver-haired bats (*Lasionycteris noctivagans*)

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Lasiurus cinereus (hoary bats) and *Lasionycteris noctivagans* (silver-haired bats) are migratory tree-roosting bats that together make-up 57% of all bats killed by wind turbines across North America (Arnett and Baerwald 2013). Fatality rates of bats differ greatly among wind energy facilities and we suspect that sites with high fatality rates may be built along bat migration routes. However, little is currently known about migration patterns and routes of bats. Furthermore, the degree of population structuring among routes and how fatalities at wind turbines will affect this structure is also unknown. Because these two species are being affected by wind turbines, data on the level of gene flow among migration routes and their overall population structure and size are urgently required to help managers determine management units. Here we describe a set of microsatellite markers that will be useful in the investigation of the genetic population structure of both *L. cinereus* and *L. noctivagans*.

Total DNA was extracted from one individual *Lasionycteris noctivagans* and one *Lasiurus cinereus,* using the Qiagen DNEasy Tissue extraction protocol. We tested forty-eight primer pairs for amplification and polymorphism using methods described in O'Bryhim et al. (2013). Touchdown thermal cycling programs (Don et al. 1991) encompassing a 10°C span of annealing temperatures ranging between 65-55°C (TD65) were used for all loci. We assessed the variability of the 18 polymorphic loci in 31 specimens of *L. noctivagans*, and the 20 polymorphic loci in 31 *L. cinereus*. Conditions and characteristics of the loci are provided in Tables 1 and 2. Tests for deviations from Hardy-Weinberg equilibrium (HWE) and for linkage disequilibrium were conducted using GENEPOP v 4.0 (Rousset 2008). After Bonferroni correction for multiple comparisons, 7 loci showed significant deviation from expectations under HWE for both species and no linkage disequilibrium was detected for 153 and 190 paired loci comparisons for *L. noctivagans* and *L. cinereus*, respectively.

These new loci will assist in examining population sizes and genetic population structure and diversity of two of the species most affected by wind turbines in North America. The loci will be used to address both basic biologic questions (e.g., learning of migration routes and movements among regions) and applied questions (e.g., population size estimation and the effect of fatalities related to wind turbines on the population structure).

Table A-1. Details for 18 polymorphic microsatellite loci developed for *Lasionycteris noctivagans* (silver-haired bats). The size indicates the range of observed alleles in base pairs and includes the length of the CAG tag; number of individuals genotyped is *N*; *k* is number of alleles observed; H_o and H_e are observed and expected heterozygosity, respectively; PI is the probability of identity for each locus, and TD refers to the touchdown protocol used for pcr (see text).

Locus	**Primer Sequence 5' --> 3'**	**Repeat motif**	**Size (bp)**	**N**	***K***	**H_o**	**H_e**	**PI**	**TD**
Lano1	F: *GGAAACAAGCTTAACATTTCAGG R: AAAGACATGAAGAAGCCCTGC	AAAG	178-194	30	5	0.700	0.709	0.13	TD65
Lano3	F:*TCATATATAGGAGTATGTCTCTCGTGG R: AATGAGCATGCTGGTTGGC	ATCT	237-265	30	7	0.833	0.774	0.085	TD65
Lano4†	F: *GCTTTGGACAGTTTCACGGG R: ACCAGATGGGAGCTCCTGC	ATCT	283-319	29	10	0.379	0.825	0.052	TD65
Lano8	F: *TCCATCTACCATCTACCTGCC R: ATGGGTGGGTGTATGAAAGG	ATGG	242-290	29	11	0.793	0.852	0.039	TD65
Lano10†	F: *GTGCAGCCATCTTGTAACGG R:CAGCCCTAGCTGGTTTGACC	ATCT	300-356	30	14	0.467	0.896	0.02	TD65
Lano13	F: *AACTGATCATCTGGTCACTTAGGG R: CCACTCTATGTACCCTCATAGCTGC	ATCT	271-395	30	14	0.900	0.887	0.023	TD65

Lano15	F: *GGGACAGCACATCCCTCC R: CCCTAAGCACCACTGTCAGG	ATCT	196-308	27	8	0.741	0.733	0.11	TD65
Lano22	F: *TGGTATCCTGTCTCCTGGTCG R: GATGCACCAGAGCCTCCC	ATCT	233-306	29	13	0.828	0.834	0.046	TD65
Lano26†	F: *CCACACATCTACCCACTCATCC R: GGCTCTTGGCTTACAGACTGG	ATGG	177-217	30	10	0.367	0.796	0.069	TD65
Lano28	F: *ATGCAAGTACGCAGTGACCC R: GGGTGCATATATTGTTTCTGACAGG	AAAG	208-224	29	5	0.621	0.683	0.15	TD65
Lano29	F: *TGTAACCCTTGCAAGAAAGATGC R: GGTCAAGGTCAACAGCCAGC	ATGG	200-240	30	11	0.733	0.852	0.039	TD65
Lano31†	F: *ATTGGTTACCATTGCTGGC R: TCCCTCTCTTTCTCACAGGG	ATCT	195-235	28	11	0.464	0.854	0.037	TD65
Lano32†	F: *ACCCACATTTCTGAGTGACATGG R: TCGAGCTTGACAAAGCCTACC	ATCT	218-262	29	10	0.414	0.800	0.066	TD65
Lano34	F: *GAGAGGCAGCACGGAAAGG R: TGAATTTGTTGTTACTGATGTGAAGG	AAAG	195-235	30	11	0.733	0.852	0.039	TD65
Lano35	F: *ATCAGTCGGGCATCAAGAGG R: TGAGAACCAGAGAGCCTGGG	AAAG	215-262	30	17	0.800	0.891	0.021	TD65

Lano40	F: *CCTTGAAACATTCATTTCTGGG R: TGTCTTAGTTTGTGCCTTCCG	AAAG	223-255	30	13	0.900	0.831	0.047	TD65
Lano41†	F: *ATCTGTCCAGTGTTGCTCCC R: TGTGGTGCATTTCCATCC	AAAG	146-190	30	6	0.267	0.794	0.074	TD65
Lano44†	F: *TCACAGTCAGATACCACTATGTACCC R: TTGTAGTATGTCTCGATACTGCTTTCC	TTCC	284-334	29	14	0.621	0.891	0.021	TD65

* indicates CAG tag (5’- CAGTCGGGCGTCATCA-3’) label;

† indicates significant deviations from Hardy-Weinberg expectations after Bonferroni corrections.

