MIGRATORY CONNECTIVITY AND WINTERING HABITAT STRUCTURE OF LOGGERHEAD SHRIKES: INFERENCES FROM STABLE HYDROGEN ISOTOPE AND MICROSATELLITE DNA ANALYSES

A Thesis
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By

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ABSTRACT

The linking of breeding and nonbreeding grounds of migratory birds is of great conservation and theoretical importance. In theory, connecting these geographically disparate areas allows for a more complete understanding of annual events, and a first step into identifying where in the annual cycle limitations to fitness may be occurring. The Loggerhead Shrike (*Lanius ludovicianus*) is a Species at Risk in Canada, and its decline is attributed to habitat loss on both the breeding and wintering grounds. In the fall, Loggerhead Shrikes from breeding areas of prairie Canada (*L. l. excubitorides*), the focus of this study, move south along the Gulf States of the United States and Mexico to wintering areas that are already occupied year-round by resident shrikes. However, the habitat structure and variability for both migrants and resident Loggerhead Shrikes have not been studied well on the wintering grounds. Thus, it has been difficult to adequately evaluate the relative contribution of changes in wintering areas to the decline of Loggerhead Shrike populations.

To identify the wintering grounds of Loggerhead Shrikes that breed in prairie Canada, I used stable hydrogen isotope (δD) analysis of feathers and claws and microsatellite DNA from feathers. In North America, δD measurements from feathers provide information on latitude of origin, while DNA can be related to an affiliation to a breeding population. Since several authors suggested that Loggerhead Shrike completed their preformative and formative molt on the breeding grounds prior to fall migrations, I reasoned that δD analysis of tertial feathers sampled on the wintering grounds would correspond to δD in precipitation from the breeding grounds. Similarly, I used DNA microsatellite markers and Bayesian clustering analysis to detect patterns of population

genetic structure within the range of Western Loggerhead Shrikes (*L. l. excubitorides*) in Canada and consequently use these to infer breeding origin of shrikes sampled in Mexico. I based the categorization of resident and migrant Loggerhead Shrikes sampled in Mexico on δD analysis of feathers.

Based on evaluation of observed and expected δD values of feathers, I determined that migrant shrikes used northeastern (63.8%) and south-central (73.7%) Mexico to winter. Microsatellite DNA and assignment tests, suggested that wintering migrant shrikes occupied north-central (18.6%) and northeastern (20.3%) Mexico. Differential habitat occupancy analyses, suggested that, in northeastern Mexico, wintering sites occupied by Loggerhead Shrike sites were structurally different from random unoccupied sites (MRPP, T = -8.04, $P < 0.001$, n = 354). An important difference was that, on average, occupied habitat contained shorter tall shrubs and huisache and fewer tall shrubs, mesquite and huisache. Similarly, residents shrikes occupied structurally different habitats (MRPP, T = -2.95, $P = 0.01$, n = 146) that had less percent cover of bare ground than those sites occupied by migrants. Based on these habitat results, I surmise that habitat availability may be a limiting factor for both resident and migratory shrike populations in northeastern Mexico.

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CHAPTER 1: GENERAL INTRODUCTION

1.1. INTRODUCTION

The Loggerhead Shrike (*Lanius ludovicianus*), once a plentiful species in the natural grasslands of North America, has been declining for the last 40 years (Sauer et al. 2005). Breeding Bird Survey (BBS) data from across North America (1966-2004), indicate an annual average rate of decline of 3.8 % (Sauer et al. 2005), while Christmas Bird Count (CBC) data (1959-1988) indicate an average decline of 1.7% per year (Sauer et al. 1996). In the United States, the species is listed as Endangered, Threatened or Concern in at least 26 states (Pruitt 2000), and in Canada, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) designated the eastern subspecies (*L. l. migrans*) as Endangered in 1991 (Cadman 1990) and the Prairie Canada subspecies (*L. l. excubitorides*) as Threatened in 1986 (Cadman 1985). Pooled BBS data from Alberta, Saskatchewan and Manitoba show a mean annual decline of 4.5% since 1968 (COSEWIC 2004).

For nearly three decades, North American researchers have investigated potential limiting factors affecting shrikes on the breeding grounds, and these include: increases in car collisions (Flickinger 1995), pesticides (Busbee 1977, Anderson and Duzan 1978, Morrison 1979), habitat change (Prescott and Collister 1993, Bjorgen and Prescott 1996), climate change (Cadman 1985), and predation (Slack 1975, Morrison 1980). However, none of these, even in combination, fully account for the rate of population decline observed across the continent (Kridelbaugh 1983, Cade and Woods 1996, Cadman 1985, 1990). It is now evident that the understanding of the ecology and biology of migratory

birds require the investigation of the extent to which individuals from the same breeding area migrate to the same nonbreeding area and vice versa.

1.1.1. IMPORTANCE OF CREATING MIGRATORY CONNECTIVITY

On average, North American long-distance migratory birds spend two to three months on the breeding grounds, six to seven months on the wintering grounds, and another two to three months *en route* (Norris et al. 2004). Migratory connectivity is the term used to describe links between breeding and nonbreeding areas (including wintering and stopover sites, Webster et al. 2002), while migratory pattern is the term used to describe spatial arrangement of populations against adjacent ones in a latitudinal sequence (Fig. 1.1). Migratory connectivity and patterns are unraveled by tracking the movements of individuals and populations as they move across large geographical scales from breeding to overwintering regions. This is important, since it is now well recognized that linking populations is critical in conservation because events occurring in these separate periods can ultimately influence lifetime fitness of individuals (Marra et al. 1998, Gill et al. 2001, Norris 2005).

Historically, migratory movements were first tracked by physically painting or tagging (e.g., neck collars, leg bands) the animal (Berhold 2000). For decades, bird banding and radio and satellite telemetry have been used to link breeding and nonbreeding grounds, but their effectiveness, in this regard, has shown insufficient, especially for small passerines (Hobson 2003). Banding is a useful mean of monitoring population trends (reviewed by Dunn and Hussell 1995) but the extremely low recovery rates, usually < 1% for non-game birds, provide little possibility for meaningful analysis

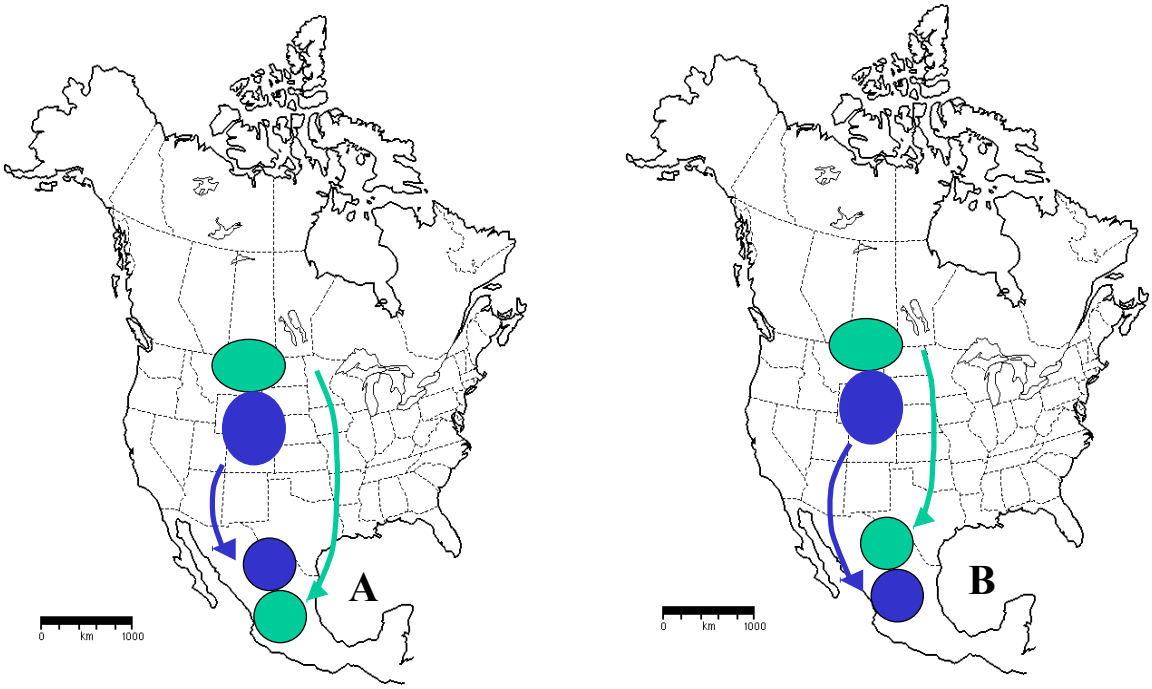

Figure 1.1. Leapfrog migration (A) occurs when the latitudinal orientation on the wintering grounds is reversed of that on the breeding grounds. That is, individuals from the northernmost parts of their breeding range winter at the southernmost parts of their wintering areas, while individuals from the southernmost parts of their breeding range winter at the northernmost portions of their wintering sites. In contrast, chain migration (B) occurs when northern and southern breeding populations maintain the same latitudinal orientation on their breeding and wintering grounds. As a result, northern breeding population winter further north than southern breeding populations (Smith et al. 2003).

(Hobson 2003). Alternatively, satellite transmitters have proved useful in tracking the movements of large-bodied birds, but they have not yet been developed to be applied to small passerines (Hobson 1999, Hobson 2003) and their financial cost limit their use, reducing sample size in studies (Hobson 1999). Ultimately, extrinsically marking passerines has not been effective to create migratory connectivity (Webster et al. 2002, Hobson 2003).The lack of reliable data for connecting breeding and wintering grounds has been both a major obstacle in understanding the causes of species declines but also the force behind inspiring the search for more sophisticated technologies involving intrinsic markers.

Intrinsic markers can be separated into two categories; those that are acquired (e.g., biogeochemical markers) and those that are inherited (e.g., molecular genetic markers, Boulet 2004). In North America, acquired intrinsic markers, such as stable hydrogen isotope (δD) values in feathers and other tissues can provide latitudinal information relevant to population delineations (Clegg et al. 2003, Mazerolle and Hobson 2005a), while inherited markers, such as morphological, behavioural, and genetic identity, can provide latitudinal and longitudinal layers of resolution depending on the distribution of populations. In combination, these two technologies are currently offering the greatest promise in creating connectivity as they provide more layers to improve resolution when delineating populations.

1.1.2. ACQUIRED STABLE ISOTOPE MARKERS

Applications of stable isotope analysis to avian ecological studies have increased exponentially in the last decade (Hobson 2005b). The application of stable isotopes of various elements (e.g., H, C, O, N, and S) to examine interactions between wildlife and

the environment started with the realization that in nature there is an isotopic relationship (shaped by various biogeochemical interactions) between tissues (animal or plant) and the environment where the tissue was grown (DeNiro and Epstein 1978). Natural abundance isotopic measurements can be used to associate single organisms to ecosystems (Lajtha and Michener 1994). The recent breakthrough in tracking movements of migratory birds in North America came with the realization that latitudinal continent-wide patterns of the abundance of deuterium (δD) in rainfall were transferred through foodwebs to consumers making it possible to track movements across latitudinal gradients (Hobson and Wassenaar 1997). Feathers are metabolically inert following formation and so lock in isotopic values typical of that source area. As a result, the moulting origin (i.e., location of replacement of feathers) of birds can be positioned on the continent based on knowledge of the δD values of mean growing-season average precipitation (Hobson and Wassenaar 1997, Meehan et al. 2004) (Fig. 1.2).

1.1.3. INHERITED MOLECULAR MARKERS

An emerging application of genetic techniques in avian conservation is the documentation of the distribution of genetic variation in populations revealed by genetic markers to track movements of migratory birds throughout the annual cycle (Clegg et al. 2003; Double et al. 2005; Bell 1996; Gibbs et al. 2000; Jones et al. 2005; Kimura et al. 2002; Milot et al. 2000, reviewed in Smith et al. 2005). The genetic markers used to identify genetic structure for a species can be used as "genetic tags" to identify the breeding origins of individuals outside their breeding range and thus elucidate migratory connectivity. Currently, the primary genetic markers used to connect breeding and non-breeding areas include, allozymes by protein electrophoresis (Williams et al. 2005),

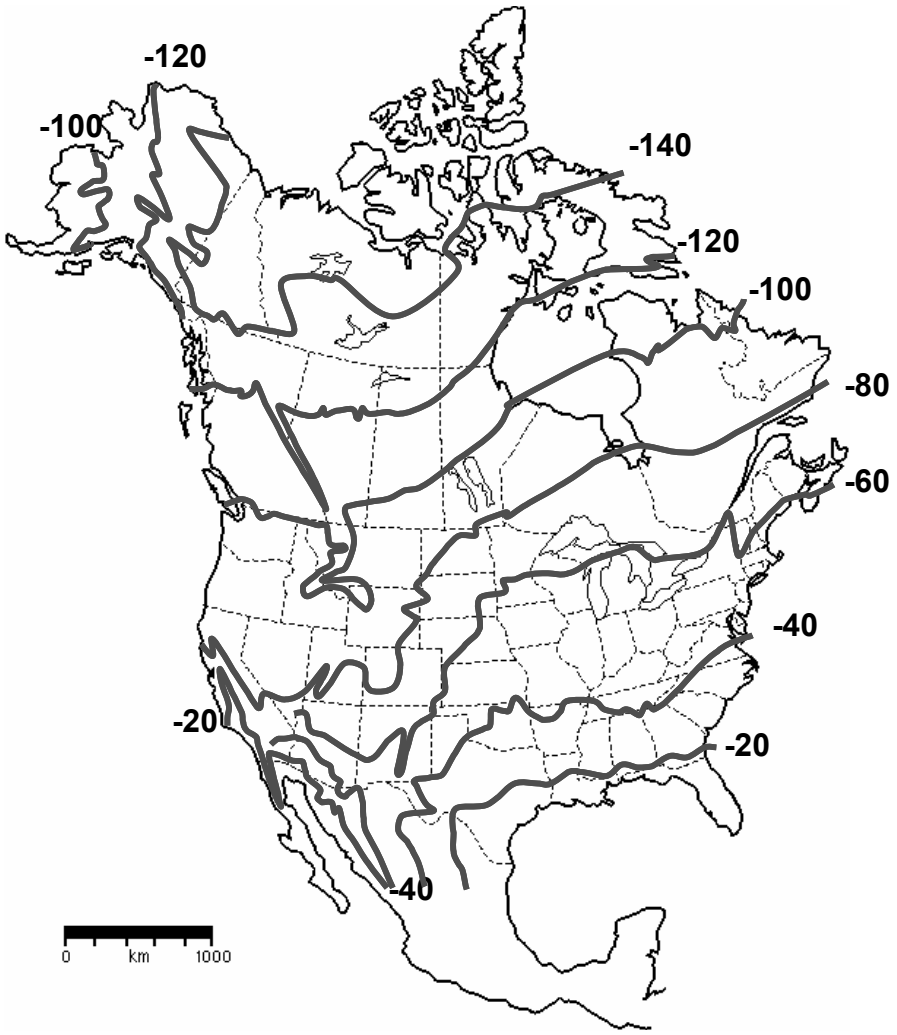

Figure 1.2. Altitude corrected growing-season δD (‰) contour lines of North American precipitation (Meehan et al. 2004). Contour lines are based on a comparison between precipitation from sampling stations across North America and an ocean water standard developed by the International Atomic Energy Agency (IAEA) and corrected for altitude using GIS-based model. In North America, naturally occurring δD becomes more deplete in an almost latitudinal direction.

mitochondrial DNA (mtDNA) (Wennerberg 2001), microsatellites (Clegg et al. 2003; Arguedas & Parker 2000; Jones et al. 2005), randomly amplified polymorphic DNA (RAPD) (Haig et al. 1997) and amplified fragment length polymorphisms (AFLP) (Bensch et al. 2002). However, to date, mitochondrial DNA haplotypes and nuclear microsatellites have become two of the most widely used genetic markers in migratory connectivity studies (Webster et al. 2002).

The inclusion of nuclear microsatellite markers in this study adds an extra layer of resolution to population delineation and provides a more complete picture of both sexes when used to identify population structure. Microsatellites are bi-parentally inherited tandem repeats of nuclear DNA that are 2-6 base pairs in length (e.g. ACACAC). They are assumed to be selectively neutral loci that typically have high mutation rates and thus high variability. They are favoured for their ability to distinguish among individuals with a very high level of certainty, especially when used in assignment tests and in combination with other markers (Webster et al. 2002).

1.1.4. IMPORTANCE OF STRENGTH OF CONNECTIVITY AND IDENTIFYING MIGRATORY PATTERNS

Migratory connectivity can either be weak or strong depending on the amount of mixing of individuals in either breeding or nonbreeding areas (Webster et al. 2002). Strong connectivity is when most individuals from a specific breeding area spend their winter together at a particular wintering location, and migratory connectivity is diffuse or weak when a particular breeding population spends their winter in separate wintering locations and vice versa (Webster et al. 2002). Linking these locations is relevant to conservation, because events and conditions in one season affect reproduction and/or

behaviour in another in a continuum (Webster et al. 2002, Webster and Marra 2005). The continuum of cause and affect interactions from biotic and abiotic events across an annual cycle is known as the Seasonal Interaction Hypothesis (reviewed by Webster and Marra 2005 and Hobson 2005b). For example, nourishment of habitats occupied in wintering and spring migration areas may influence body condition, migration rates and subsequent breeding success (Marra et al. 1998, Newton 2004, Webster and Marra 2005, Hobson 2005b). Henceforth, to fully understand the limitations, either isolated or compounded, affecting migratory species is crucial to connect all stages of the annual cycle in time and space. Furthermore, understanding seasonal interactions and migratory connectivity provides insights into degrees of species' adaptation, speciation and demographic changes (Webster and Marra 2005, Hobson 2005b).

1.2 METHODS

1.2.1 STUDY SPECIES

My research focuses on Loggerhead Shrikes (*Lanius ludovicianus*), one of two true shrike species in North America and the only lanid that occurs exclusively in the New World. The distribution of the Loggerhead Shrike presently extends from coast to coast in North America and from southern Canada to the Isthmus of Tehuantepec in Mexico (Fig. 1.3). The loggerhead shrike is generally recognized as 11 subspecies in North America; and in two of them, their breeding range extends into southern Canada; *L. l. migrans* primarily in eastern Canada, and *L. l. excubitorides* in the southern regions of Alberta, Saskatchewan and Manitoba. (Miller 1931) (Fig. 1.4). Loggerhead Shrikes are predominantly associated with open grassland and agricultural landscape throughout their annual cycle, but also occur in semi-deserts, scrubland, wooded savannas and orchards.

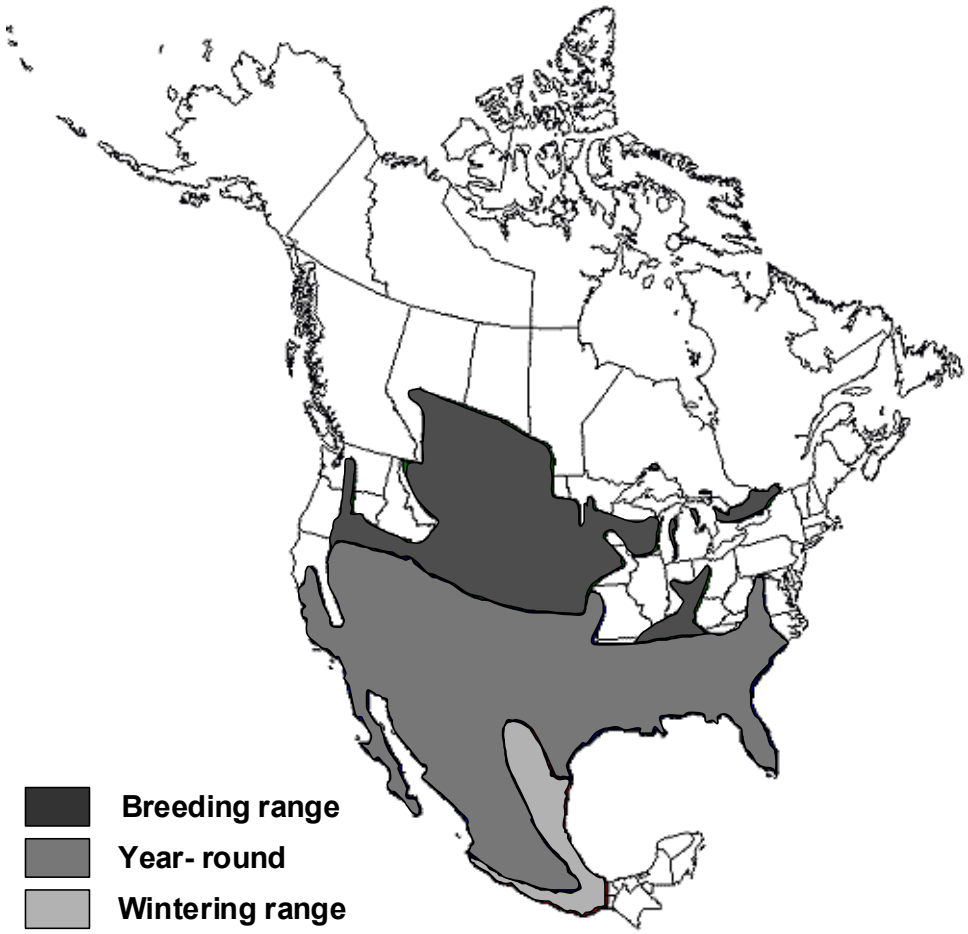

Figure 1.3. Distribution of the Loggerhead Shrike across North America (Yosef 1996). Ranges of eastern and western Canadian populations are currently contracting.

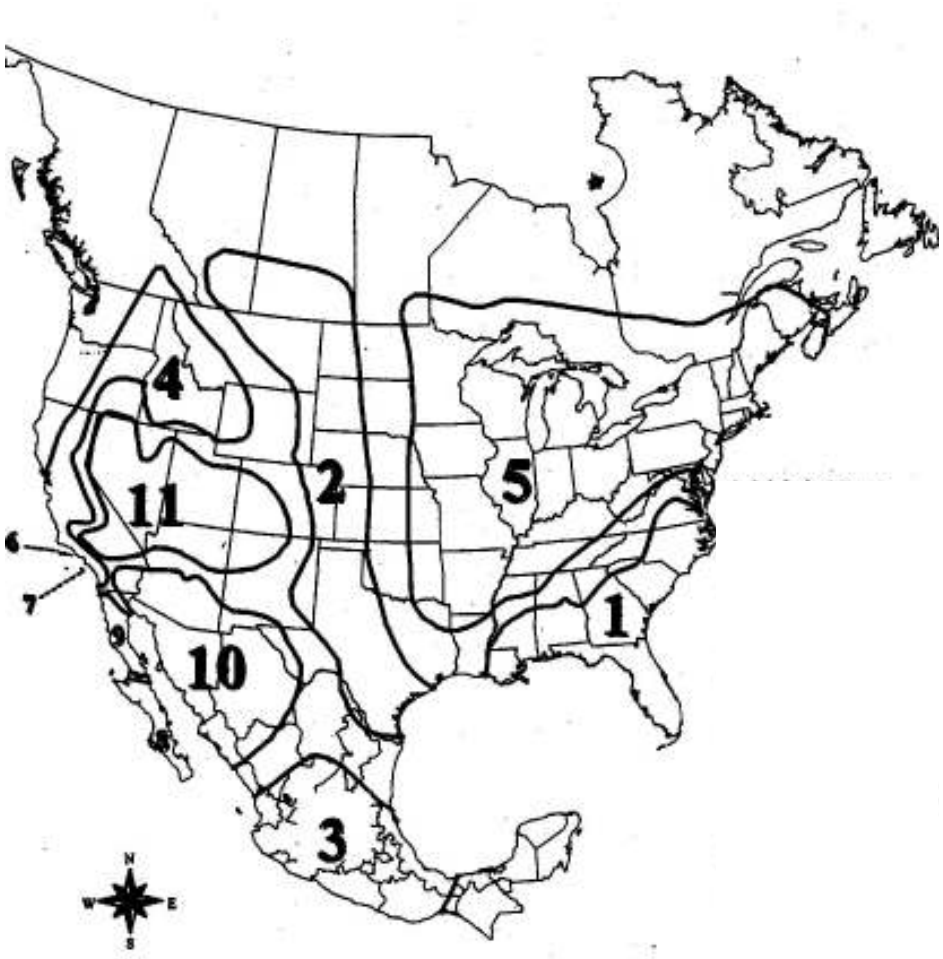

Figure 1.4. Most recognized subspecies delineation of Loggerhead Shrikes in North America as described by Miller (1931) *in* Pruitt (2000). Putative subspecies ranges are described as follow; 1) *L .l. ludovicianus*, 2) *L. l. excubitorides*, 3) *L. l. Mexicanus*, 4) *L. l. gambeli*, 5) *L. l. migrans*, 6) *L. l. anthonyi*, 7) *L. l. mearnsi*, 8) *L. l. nelsoni*, 9) *L. l. grinnelli*, 10) *L. l. sonoriensis*, 11) *L. l. nevadensis*. Areas between subspecies are putative hybrid zones.

Shrikes are a sit-and-wait predator of small mammals, birds and insects (Morrison, 1980). They use perches in the form of thorny shrubs, trees, barbwire fences and electrical wires (Miller 1931, Yosef 1996, Lefranc and Worfolk 1997, Pruitt 2000), as vantage points, as well as for impaling prey for shredding and storage (Yosef and Pinshow 2005)

1.2.2. STUDY AREA

Field work was conducted during winter (Jan-March) in 2003 and 2004 in Mexico. Total sampling area extends over the Mexican States of Chihuahua, Durango, Coahuila, Nuevo Leon, Tamaulipas, Aguascalientes, Jalisco and Michoacan (Fig. 1.5). In 2003, I sampled in 6 localities in Mexico; three in north-central Mexico (States of Chihuahua and Durango) and three in south-central Mexico (States of Jalisco, Michoacan and Aguascalientes). In 2004, I sampled in 5 localities in northeastern Mexico in the States of Coahuila, Nuevo Leon and Tamaulipas. In general, sampling took place in a variety of biomes from desert and xeric shrublands in northern Mexico to flooded grasslands and savannas in more southern states.