Table A-2 Details for 20 polymorphic microsatellite loci developed for *Lasiurus cinereus.* The size indicates the range of observed alleles in base pairs and includes the length of the CAG tag; number of individuals genotyped is *N*; *k* is number of alleles observed; H_o and H_e are observed and expected heterozygosity, respectively; PI is the probability of identity for each locus, and TD refers to the touchdown protocol used for pcr (see text).

Locus	**Primer Sequence 5' --> 3'**	**Repeat motif**	**Size (bp)**	**N**	***K***	**H_o**	**H_e**	**PI**	**TD**
Laci 1†	F: *AGGGCTCAGTAAGCACCAGC R: AAGCTAGCAGGAGCCAGAGC	TTCC	205-309	29	32	0.724	0.944	0.0058	TD65
Laci 15	F: TGAACAATCAGTAGGTATGTAAACTGC R: CCATATGAAATTGCTGACAGTGC	ATCT	338-366	30	8	0.900	0.828	0.052	TD65
Laci 20	F: *GAGTGTTCCAATTAGAAGATAAGGC R: CTCACCTGCTTCACAATCCC	ATCT	217-249	31	8	0.774	0.766	0.089	TD65
Laci 22	F: *CGCAGAAGGTAGCCAAGTAGG R: GAAGGGCTATTCTCCATTCCC	AAAG	161-217	31	12	0.839	0.844	0.042	TD65
Laci 25†	F: *TTGCAGATGGCAGATCTTGG R:AAATATATGAGAGCAAGTGTATTAGCC	ATCT	204-224	31	6	0.290	0.683	0.14	TD65
Laci 26	F: *CTTGACATAGACTCATGTTTCACTCC R: GCACACATAGAGTCCGATGAGG	TTCC	271-321	30	18	0.933	0.894	0.02	TD65

Laci 28†	F: *TTCTGTTATTTGTCCTGTGAATTGC R: GTCCGGGTCTGTGAGAGGG	ATATCT	294-342	30	10	0.733	0.859	0.036	TD65
Laci 29†	F: *GTGTGCCCTTGAGGAACAGC R: GTCTCTGTGAGCAGAGGAGGG	ATCT	197-251	31	12	0.742	0.883	0.025	TD65
Laci 31	F: *GAGCATATGTCAGGAAGGAGG R: TATGCTCTTGAGGAGCTTGC	AAGAG	309-387	31	13	0.774	0.872	0.029	TD65
Laci 33	F: *TGACTAGGGCTGGTTGAGC R:CAGAATATATTTGTGTACAGAACTGGG	AATAAG	279-379	31	17	0.871	0.895	0.02	TD65
Laci 34	F: *GGCTTTCTAACCCTGGGACC R: TCAGGATTCTTTAGAGAAACAGAAGC	ATCT	259-299	31	13	0.935	0.894	0.02	TD65
Laci 35	F: *CAGTTGATTGAACTTCCAAGGG R: TCATCAGAAGTCCTGGTTGGG	ATATCT	211-337	31	31	0.968	0.949	0.0049	TD65
Laci 36	F: *CCTTTCTCTGTCCCTCAGTGC R: TCCACCTCCCACCATTTCC	AAAG	175-230	31	13	0.871	0.853	0.038	TD65
Laci 37†	F: *CACGACTTCCTGGTACTTCTCG R: TCTCCTGTTCTTTGCATCCTAGC	TTCC	244-349	27	18	0.222	0.911	0.015	TD65
Laci 38†	F: *TCACCTAATATGCAGCTCTGGG R: TGAAAGAGTAACATACATCCGACCC	AAAG	266-342	30	22	0.533	0.923	0.011	TD65

Laci 39	F: *GCTCTTGTTGATGTTTCTCTTTCTCC R: TGATGTTTCAATTGCATGATTCC	AAAG	283-347	31	14	0.935	0.866	0.03	TD65
Laci 41	F: *CCAATACAGGCAGCATTAGCC R: TGCAGTTGGTAATTACTGGAGGG	AAAG	197-321	30	17	0.767	0.885	0.023	TD65
Laci 42†	F: *TGTTTACCATGTCATCCAGGG R: TGCATGTGGTATCCCTCCC	TTCC	182-234	31	10	0.516	0.823	0.054	TD65
Laci 43	F: *GACCGCTTTGTAAAGTATGTGGG R: TGCCTGTGGCCTTTATGACC	TTCC	172-224	31	10	0.645	0.850	0.04	TD65
Laci 48	F: *TTCCACAAATGCTGAAAGGG R: TTTCTCTGCGATGGAAGAACC	AAAT	177-217	31	12	0.645	0.843	0.041	TD65

* indicates CAG tag (5’- CAGTCGGGCGTCATCA-3’) label;

† indicates significant deviations from Hardy-Weinberg expectations after Bonferroni corrections.

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