1.3 RATIONALE AND OBJECTIVES

Currently, it is not clear where northernmost populations of Loggerhead Shrikes from the North American Great Plains winter or of the habitat structure those shrikes may be using there. Prairie Canada serve as a northernmost fringe for some avian populations, including shrikes; therefore, identifying and delineating the wintering locale(s) of these northernmost populations is becoming one important objective in the recovery of endangered species in Canada (e.g., The Loggerhead Shrike Recovery Team). This species is an appropriate model because it occurs throughout a clear hydrogen isotopic

Figure 1.5. Sampling locations for Loggerhead Shrikes in Mexico. Regions were categorized as follow; Region A: covered the States of Chihuahua and Durango in north-central Mexico; Region B covered the States of Coahuila, Nuevo Leon, Tamaulipas in northeastern Mexico; and Region C covered the States of Aguascalientes, Jalisco, Michoacan in Mexico.

gradient with latitude and both resident and migrant individuals mixed in Mexico should be isotopically different. Similarly, genetic inherited markers should potentially distinguish among eastern Loggerhead Shrikes versus prairie Canada shrikes versus shrikes from other source populations and resident shrikes. This study is the second to measure stable isotopes in tissues of the Canadian prairie loggerhead shrike, and the first to use genetic profiles to create migratory connectivity in this species. I chose the sampling of feathers and claws because is non-intrusive and it does not require previous capture and marking of individuals (Hobson 2005a). Feathers S9 and R1 were collected, because of their high likelihood of being grown prior to fall migration on the breeding grounds (Miller 1928, Pyle 1997). Even though Loggerhead Shrikes display an eccentric moult pattern, hatch-year (HY) birds undergo a partial-incomplete preformative moult which usually includes 1-3 tertials in ~93% of birds and all 12 rectrices in ~73% (Pyle 1997), and after-hatch-year (AHY) birds undergo a complete basic moult on the breeding grounds (Pyle 1997). Thus, the isotopic analysis of these feathers, obtained on the wintering grounds, should provide a minimum estimate of the percentage of birds originating from northern natal or breeding areas. Feather vanes and claw tip were used for stable isotope analyses, while feather calami were used for genetic analysis. Habitat features of all wintering Loggerhead Shrikes were recorded as well as at random points to assess differences in habitat structure of occupied versus unoccupied sites, as well as sites occupied by residents versus migrants.

Here, I built on previous connectivity study using stable isotopes (Hobson and Wassenaar 2001) and on previous shrike phylogeographic work conducted at Queen's University which showed genetic structure between eastern and western populations of

the northern part of the breeding range (Vallianatos et al. 2001, Vallianatos et al. 2002). My main objectives were to: (1) investigate migratory connectivity of northern Prairie loggerhead shrike populations using intrinsic acquired markers; (2) Compare the genetic structure of Loggerhead Shrike populations from Prairie Canadian with those wintering in Mexico; (3) Describe the structure and variability of utilized habitat, and differences between utilized habitats from randomly available habitat, as well as between those utilized by residents versus migrants.

This thesis is organized into 5 chapters that are formatted for scientific journals except for chapters 1 and 5, the general introduction and synthesis, respectively.
In Chapter 2, "Migratory connectivity in western populations of the Loggerhead Shrike (*Lanius ludovicianus excubitorides*)" addresses the first objective. This chapter evaluates the reliability of feathers S9 and R1 to provide a more accurate breeding grounds signature. I compared the isotopic results of birds from unknown origins with expected isotopic values as shown in Meehan et al. (2004) and with isotopic values of museum samples of Mexican breeding shrikes. By this approach, I obtained a minimum estimate of the percentage of wintering birds from northern latitudes. I also evaluated the levels of concordance between δD values in feathers and in claws. In theory, because of the different metabolic rates of feathers and claws, I hypothesized that different tissues will provide different temporal and spatial information (see Bearhop et al. 2003).

In Chapter 3, "Genetic structure and migratory connectivity of the western subspecies of the Loggerhead Shrikes (*Lanius ludovicianus excubitorides*)" addresses the second objective. This chapter covers the genetic identification of prairie Loggerhead Shrikes within the genetic mix of shrike subspecies wintering in Mexico. Assignment

tests took the information from given genotypes and probabilistically assigns individuals to a user-defined number of anonymous genetic clusters (*K*), thus elucidating genetic structure. My interest in using assignment test lay in the identification of the number of Loggerhead Shrike populations (*K*) that best fit the data, in both Mexico and Prairie Canada.

In Chapter 4, "Structure and variability of wintering habitats used by resident and migrant Loggerhead Shrikes (*Lanius ludovicianus*) in Mexico" addresses the fourth objective. This chapter uses habitat features of all wintering shrikes in Mexico to assess whether differential habitat occupancy of presence vs. absence and resident vs. migrant differ. Additionally, I compared the habitat characteristics between residents and migrants.

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CHAPTER 2: MIGRATORY CONNECTIVITY IN WESTERN POPULATIONS OF THE LOGGERHEAD SHRIKE (*Lanius ludovicianus excubitorides*)

2.1. INTRODUCTION

Establishing connectivity between breeding and wintering populations of migratory birds is now recognized as an important component of the effective conservation of populations (Webster et al. 2002, Newton 2004, Hobson 2005 Webster and Marra 2005). On average, North American long-distance migratory birds spend two to three months on the breeding grounds, six to seven months on the wintering grounds, and another two to three months en route (Norris et al. 2004). Understanding components of the life history of migratory songbird populations thus requires the investigation of factors operating during all periods of their annual cycle and all regions of the species distribution (Myers et al. 1997). Such information is useful since productivity and recruitment is influenced by conditions at several locations and periods in the life cycle of individuals (Webster et al. 2002, Newton 2004, Webster and Marra 2005, Hobson 2005). Ultimately, connecting these disparate locations and events allows for more in-depth ecological and biological investigation and monitoring of populations throughout their annual cycle.

In recent years, changes in population size and distribution of many migratory bird species has inspired the investigation of migratory connectivity using the measurement of naturally occurring stable isotopes as markers of origin in bird tissues (e.g., feathers, blood, claws) (Hobson and Wassenaar 2001, Bearhop et al. 2003 Mazerolle and Hobson 2005). This approach is based on the fact that isotopic measurements of animal tissues reflect those in their diets, and foodweb isotope signatures can vary spatially (DeNiro and Epstein 1978, Hobson 2005). A major

advance in tracking animal movements came with the realization that naturally occurring deuterium (^2H, measured as δD) concentrations in tissues of living organisms are correlated with mean deuterium abundance in growing-season precipitation (Cormie et al. 1994; Hobson and Wassenaar 1997, Meehan et al. 2004, Chamberlain et al. 1997). Fortunately, in North America, δD patterns in precipitation are closely related to latitude (Hobson and Wassennar 1997). By measuring δD values in animal tissues, information on approximate latitude where those tissues were grown is possible (see Kelly et al. 2002 Rubenstein et al. 2002, Hobson et al. 2004b; Mazerolle et al. 2005).

The Loggerhead Shrike is a songbird with raptorial habits consuming small mammals birds, reptiles, amphibians and insects. Eleven subspecies occur in North America (Miller 1931), two of which breed regularly in Canada, *L. l. migrans* in eastern Canada, considered endangered and *L. l. excubitorides* in prairie Canada, considered threatened (COSEWIC 2004). The species is a partial migrant, with northern populations being migratory, while southern populations appear sedentary (Miller 1931, Burnside 1987). However, knowledge of the migration pattern of the Loggerhead Shrike is limited (Miller 1931, Yosef 1996). Less than 1% of band returns from 19,559 birds banded from 1955 to 1998 across North America suggested that birds east of the Rocky Mountains winter, in part, in southeastern United States (Sauer et al. 2005). Similarly, Burnside (1987) found that birds from Alberta and Saskatchewan migrated south to southern Texas. Recently, stable carbon and hydrogen isotope analysis of tail feathers revealed that, in winter, northern Mexico attracted a higher proportion of migratory shrikes than Texas (Hobson and Wassenaar 2001).

Most migratory Loggerhead Shrikes moult prior to fall migration (Miller 1928). Therefore, the isotopic analysis of flight feathers from shrikes examined in winter in Mexico should provide estimates of breeding origins of migrants and distinguish them from residents.

The objectives of this study were to examine patterns of stable-hydrogen isotope distributions in feathers of Loggerhead Shrikes in winter in Mexico in order to further elucidate the proportion of residents and migrants wintering in three different regions in Mexico and, among migrants, to determine their likely origins. I also was interested in detecting potential movements of resident shrikes within Mexico.

2.2. METHODS

2.2.1. SAMPLE COLLECTION

Loggerhead Shrikes were captured using walk-in traps baited with protected live mice during the winters (January-March) of 2003 and 2004 in the Mexican States of Chihuahua, Durango, Coahuila, Nuevo León, Tamaulipas, Aguascalientes, Jalisco and Michoacán (approximately 20^{o} to 31^{o} latitude and 98^{o} to 107^{o} longitude, Figure 2.1). In 2003, inner secondary (s9) feather was plucked from 238 individuals in 6 localities in Mexico; three in north-central and three in south-central Mexico. In 2004, the same feather was plucked from 173 new individuals at 5 localities in northeastern Mexico. We decided on s9 based on the moulting account provided for this species (Yosef 1996). However, since my study, it has been determined that shrikes breeding at their northernmost distribution in North America may interrupt flight feather moult and sometimes moult s9 on their wintering grounds (Pérez and Hobson 2006). Thus, my estimates of the proportion of northern migrants in our Mexican samples should be

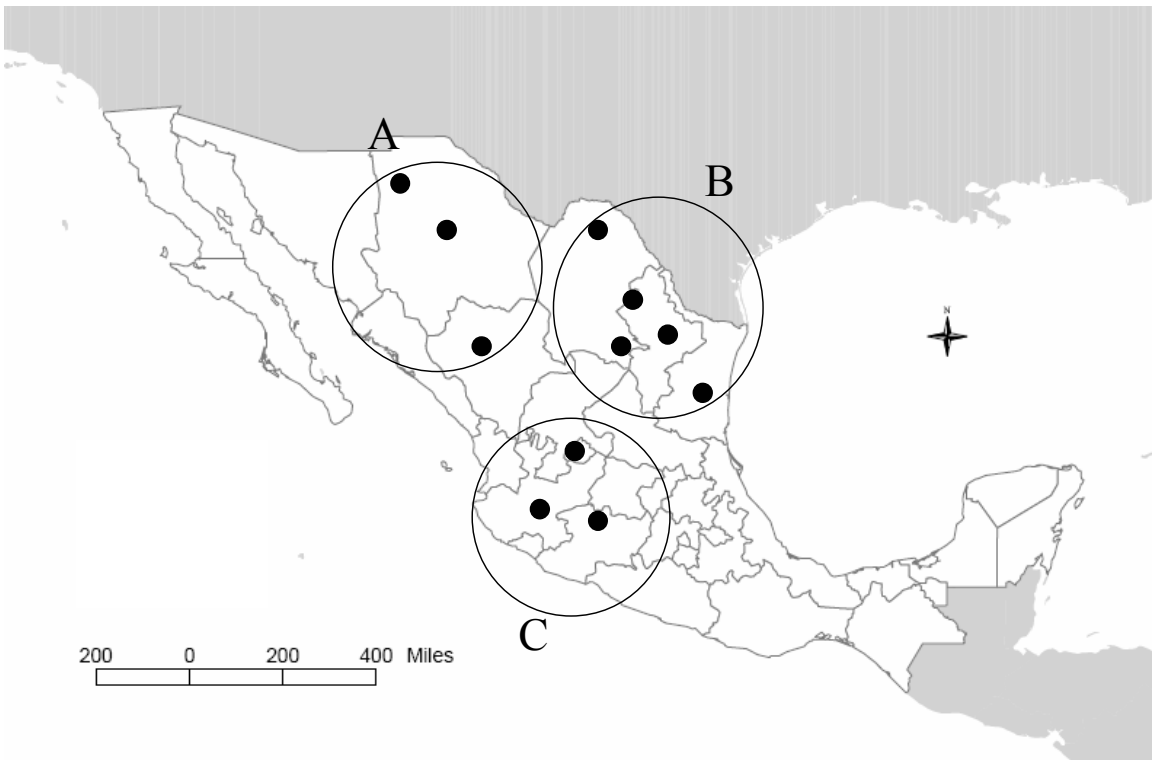

Figure 2.1. Sampling locations of Loggerhead Shrike in Mexico. Region A includes Chihuahua and Durango States in north-central Mexico; Region B, in northeastern Mexico, includes the States of Coahuila, Nuevo León and Tamaulipas; and Region C includes the States of Aguascalientes, Jalisco, and Michoacán in south-central Mexico.

considered a conservative estimate.

For general analysis and interpretation of the results, the overall 11 sampling sites were grouped into three more general sampling regions; north-central (Region A), northeastern (Region B), and south-central (Region C) (Figure 2.1). In 2004, birds were aged to second-year (SY) and after-second-year (ASY) based on criteria outlined in Pyle (1997). Sexes were not obtained because it is currently unknown how to sex live shrikes visually outside the breeding season (Collister & Wicklum, 1996). To survey in Mexico, a collection permit was obtained through the Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT). All captured birds were banded with a U.S. Fish and Wildlife Service aluminum leg band under a Canadian Wildlife Service banding permit. Feathers were placed in a labeled paper envelope.

Depictions of expected feather deuterium values (δD_f) based on mean annual growing-season precipitation (δD_p) have been developed for raptors in North America (Lott and Smith 2006) including northern Mexico, but are not yet available for central and southern Mexico. Therefore, using museum specimens of shrikes from known summer provenance, a δD_f base map for Mexico was developed from isotopic measurement of feathers of resident shrikes as suggested in Hobson (2005). I based my approach of depicting contour lines for Mexico by combining information from the median δD_f values from the museum feather samples, the isoclines depicted by Lott and Smith (2006) for northern Mexico, and the general landscape patterns of relief in Mexico from freely available satellite imagery on the web. For the museum samples, approximately 1 cm of the second secondary (s2) feather was clipped from 40 Mexican resident shrikes specimens kept at the Universidad Autónoma de Mexico. The

interpolation of δD_f contour lines occurring in Mexico allowed the estimation of expected deuterium feather values for Region C.

2.2.2. STABLE ISOTOPE ANALYSIS

Feather samples were bathed in 2:1 chloroform:methanol solution overnight, drained and air dried under a fumehood for at least 24 h before being analyzed at the National Water Research Institute in Saskatoon, Canada. Distal end of feather vane were then cut and 350±10μg weighed into 4.0 x 3.2 mm silver capsules. Stable isotope analysis of the non-exchangeable hydrogen was conducted using online continuous-flow isotope-ratio mass spectrometry (CFIRMS), as described by Wassenaar and Hobson (2003). Estimates of deuterium concentration were expressed in delta notation in parts per thousand (‰) as the non-exchangeable hydrogen portion of samples normalized on the VSMOW-SLAP (Vienna Standard Mean Ocean Water-Standard Light Antarctic Precipitation) scale.

For collection regions A and B, expected δD_f values of s9 were derived using the altitudinally-corrected continental pattern of δD_f for North American raptors depicted in Lott and Smith (2006). For Region C, measured δD_f values were compared to the deuterium base map for shrikes established using the museum skins of resident Mexican shrikes. Based on Lott and Smith (2006), resident birds that grew their feathers in Region A were expected to have feather δD between -50 and -20‰ (Figure 2.2), whereas migratory birds that grew their feathers at more northern latitudes than Region A were expected to have feather δD values < -50‰. Resident Loggerhead shrikes from Region B were expected to have δD feather values of -35‰ and higher. Based on the δD_f values

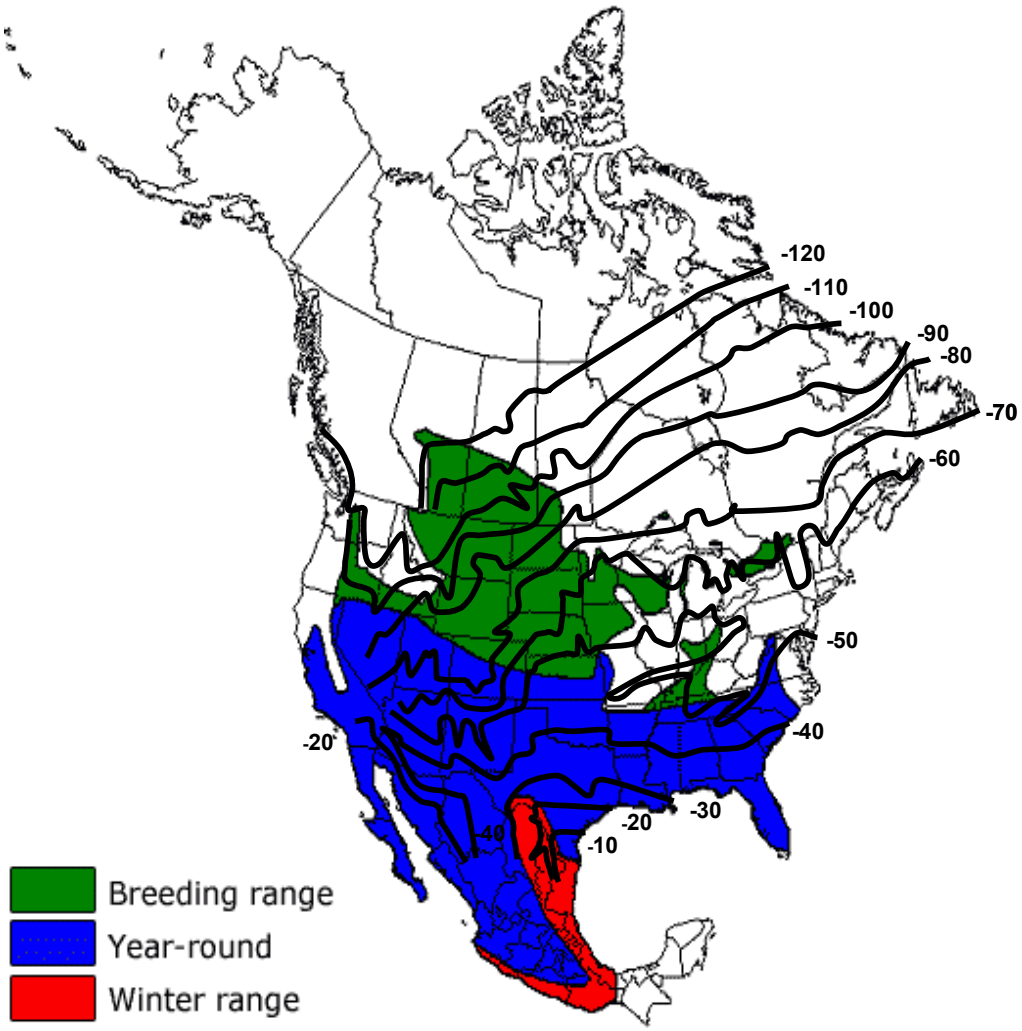

Figure 2.2. Distribution of the Loggerhead Shrike (Yosef 1996). Solid grey lines represent the expected continental pattern of deuterium concentration in feather (δD_f) for North American raptors. Estimates of deuterium in feathers were expressed in delta notation in parts per thousand (‰). Expected deuterium values for regions A and B (Figure 2.1) were estimated based on this map. Expected feather deuterium values for Region C could not be estimated from this map and were derived from Figure 2.3. This figure has been reproduced from isoclines in Lott and Smith (2006).

from museum samples, resident birds from Region C were expected to have values in the range of -55 to -20‰ (Figure 2.3).

2.2.3. STATISTICAL ANALYSIS

I used a Kolmogorov-Smirnov test (Sokal and Rohlf 1995) to assess normality in each of the three sampling regions. If non-normal, I opted to use the non-parametric Mann-Whitney *U*-test to test for differences in δD_f values between sampling regions. Pearson chi-square test was used to investigate whether proportions of resident and migratory shrikes differed between regions A, B, and C. We also used Mann-Whitney *U*-test to test effects of age on δD_f values within study regions from 2004. All analyses were two-tailed and performed with SPSS version 12.0 for windows (SPSS inc. Chicago, IL).

2.3. RESULTS

Distribution of deuterium values in north-central and south-central Mexico were normal (Region A: $D = 0.63$, $P = 0.83$, n = 139; Region B: $D = 0.63$, $P = 0.83$, n = 99); however, northeastern Mexico was not (Region C: $D = 2.22$, $P < 0.001$, n = 173). Based on δD_f values of shrikes sampled in Region A, 28.1% of birds were more depleted than expected for a resident bird, 64% were within the expected values for resident birds and 7.9% were more enriched than expected; Figure 2.4). In Region B, 63.8% of birds had δD_f values more depleted than expected for a resident while 36.2% were not. Two δD_f values were considered outliers and removed from further analysis because they were too enriched (i.e., positive values). In Region C, 73.7% of birds had δD_f values more

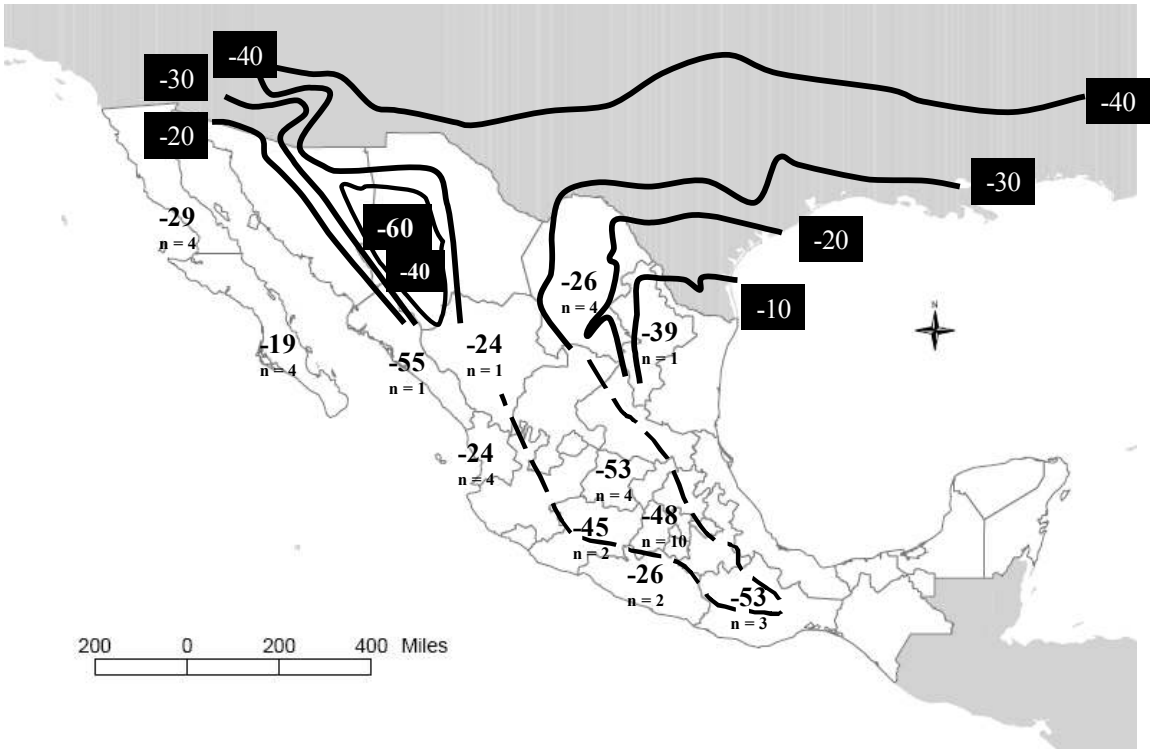

Figure 2.3. Expected pattern of deuterium values in feathers (δD_f) for Loggerhead Shrikes in Mexico based on museum specimens. Deuterium values represented in the black squares and by black solid lines are those presented in Lott and Smith (2006) (Figure 2.2). Dotted lines represent the interpolation of expected δD_f from resident shrikes. Estimates of deuterium concentration were expressed in delta notation in parts per thousand (‰) and n is the number of museum specimens sampled in each State of Mexico.

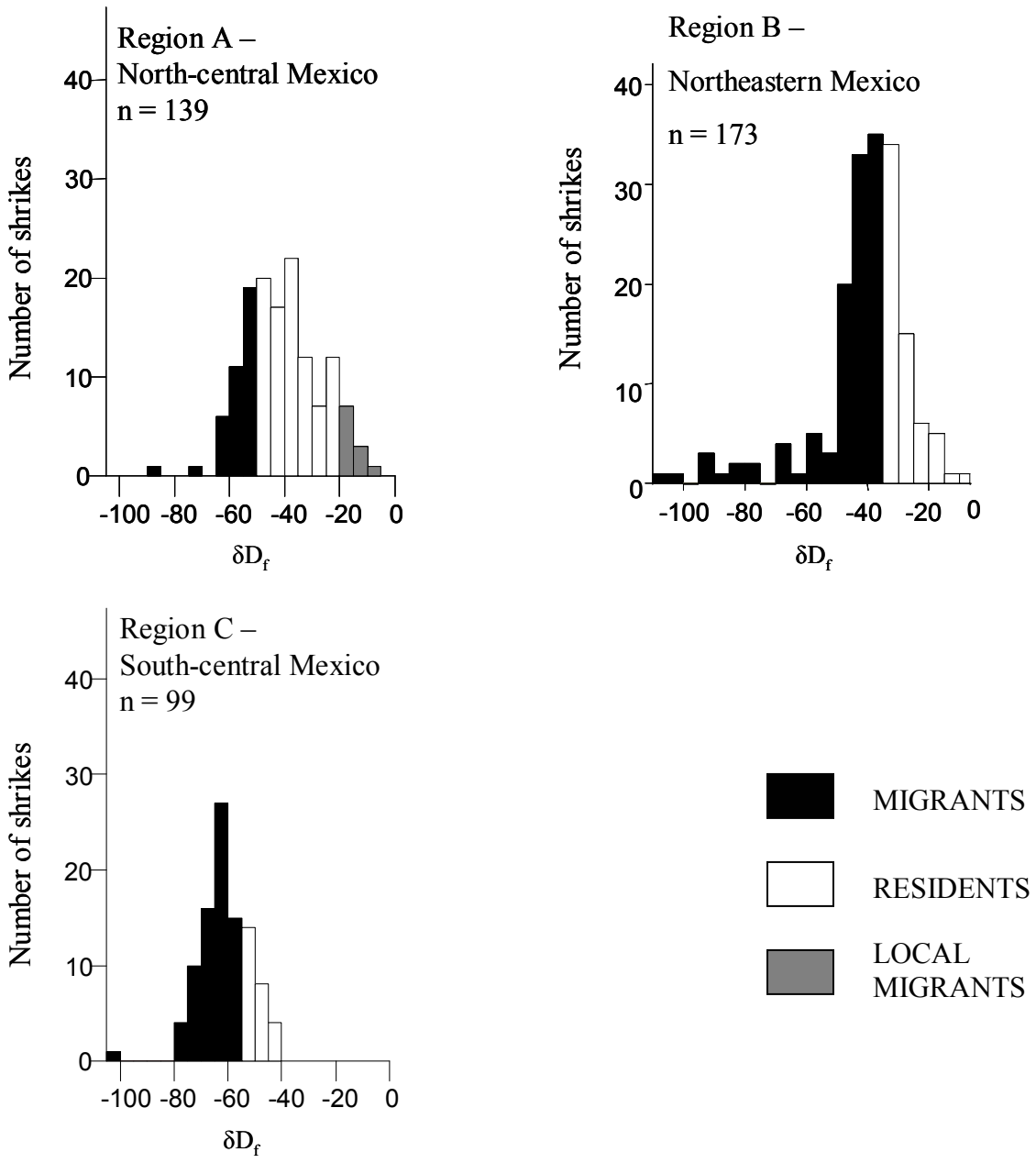

Figure 2.4. Frequency distributions of δD_f of migratory, resident and local migratory Loggerhead Shrikes during winter in three areas in Mexico. North-central (Region A), Northeastern (Region B) and south-central (Region C) sites in Mexico consisted of 28.1%, 63.8% and 73.7% of migrant individuals from northern breeding grounds, respectively. A small number of individuals wintering in north-central Mexico (Region A) were local residents that moved there possibly from areas closer to the Gulf coast. N is the sample size.

depleted than local expected values, and 26.3% had δD_f values within the local expected values.

Feather δD values differed significantly among regions A and C ($U = 1271.5$, $P < 0.001$, n = 239) and regions B and C ($U = 1665$, $P < 0.001$, n = 272). Regions A and B did not differ in feather δD values ($U = 10834.5$, $P = 0.13$, n = 312). The proportion of derived migratory and resident birds differed significantly between regions A and B ($\chi^2 = 32.8$, $P < 0.0001$) and A and C ($\chi^2 = 41.8$, $P < 0.0001$). The was no difference in proportion of resident and migrants between regions B and C ($\chi^2 = 2.8$, $P = 0.09$) Feather δD values within sampling sites did not differ among age classes in Region C (U-test = 2955, $P = 0.17$, n = 168).

2.4. DISCUSSION

Stable hydrogen isotope analyses of inner secondary feather (s9) of all wintering Loggerhead Shrikes examined in Mexico during winter indicated that migrant shrikes occurred together with residents in all areas I investigated (A: north-central, 28.1%; B: northeastern 73.7% , C: south central 63.8%) As expected, s9 δD_f values from migrants represented a range of latitudinal origins from southern Canada to Mexico, all well within the overall continental range of the Loggerhead Shrike. Based on Yosef (1996), the southernmost limit of migratory versus resident western Loggerhead Shrikes is approximately around the -50‰ isocline (see Figure 2.2). Using this criteria, I found that 28.1%, 13.3%, and 87.9% of shrikes from regions A, B, and C, respectively, grew their feathers at latitudes occupied by migratory shrikes (i.e. above the -50‰ isocline). The higher proportion of migrant shrikes with lower δD_f values at Region C suggests a possible leapfrog migration pattern whereby northern migrants winter farther south in

Mexico than more southern migrants. However, further research is required to determine if this pattern remains consistent among years.

The wide distribution in feather δD values in Region A showed that, in winter, north-central Mexico is occupied by a mixture of local resident and migrant shrikes likely from coastal environments. The high δD_f values (> -20‰) I measured in Region A, were expected from the Gulf States of Mexico and the southern U.S. Such movements have not been documented for so-called nonmigratory populations of Loggerhead Shrikes in North America. Possibly, some populations use a step migration strategy shown in Old World shrikes (Safriel 1995, Curry-Lindahl 1981) whereby migratory individuals have more than one wintering site, and they move among them during winter (Lovei 1989).

Currently, there are several assumptions inherent in the application of the stable hydrogen isotope approach for establishing origins of migratory shrikes in North America including Mexico. First, I have established that our choice of s9 for analysis will likely provide a minimum estimate of the number of actual migrants at any sites in Mexico (Pérez and Hobson 2006). However, the occurrence of interrupted moult, whereby some individuals delay the moult of s9 until near their wintering grounds, is likely true only of those individuals at the northernmost extent of their range (e.g. Miller 1931) and so may represent a comparatively small error in assignment of residents and migrants in Mexico. Secondly, the feather basemap based on the long-term IAEA dataset for precipitation will have associated error in assignment (Hobson 2005, Lott and Smith 2006). Since shrikes are raptorial, I was fortunate to be able to use the raptor deuterium feather basemap established specifically for this group of birds and so argue that the extended basemap I established for Mexico is the best possible. Nonetheless, there will of course be errors in

assignment. Wassenaar and Hobson (2006) recently established that the absolute minimum error that could be expected for deuterium analyses of feathers was of the order of 3‰ and suggested a more realistic variance to expect was of the order of 6‰. My general approach was based on considerations of a general error of 6‰ and the nature of the isotopic contours in each region. I used an isotopic range of at least 30‰ to correspond to residents in each of the areas I examined. No information currently exists on how much local mean annual precipitation may vary from the long-term average values represented in the basemap and this information will be particularly difficult to interpret for Mexico which has only two IAEA recording sites.

Although the ability to assign wintering individuals back to their breeding grounds is still limited, δD_f values have provided information on origins of long-distance migrant shrikes that far exceed that gained from 50 years of banding effort. Overall, these results have provided new evidence that a significant proportion of *Lanius ludovicianus*, most likely *L. l. excubitorides*, from the northern and central parts of their range winter in south-central and northeastern Mexico. Additionally, the isotopic evidence suggested local migration within previously designated nonmigratory populations, especially of birds moving into the Chihuahuan desert (Region A) from southwestern U.S. and northeastern Mexico to winter.

My results have implications for the conservation of this declining species. First, Mexico is clearly an important overwintering site for shrikes that breed north of the U.S. Mexico border. My analyses show even greater proportions of migrants in Mexico than previously established isotopically by Hobson and Wassenaar (2001). Further studies are now required to evaluate the availability of suitable habitats for both resident and migrant

shrikes in Mexico and whether these differ (Pérez and Hobson, in prep) or are declining. Currently, we also have no information on population trends of shrikes in Mexico. The combined use of roadside shrike surveys with stable isotope analyses (Hobson and Wassennar 2001) to monitor resident vs. migrant populations would greatly assist in interpreting population trends throughout the species' range.

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CHAPTER 3: GENETIC STRUCTURE AND MIGRATORY CONNECTIVITY OF THE WESTERN SUBSPECIES OF LOGGERHEAD SHRIKE *Lanius ludovicianus excubitorides*

3.1 INTRODUCTION

One of the most daunting challenges in avian conservation is identifying wintering grounds and stopover sites for migratory species (Webster et al. 2002, Hobson 2005, Skagen 2006). Approximately 361 species of Neotropical migratory bird that breed in the United States (U.S.) and Canada, winter in the Caribbean, Mexico and southward (DeGraff and Rappole 1995), and for the vast majority of these migrants at best there is only fragmentary evidence as to where they winter. A major obstacle in identifying wintering grounds of discrete breeding populations has been the difficulty of tracking individuals during migration and onto their wintering areas (Webster et al. 2002).

Traditional techniques that rely on mark and subsequent recapture, such as leg banding, have proven ineffective because few passerine birds, which comprise the majority of migratory species, are ever recovered and satellite transmitters are not yet available for the small passerine birds (Hobson 2003). Other traditional methods such as population delineation by plumage coloration or morphological measurements can be effective for some species (Escalante-Pliego and Peterson 1992, Brumfield and Remsen 1996), but may also be unreliable for discriminating among populations, because many populations within a species show overlap in these attributes between sexes and/or populations (e.g., Miller 1931, Collister and Wicklum 1996, Smith et al. 2005).

The persistence of migratory birds is influenced by events occurring at different times and locations outside the breeding season, which may affect both fecundity and recruitment, and ultimately adaptation and speciation (reviewed in Webster and Marra

2005). Thus, connecting breeding and wintering populations of a species allows for a more complete investigation of the factors that may limit or cause the diminution of breeding populations.

Fortunately, population-specific genetic markers can be used successfully to identify breeding origins of individuals during all stages of the annual cycle (Haig et al. 1997, Webster et al. 2002). This approach relies on identifying patterns of population genetic structure over a wide distribution of individuals. When genetic variation is structured geographically on the breeding grounds, the breeding provenance of individuals sampled during migration or on wintering sites can be identified based on similarity of allele frequency (Haig et al. 1997). DNA microsatellites have proved useful to identify population genetic structure over broad geographical areas and trace migratory movements (Clegg et al. 2003, Jones et al. 2005), because they are bi-parentally inherited, presumably neutral, fast evolving and allow for recombination, integrating several genealogical processes. Hence, microsatellites can exhibit differentiation over fine geographical and temporal scale and thus is the marker of choice when whishing to find population-specific markers (e.g., Gibbs et al. 2000, Högland and Shorey 2003, Jones et al. 2005).

This study encompasses Loggerhead Shrikes (*Lanius ludovicianus*), one of two true shrike species in North America and the only lanid that occurs exclusively in the New World. The Loggerhead shrike provides a particularly compelling case study for creating migratory connectivity and its importance in conservation, and for applying genetic methodologies to identify wintering grounds for northern breeding populations. Eleven subspecies are generally recognized in North America; and in two of them, their

breeding range extends into southern Canada; *L. l. migrans,* classified as Endangered, and *L. l. excubitorides*, classified as Threatened (COSEWIC 2004). Habitat limitations occurring on both the breeding (Prescott and Collister 1993) and the wintering grounds (Brooks and Temple 1990, Lymn and Temple 1991, Temple 1995) are the most frequently cited causal mechanisms of their decline; however the ultimate cause is still unknown.

Prairie Canada serves as a northernmost breeding fringe for *L. l. excubitorides* Swainson, the focus of this study, and it is not clear where these populations winter. Hence, identifying and delineating the wintering locale(s) of these northernmost populations is important to concentrate future investigations of potential limiting factors that may be affecting shrikes there. This species is an appropriate model because species-specific genetic inherited markers have been developed (Chabot et al. 2005) to be used in distinguishing among eastern versus western (prairie) Canada Loggerhead Shrikes. The main objective of this study was to use DNA microsatellite markers and Bayesian clustering analysis (Pritchard et al. 2000) to detect patterns of population genetic structure within the range of Western Loggerhead Shrikes (*L. l. excubitorides*) in Canada and use these to infer breeding origin of shrikes sampled in Mexico. Although shrikes from the northern parts of their range are known to winter in various areas around the Gulf of Mexico (Burnside 1987), I focused my study in Mexico because Hobson and Wassennar (2001) showed evidence that northern Mexico contained a higher proportion of migratory shrikes than southern U.S., most possibly belonging to *L. l. excubitorides* populations. Assignment tests takes the information from given genotypes and probabilistically assigns individuals to a user-defined number of anonymous genetic

clusters (*K*), thus elucidating genetic structure. My interest in using assignment test lay in the identification of the number of Loggerhead Shrike populations (*K*) that best fit the data, in both Mexico and Prairie Canada. This approach will ultimately help to provide an objective assessment of coupling microsatellite markers and Bayesian analysis to inferring migratory connectivity of Loggerhead Shrikes.

3.2 MATERIALS AND METHODS

3.2.1. TISSUE COLLECTION

Feather samples were collected from individual Loggerhead Shrikes on the breeding and wintering areas (Fig 3.1, Table 3.1) from 2002 to 2004. Loggerhead Shrikes were captured using modified versions of Potter traps (Blake 1951) baited with protected mice. From each bird, the inner secondary (S9) and inner rectrix (R1) feathers were plucked. All captured birds were banded with a U.S. Fish and Wildlife Service aluminum leg band under a Canadian Wildlife Service banding permit. Feathers were placed in a labeled paper envelope for transport to the laboratory. In Canada and Mexico, populations were defined operationally as samples falling within a province, a state or a putative hybrid area. In prairie Canada, 144 feather samples from 4 localities (hereafter referred as sub-populations), were collected in southern Alberta (n = 36), south- western and central Saskatchewan (n = 62); southeastern Saskatchewan (n = 17) and southwestern Manitoba (n = 29) (Fig 3.1). In Mexico, a total of 309 samples from 11 localities were collected in the States of Chihuahua, Durango, Aguascalientes, Jalisco, Michoacán, Coahuila, Nuevo León, and Tamaulipas. For general analysis and interpretation of the Bayesian results, the overall 11 Mexican sampling sites were

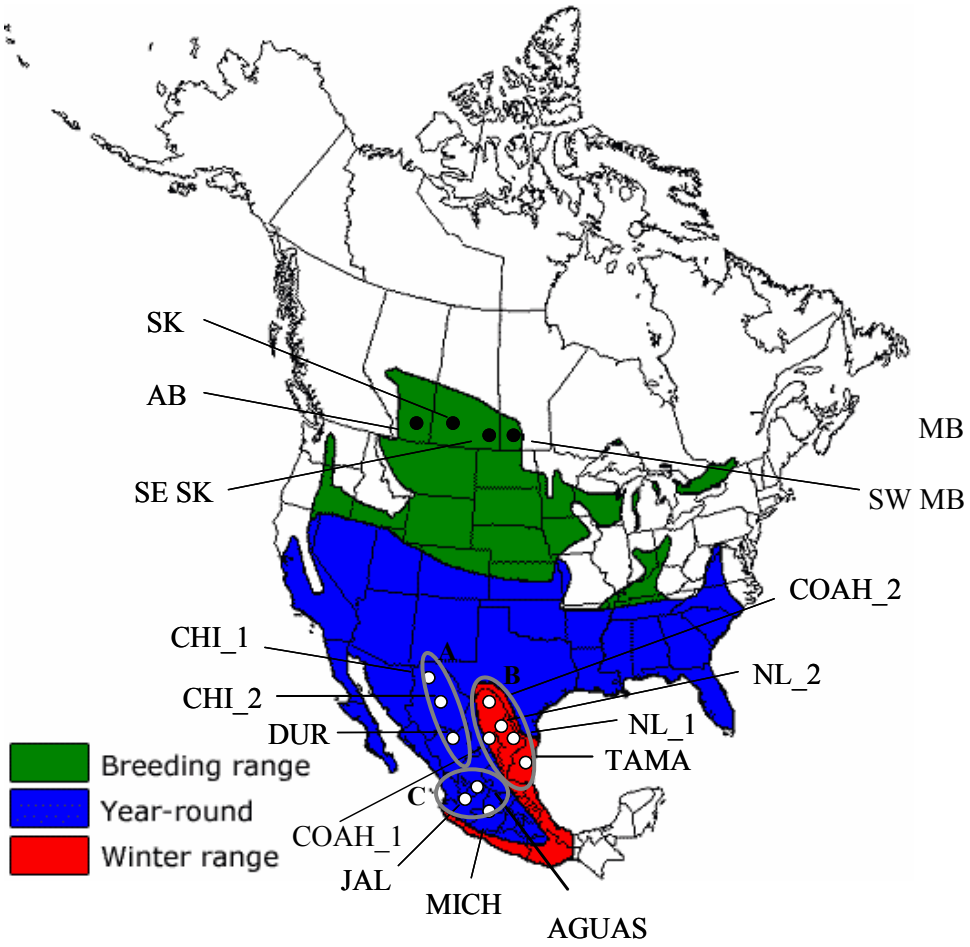

Figure 3.1. Distribution of Loggerhead Shrikes in North America (Yosef 1996) and location of sampling sites on breeding and wintering grounds. In Mexico, the 11 collection sites were grouped into regions A, B and C. See Table 3.1 for location codes.

grouped into three more broader sampling regions; north-central (Region A, *n* = 97), northeastern (Region B, *n* = 126), and south-central (Region C, *n* = 84) (Fig 3.1).

On the breeding grounds, adults (second-year; SY, and older), as determined by the presence of either a cloacal protuberance (males) or brood patch (females) (Pyle 1997), were sampled between May and August. Overwintering sampling was done on birds between January 16th and March 3rd in 2003 and 2004.

3.2.2. DNA EXTRACTION

Total genomic DNA was extracted from the proximal 4-5 mm of feather rachis' using a GeneClean II kit (Qbiogene, Inc.) following the manufacture's instructions. Each feather base was minced into slivers with a sterile scalpel then placed in a sterile 1.5ml Eppendorf tube. DNA extraction began by incubating each feather rachis in a 55^{o}C for four hours in 180µl of lysis buffer (500ml of lysis buffer comprised of 50ml of 1M Tris-HCL, 10ml of 5M NaCl, 50ml of 0.5M EDTA, 100ml of 10% SDS and 290ml of double distilled water (ddH_2O), and an aliquot of 20µl of 20mg/ml Proteinase K solution. An additional aliquot of 5µl of 20mg/ml Proteinase K was added to each sample and incubated in a water bath overnight at 37^{o}C. The following day, mixture were centrifuged for five minutes at 21,000 g and the supernatant was transferred to a clean 1.5ml Eppendorf tube to avoid including particulate matter in the extraction. Isolation of DNA was performed used a Geneclean II kit (BIO 101 Inc.), with two modifications from the manufacturer's protocol: a 10µl of glass milk was added to bind the DNA and two final elutions were performed in 10µl of ddH_20. The approximately 20µl of eluted DNA was centrifuged a final time to remove all traces of glass milk and transferred to another sterile tube and stored at -20^{o}C until subsequent genotyping.

3.2.3. MICROSATELLITE AMPLIFICATION AND DNA GENOTYPING

Microsatellite loci were amplified using nine primer pairs: LLU011, LLU11, LLU020, LLU 040, LLU045, LLU89, LLU 133 (Lougheed et al. 2001), LS4 (Mundy et al. 1997a) (all Loggerhead Shrike-specific) and SJR4, developed for Florida Scrub-Jay *Aphelocoma coerulescens* (D. B. McDonalds and W. K. Potts, unpublished work; see Hansson et al. 2000). For each sample, a 0.3µl volume end-labeled was added, including 0.05µl of 10nM Forward Primer, 0.03µl of 10X MBI Fermentas PNK buffer, 0.10µl of ddH_20, 0.05µl of γ-P^{33} ATP and 0.07µl of MBI Fermentas 10u/µl PNK). To stop kinase activity, reactions were incubated for 30 minutes at 37°C and then for 10 minutes at 65°C. Each microsatellite PCR reaction contained 1µl DNA template, 6.35µL sterile double-distilled water, 10X Oliver's Buffer (25µl $MgCl_2$, 50µl Tris – pH 8.0, 50µl Tris – pH 8.8, 500µl KCl, 200µl of 20 mg/ml, 100µl of 1% gelatin, and 75µl ddH_20), 0.5µl dNTPs (10mM), 0.5µl reverse primer (10mM), 0.25µL forward primer (10µM), 0.1µL Taq (5 units) and 0.3µl end-labeled forward primer (10µM). Solutions were run for 2 minutes at 94°C and 35 cycles at 92°C for 30 seconds, 56.5 or 65°C (depending on primer) for 20 seconds and at 72°C for 25 seconds. To visualize the genotype, 10µl of stop solution containing brophenol blue was added to the PCR solution, heated to 60°C and loaded into a 6.5% polyacrylamide gel. Size standards were also loaded in each gel to facilitate scoring of alleles. Gels were electrophoresed using a standard PAGE gel rig in 0.5X TBE buffer (0.45M Tris-Borate, 0.01M EDTA) at 65 watts for 2.5 hours. Gels were transferred to blotting paper, vacuum dried, and exposed to X-ray film for 24 to 96 hours. Scoring of each individual's genotype was done by hand.

3.2.4. ANALYSIS OF MICROSATELLITE GENETIC DIVERSITY

For each population sample I calculated observed (H_{obs}), and expected heterozygosity (H_{exp}), and mean number of alleles per locus using the program ARLEQUIN 3.01 (Schneider et al. 2000). For each locus/population combination, I tested for departures from expected genotype frequencies Hardy-Weinberg equilibrium using the Markov chain simulation method of Guo and Thompson (1992). For each population, I tested for departures from linkage equilibrium between all pairs of loci using a likelihood-ratio test according to the permutation procedure of Slatkin and Excoffier (1996). Bonferroni corrections were applied to correct for multiple comparisons (Sokal and Rohlf 1995).

3.2.5. ANALYSIS OF POPULATION GENETIC STRUCTURE

To assess whether there is significant differentiation within and among the four prairie Canada sub-populations, analysis of molecular variance (AMOVA) and degree of genetic differentiation (F_{ST}) were calculated using the program ARLEQUIN 3.01. A F_{ST} value of < 0.05 was considered negligible genetic differentiation; Wright 1978). The significance of F_{ST} from zero was assessed using 1000 bootstrap replicates. Additionally, locus-by-locus AMOVA analyses were performed. Pairwise F_{ST} (Weir and Cockerham 1984) analysis and estimate of number of migrants per generation, gene flow (N_m), between the four Canadian sub-populations were estimated in GenAlEx (Peakall and Smouse 2003).

Alternatively, analyses of genetic differentiation among sub-populations were also undertaken using the Bayesian-clustering program STRUCTURE (Pritchard et al. 2000; www.http://pritch.bsd.uchicago.edu). The program STRUCTURE assumes that

within populations, loci are at Hardy-Weinberg (HW) and linkage equilibrium. Traditionally, more conventional statistical approaches (e.g. F_{ST}) need to identify *a priori* the geographic limits of the populations that are sampled, usually by designating different sample locales as different sub-populations. These sub-population definitions may not reflect true genetic and demographic independence. As such, different designations of sub-populations may affect calculations of N_m (Neigel 2002). Fortunately, newly developed Bayesian analyses in genetic studies combine information from several loci into a single probability model, instead of simple average (e.g., F_{ST}) (Corander et al. 2003), so that estimates of the amounts of genetic partitioning among individuals and sub-populations are tabulated directly (Pritchard et al. 2000, Corander et al. 2003). In STRUCTURE, individuals can be assigned to a number of genetic clusters, *K*, probabilistically or jointly to two or more clusters if their membership coefficient (*Q*) indicates that they are admixed (Pritchard et al. 2000, Rosenberg et al. 2002). With this approach, populations can be designated as reflected by their genetic distinctiveness. Further, STRUCTURE allows known origin individuals to then be used as training samples to classify individuals of unknown origin to population(s) (e.g., Beaumont et al. 2001).

Using no *a priori* population information, STRUCTURE was used to classify individuals from prairie Canada into *K* genetic clusters of random mating individuals that minimize HW and linkage disequilibrium (LD). The purpose of this approach was to assess whether genetic structure exist in the Canadian *L. l. excubitorides* population, with the intention to using these results to classify shrikes sampled in Mexico. Preliminary tests for convergence of ln likelihoods and α levels recommended a burn-in of 50,000

iterations and a subsequent run of 100,000 iterations. Data were analyzed under these settings, with the admixture (i.e., allowing mixed ancestry) and correlated (similar) allele frequency models (Falush et al. 2003), three times for each value of *K*, varying *K* from 1 to 6. Posterior probabilities for each *K* were computed for each set of runs using the formula in Pritchard et al. (2000).

Genetic profiles found in shrikes from prairie Canada from the latter analyses were used to classify birds sampled in Mexico into being migratory from Canada, or either migratory from elsewhere or Mexican residents. To determine the most useful and accurate level for genetic differentiation or identification when using assignment tests, genetic data were tested at various spatial scales (e.g., Haig et al. 1997). Consequently, two approaches were used to test assignment performances when using prior population information. To assess the accuracy of assignments of unknown individuals to Canadian genetic clusters, 4 known Canadian individuals, each one representing one of the 4 genetic clusters (Fig. 3.2), were treated as 'unknowns'. These 4 individuals were placed in the group of shrikes sampled in Mexico (unknown origin individuals) and were the last 4 individuals of each dataset of each region in Mexico (see Fig. 3.3). The best assignment approach for prairie Canada was subsequently used to classify shrikes examined in Mexico into clusters. I first used the *K* with the highest posterior probability, as prior population information, to assign individuals of unknown origin (i.e. all individuals sampled in Mexico and the 4 'unknown' previously described individuals) to source populations (e.g., Beaumont et al. 2001). The second assignment approach was to group individuals based on estimates of membership (*Q*'s >= 0.90), irrespective of *K*. It has been shown that, in some instances, the number of clusters, or populations that are well

supported by the estimate of membership (*Q*) are of much more interest, when genetic structure is not apparent, than the posterior probability of the parameter *K* (e.g., Dawson and Belkhir 2001, Adeyemo et al. 2005, Lecis et al. 2006). While exploring the data using no prior population information, I noted that with each increase in *K* (i.e., *K* = 3, *K* = 4, *K* = 5, *K* = 6) some individuals did not show signs of admixed ancestry and were consistently, and with high *Q*, being assigned to different clusters, regardless of sampling location. Thus, four groupings were selected as pre-defined prairie Canada clusters (Fig 3.2). For the assignment tests, all individuals showing admixed ancestry were removed because, as dispersal appears common in Loggerhead Shrikes (Haas and Sloane 1989, Brooks and Temple 1990, Collister and De Smet 1997), it would be difficult to assess (based on ancestry) if an individual had dispersed from another population or had a genotype that it is less common than average. Assignment tests were set at *K* = 5 to allow assignment to one of the 4 prairie Canada genetic clusters and one extra cluster. Cut-off of *Q* values were set at 90%, because all 'unknown' prairie Canada individuals were assigned back to their respective clusters with *Q*'s of >= 90%.

3.3. RESULTS

3.3.1. MICROSATELLITE ANALYSIS

Genotypic data were obtained for a total of 438 individuals for nine microsatellite loci of Loggerhead Shrikes from both prairie Canada and Mexico. All loci were polymorphic in the 144 genotyped Loggerhead Shrikes from prairie Canada (Table 3.1),

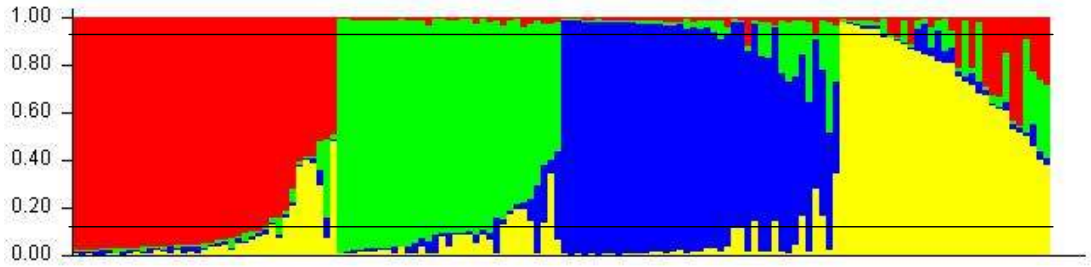

Figure 3.2. Estimates of membership (*Q)* of Loggerhead Shrikes from prairie Canada (Alberta to southwestern Manitoba) using no prior population information at $K = 4$, in STRUCTURE (Pritchard et al. 2000). Each individual is represented as a vertical bar, whose length is proportional to the estimated membership into four clusters. The horizontal black lines indicate values of individual proportion of membership $Q = 0.10$ or 0.90. Individuals with $Q >= .90$ were selected for assignment tests, here represented as Cluster 1, 2, 3, and 4.

Table 3.1. Sampling site information for microsatellite data. Diversity measures for each population were calculated across 9 loci – *n:* sample size, N_a: mean number of alleles, H_O: observed heterozygosity, H_E: expected heterozygosity, F_{ST}: degree of genetic diversity

Site, Province/State, Country	**Abbrev.**	***n***	N_A	H_O	H_E	F_{ST}
Alberta, Canada	AB	36	11.2	0.73	0.66	0.009
Saskatchewan, Canada	SK	62	14.4	0.62	0.74	0.010
Southeastern Saskatchewan	SE SK	17	6.2	0.58	0.74	0.011
Southwestern Manitoba	SW MB	29	10.8	0.65	0.78	0.010
Janos, Chihuahua, Mexico[§]	CHI_1	27	-	-	-	-
Aldama, Chihuahua, Mexico[§]	CHI_2	26	-	-	-	-
Lago Santiaguillo, Durango, Mexico[§]	DUR	44	-	-	-	-
Lago Chapala, Jalisco, Mexico[×]	JAL	25	-	-	-	-
Lago Cuitzeo, Michoacan, Mexico[×]	MICH	32	-	-	-	-
Aguascalientes, Aguascalientes, Mexico[×]	AGUAS	27	-	-	-	-
Saltillo, Coahuila, Mexico[ŧ]	COAH_1	35	-	-	-	-
San Fernando, Tamaulipas, Mexico[ŧ]	TAMA	25	-	-	-	-
China, Nuevo Leon, Mexico[ŧ]	NL_1	23	-	-	-	-
Anahuac, Nuevo Leon, Mexico[ŧ]	NL_2	22	-	-	-	-
Allende, Coahuila, Mexico[ŧ]	COAH_2	23	-	-	-	-

Region A: [§], Region B: [×] and Region C: [ŧ], in Mexico.

showing between 4 to 30 alleles per locus overall. On average, observed heterozygosity was lower than expected in three out of the 4 prairie Canada sub-populations. After Bonferroni correction ($P = 0.05/9 = 0.005$), when prairie Canada was analyzed as one population, significant deviations from Hardy-Weinberg equilibrium were found in two loci (LLU#011 and LLU#40, $P < 0.005$). When the prairie Canada population was divided into 4 sub-populations, 4 loci deviated from HW equilibrium; including LLU#20, LLU#011, LLU#40, and LLU#089 (all $P < 0.005$; Table 3.2). Although deviations from HW equilibrium were found and genotyping errors or null alleles could not be completely ruled out as a cause, as the deviations increased when prairie Canada was divided into 4 sub-populations, the most likely explanation is an over-representation of homozygosity caused by sampling sub-populations (i.e.,Wahlund effect; Wahlund 1928). Population genetic theory suggests that if a population contains multiple genetic sub-populations, an excess homozygosity is likely to be found (Wahlund 1928).

Fisher's exact test after Bonferroni correction (0.05/36 = 0.001), found that ten loci-pair comparisons (out of 36) showed highly significant linkage disequilibrium (LD) in prairie Canada which can lead to association between alleles in a population. Although, at this time it is impossible to identify the cause, based on the results of traditional statistics, admixture and gene flow are the likely causes (Ardlie et al. 2002).

3.3.2. ANALYSIS OF POPULATION GENETIC STRUCTURING

Analysis of molecular variance (AMOVA) showed that most of the variance (98.98%, $P < 0.001$) in the prairie Canada sample was attributed to within-shrike sub-populations and only 1.02% is attributed to differentiation among shrike sub-populations. Locus-by-locus AMOVA showed that this same pattern of partitioning of variance

Table 3.2. *P*-values of test for loci (9) deviations from Hardy-Weinberg equilibrium in prairie Canada (as one population) and the 4 sub-populations.

	LOCI								
	LLU20	**SJR4**	**LLU11**	**LS4**	**LLU011**	**LLU40**	**LLU45**	**LLU89**	**LLU113**
Prairie Canada	0.06	0.50	0.22	0.24	0.000 *	0.000 *	0.02	0.21	0.01
Alberta	0.06	1.0	0.71	1.0	0.03	0.008	0.75	0.64	0.03
Sask.	0.15	0.39	0.62	0.34	0.001 *	0.001 *	0.09	0.90	0.02
SE Sask	0.003 *	1.0	0.03	1.0	0.15	0.005 *	0.12	0.41	0.97
SW MB	0.04	0.04	0.01	0.01	0.003 *	0.07	0.16	0.00 *	0.31

* Loci deviating significantly from HW equilibrium after Bonferroni correction ($P = 0.05/9 = 0.005$).

within- and among- population variations is consistent across all loci. Averaged over 9 loci, 99.3% and 0.7% of differentiation is attributed to within- and among-shrike sub-population in prairie Canada, respectively, $P < 0.001$).

All pairwise F_{ST} values were relatively low (all $F_{ST} < 0.031$), and none were significantly different from zero following table-wide corrections for multiple comparisons. In general, F_{ST} values varied little between the 4 sampling areas in prairie Canada (Table 3.3) indicating negligible genetic differentiation in shrikes in prairie Canada. The largest pairwise F_{ST} value was between southeastern Saskatchewan and southwestern Manitoba ($F_{ST} = 0.031$), while the lowest was between southern Alberta and Saskatchewan ($F_{ST} = 0.006$). As the low F_{ST} estimates suggested, gene flow estimates (N_m), indicated a high rate of gene flow between all sampling areas in prairie Canada, with the lowest rate being 6.4 between southeastern Saskatchewan and southwestern Manitoba (Table 3.3), while the highest migration rate is 33.2 between Saskatchewan and southwestern Manitoba.

The posterior probability under the admixture and correlated allele frequency model suggested that a K of 1 was the most parsimonious model, implying little genetic structure within prairie Canada (Table 3.4). However when using prairie Canada as one cluster and $K = 2$, the assignment probabilities of the 4 'unknown' prairie Canada individuals to the prairie Canada genetic cluster were of 19.1%, 97.8%, 3.7%, 78.4%, respectively. Only one individual was assigned back to prairie Canada with a $Q > 90\%$. When using the 4 identified clusters described in the methodology, as prior population information and using $K = 5$, the 4 'unknown' prairie Canada individuals representing each of the identified genetic clusters were assigned back to prairie Canada with high Q's

Table 3.3. Pairwise estimates of F_{ST} (lower) and N_m (upper) between Loggerhead Shrike populations in prairie Canada based upon microsatellite genotypes, (AB = Alberta, SK = Saskatchewan, SE SK = Southeastern Saskatchewan and SW MB = Southwestern Manitoba).

	AB	SK	SE SK	SW MB
AB	-	11.831	7.578	8.743
SK	0.006	-	7.068	33.217
SE SK	0.027	0.024	-	6.396
SW MB	0.010	0.010	0.031	-

Table 3.4. Estimates of log and posterior probability of data under various assumption for K = 1-6, to infer the number of genetic clusters (K) for prairie Canada.

	Admixture and correlated model	
	log P (X|K)	posterior probability
1	4877.6	~1.0
2	4644.3	0
3	4512.3	0
4	4579.0	0
5	4544.0	0
6	4421.9	0

(> 90%, in all cases). Using these genetic cluster designations for prairie Canada populations as training samples 18.6% of shrikes sampled in Mexico Region A, 20.3% of shrikes sampled in Region B and 8.3% of samples from Region C had a high *Q* and similar allele frequency as to those selected for prairie Canada (Fig 3.3).

3.4. DISCUSSION

Results of the Bayesian assignment tests indicated that regions A and B were preferred wintering areas of migratory Loggerhead Shrikes within the sampled areas in Mexico; consisting of 18.6% and 20.3% of all captured shrikes within these regions, respectively. These results agreed with previous accounts that northeastern (Howell and Webb 1995, Yosef 1996) and north-central (Hobson and Wassenaar 2001) Mexico are used by wintering migratory shrikes, most likely *L. l. excubitorides*.

The relatively low proportion of migratory individuals (8.3%) found in Mexico sampling Region C, provides preliminary evidence of a chain migration pattern. That is, Loggerhead Shrikes from northernmost breeding areas winter at more northerly wintering latitudes than individuals from more southerly breeding areas. Similar to leapfrog migration (Berthold 2001), chain migration may have evolved to counter intraspecific competition (Cox 1968). Historically, Loggerhead Shrikes were believed to occupy the Gulf Coast of Mexico only in winter (Miller 1931, Yosef 1996, Lefranc and Worfolk 1997). However, the northern parts of the Gulf Coast of Mexico appear to be occupied by both residents and migrants in winter (see below). If resident shrikes occupy those areas year-round, intraspecific competition in winter may occur.

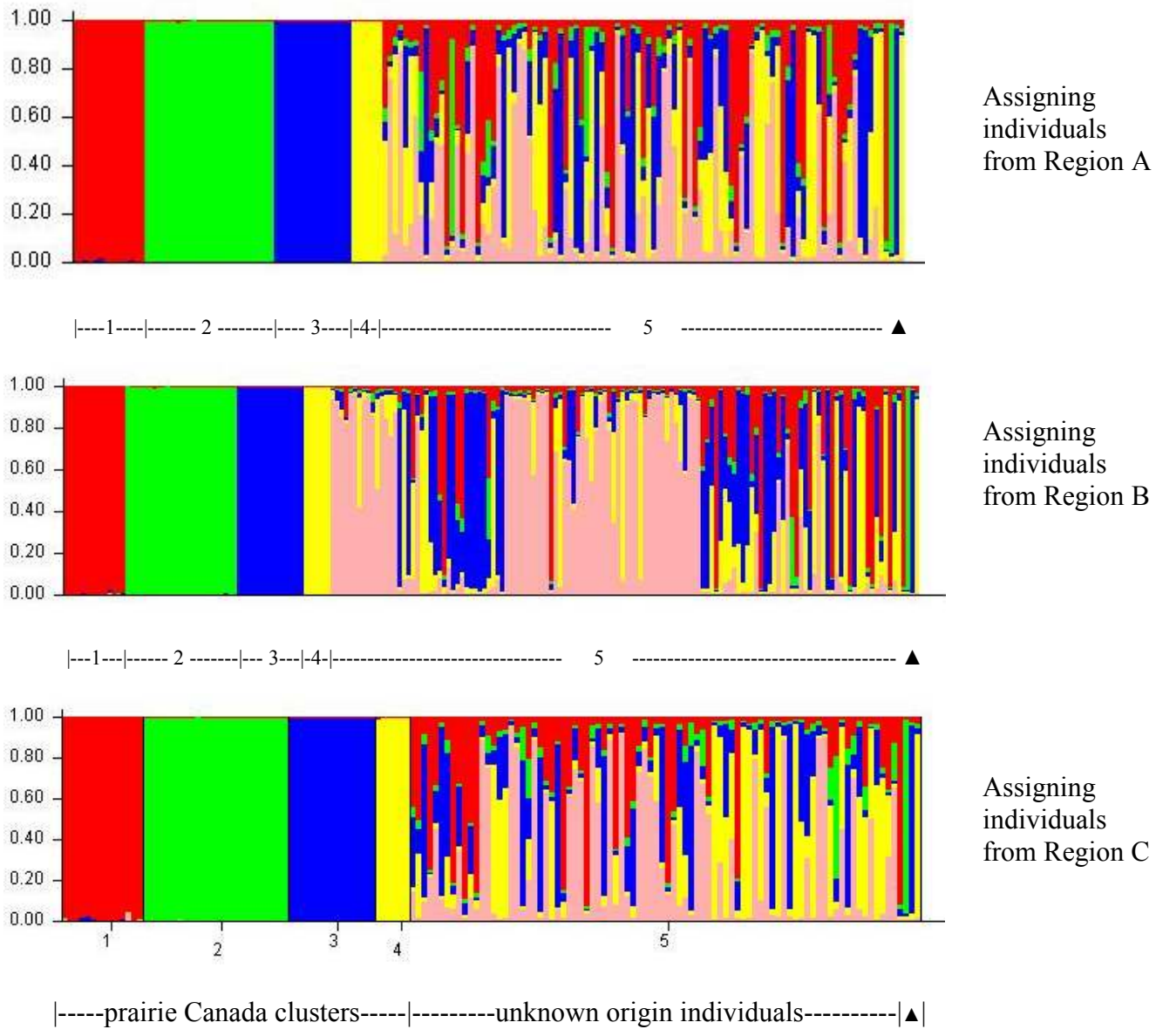

Figure 3.3. Bayesian analysis of unknown individuals sampled in 3 sampling regions in Mexico to source clusters from prairie Canada and using $K = 5$. Each bar represents an individual. The y-axis is the estimate of membership (Q) of an individual being assigned to one of 5 genetic clusters. Last 4 individuals, labeled ▲, are prairie Canada individuals concealed as 'unknowns' to test assignment capabilities at each run.

Interestingly, Region B was the only Mexican sampling area that assigned a considerable number of individuals (24%) to a genetic cluster other than those identified for prairie Canada. This suggested that, in winter, Region B was an area occupied by migrant shrikes most likely from prairie Canada, as well as by, either, a putative resident population or perhaps another migratory population. This is important because previous accounts (Howell and Webb 1995, Yosef 1996, Lefranc and Worfolk 1997) suggested that areas around the Mexican Gulf Coast were only occupied by migrant shrikes in winter. Alternatively, these results suggested that perhaps there is a local resident population there year-round, or that resident populations are expanding their wintering ranges, as noted in Pennsylvania by Hunter et al. (1995). In contrast, in winter, regions A and C appeared to mostly harbour individuals with various levels of admixed ancestry which were, in part, traceable to those clusters identified for prairie Canada. Possibly, these levels of admixed ancestry represent levels of gene flow among the species or traces of post-glacial northern range expansion of the species (Mundy et al. 1997b). I surmise that a more accurate classification of these individuals showing admixture, could be done when more source populations can be used as prior population information. Puzzling, however, was the fact that no shrike sampled in Mexico assigned strongly to Cluster 2 (from prairie Canada), even though it was the cluster with the most representatives. Currently, I have no explanation for this result. However, perhaps, most individuals from prairie Canada winter in areas other than the ones sampled.

Posterior probability of Bayesian structure analysis of microsatellite DNA using no *a priori* information revealed that Loggerhead Shrikes from Alberta to southwestern Manitoba form a single genetic cluster (i.e., K =1). These findings were supported by

traditional statistics. Pairwise estimates of genetic diversity, F_{ST}, suggested little genetic diversity in shrikes between all sampling areas in prairie Canada (all $F_{ST} < 0.031$). These results are in agreement with other shrike genetic studies (Chabot et al. unpublished) and studies of other species (Greater Prairie Chicken, *Tympanuchus cupido pinnatus*; Johnson et al., 2003, Sandhill Cranes, *Grus canadensis*; Jones et al. 2005).

A likely explanation for the low levels of genetic differentiation in prairie Canada populations was attributed to dispersal as expressed by the high levels of gene flow (i.e., all $N_m > 6.40$). These high levels of gene flow suggested that shrikes from Alberta to southwestern Manitoba sustain similar allele frequencies which indicated that no barrier, such as fragmentation, is restricting gene flow. Studies of marked Loggerhead Shrikes typically exhibit very low levels (< 2% return rates) of natal philopatry (Haas and Sloane 1989, Collister and De Smet 1997). Natal dispersal results in long-distance gene flow over short ecological time scales by long-distance dispersal of individuals or incrementally by shorter distance dispersal over several generations. Regardless, it is important to remember that high levels of gene flow would tend to reduce genetic differentiation making demographic structure difficult to evaluate using molecular techniques (Kimura et al. 2002).

Analysis of molecular variance (AMOVA) of populations sampled in prairie Canada revealed that most of the genetic diversity found there was attributed to within-population differentiation (98.98%, $P < 0.01$). Both traditional F_{ST} and Bayesian analysis of microsatellite DNA data did not reveal any evidence of a putative hybrid zone occurring in southeastern Saskatchewan – southwestern Manitoba, as suggested by Miller (1931) and Vallianatos et al. (2001), based on morphology and mitochondrial and nuclear

intron DNA data, respectively.

Bayesian clustering methods using microsatellite DNA data from feathers of Loggerhead Shrikes clearly indicated that grouping individuals according to their estimate of membership (*Q*), as opposed to using a single *K*, was far superior at assigning known Canadian shrike individuals (concealed as unknowns) to sources. Although I can not be certain of the biological significance of the 4 genetic clusters uncovered in prairie Canada, I surmise that they could be indicative of ancestry, from post-glacial northern range expansion of the species (Mundy et al. 1997b), and subsequent dispersal. When a single genetic cluster from prairie Canada was used as a training cluster to assign unknown Mexican individual to prairie Canada, the prairie Canada individuals placed as unknowns were not consistently assigned back (i.e., *Q*'s of 19.1%, 97.8%, 3.7% and 78.4). Several reasons for this are possible. First, as admixed individuals appear in both source and unknown origin populations, they could be acting as 'outliers' in the analysis (e.g. Haig et al. 1997). Second, the data did not completely respect the assumptions of complete HW and linkage equilibrium. Third, when allele frequencies vary gradually across a region (i.e., isolation by distance), the Bayesian approach is not well suited to identify discrete population structure (Pritchard and Wen 2004). Because most alleles are widespread, perhaps genetic differences among Loggerhead Shrikes individuals in prairie Canada, perhaps derive mainly from a cline in allele frequency rather than from distinctive "diagnostic" genotypes (e.g., Rosenberg et al. 2002). As a result of the inconsistencies in assignment of known origin individuals, this approach was abandoned.

Although this study identified specific genetic markers for northernmost Loggerhead Shrike prairie Canada populations, it did not have complete sampling across

all putative genetic subsets. Therefore, I strongly recommend that future connectivity studies should first identify all potential source clusters, as it will likely greatly increase assignment accuracy. The ability to assign unknown individuals to sources is likely limited by such incomplete sampling; however, we did successfully employ microsatellite and Bayesian clustering methods to link breeding with wintering grounds of migratory Canadian Loggerhead Shrikes and I thus have made a significant advance to the sparse knowledge previously gained from 50 years of banding. The genetic evidence provided here revealed that a significant proportion of *L. l. excubitorides* from northern parts of the Great Plains winter in regions A and B in Mexico. Additionally, a single genetic cluster occupies prairie Canada, which most likely extends into the Great Plains of the United States. This result disagrees, in part, with the putative subspecies designation recognized by Miller (1931), who believed that southwestern Manitoba was part of a hybrid population. I provided some tantalizing evidence of a chain migration pattern although fully describing this pattern will require further research, as the southernmost areas inhabited by shrikes remain unsampled (e.g., Chiapas). Finally, the results presented here showed that DNA microsatellite markers coupled with new Bayesian assignment methods may be a powerful tool in studies of bird migration to identify breeding ancestry in different geographical areas and parts of their annual migratory cycle.

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CHAPTER 4: STRUCTURE AND VARIABILITY OF WINTER HABITATS USED BY RESIDENT AND MIGRANT LOGGERHEAD SHRIKES (*Lanius ludovicianus*) IN MEXICO

4.1. INTRODUCTION

Similar to several North American grassland-associated species, the Loggerhead Shrike (*Lanius ludovicianus*) is declining throughout much of its range (Yosef 1996, Cade and Woods 1997). In Canada, the Loggerhead Shrike is a Species at Risk; eastern populations (*L. l. migrans*) are considered "Endangered", while western, prairie Canada populations (*L. l. excubitorides*) are considered "Threatened" (Committee on the Status of Endangered Wildlife in Canada, COSEWIC; Cadman 1990, Cadman 1985, respectively). Such population declines are undoubtedly associated with loss of breeding habitat through agriculture and loss of grasslands (Prescott and Collister 1993, reviewed by Pruitt 2000). However, little is known about habitat loss on the wintering grounds where the species spends most of its annual cycle (John et al. 1994, Yosef 1996, Hobson and Wassenaar 2001).

In the fall, shrikes from northern breeding areas move south along the Gulf States of the United States (U.S.) (Burnside 1987) and Mexico (Miller 1931, Hobson and Wassenaar 2001) to wintering areas that are already occupied year-round by resident shrikes (Miller 1931, Temple 1995). There, it has been suggested that Loggerhead Shrikes compete intraspecifically (Miller 1931) and interspecifically (Kim et al. 2003). However, previously, it has been difficult to impossible to distinguish resident from migratory shrikes using conventional approaches. Fortunately, the use of biogeochemical tracers (i.e. stable-hydrogen isotopes) has enhanced my ability to separate migrants from

residents on the wintering grounds (Webster et al. 2002, Hobson 2005). This approach is based on the fact that naturally occurring deuterium (^{2}H, measured as δD) abundance in growing-season precipitation are correlated with deuterium concentrations in feathers (Hobson and Wassenaar, 1997; Chamberlain et al. 1997). To the advantage of this study, in North America, δD patterns in precipitation are closely related to latitude (Hobson and Wassennar, 1997), so it is possible to categorize those birds that grew their feathers locally from those that grew their feathers farther north.

As a first step in identifying habitat requirements of Loggerhead Shrikes wintering in northeastern Mexico, I captured wintering individuals and used stable-hydrogen isotope analysis of feathers to distinguish migrants from more northern areas from local Mexican residents. This allowed me to determine if habitat requirements of northern birds on the wintering grounds differed from local birds. I reasoned that if habitat occupancy differed between these two groups, that would provide evidence for potential competition between residents and migrants on the wintering grounds. Finally, by describing such habitat requirements, I sought to understand if habitat loss on the wintering grounds was a potential factor in continental population declines in this species.

4.2 METHODS

4.2.1 STUDY AREA

I conducted roadside searches (Bjorge and Prescott 1996, Bohall-Wood 1987, Hobson and Wassenaar 2001) to survey wintering shrikes in Mexico. Fieldwork was conducted from 3 February to 2 March 2004 in five sites in northeastern Mexico in the States of Coahuila, Nuevo Leon and Tamaulipas (Fig. 4.1). The ecological zones

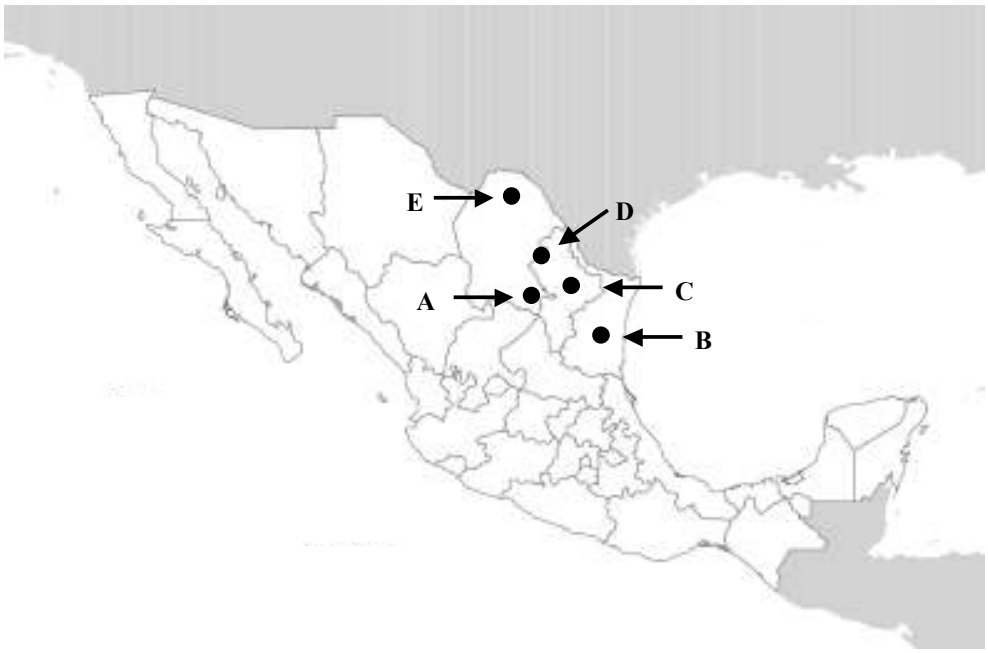

Figure 4.1. Map of Mexico showing the localities where Loggerhead Shrikes habitat surveys were conducted during the winter of 2004. Site A corresponds to Sierra de Zapalinamé, south of Saltillo, Coahuila. Site B corresponds to areas between Ciudad Victoria and San Fernando, Tamaulipas. Site C corresponds to China, Nuevo León. Site D corresponds to areas between Sabinas de Hidalgo and Anahuac, Nuevo León, and site E corresponds to Morelos, Coahuila.

surveyed were the mesquite-grasslands, deserts, chaparral regions (Leopold 1950, Rzedowski 1981) and agricultural regions. The most common grassland cover types included a mix of mesquite (*Prosopsis glandulosa*), huisache (*Acacia farneasiana*), *Yucca* species (spp.), *Opuntia* spp., gatuño (*Mimosa* spp.), gobernadora (*Larrea tridentata*), *Agave* spp., granjeno (*Celtis* spp.), hojacen, (*Flourensia cernua*), and cenizo (*Leucophyllum frutescens*).

The search for Loggerhead Shrikes was conducted by driving secondary roads within ~100 km of a field-base. When a shrike was detected, habitat features were recorded within a 100 meter radius (3.1 ha) for height and percent cover class. The habitat features from a given circular plot were used to describe an "occupied" site regardless of the number of birds on the site. To characterize the structure and composition of a particular site, habitat features were averaged across sample plots (e.g. I lumped all tall shrubs regardless of species). Habitat features considered were grass, shrubs (including trees), cultivated land, bare ground (unvegetated soil, roads, railroads, and water), mesquite and huisache. The height of the dominant vegetation was measured at each point (± 5 cm for vegetation of < 2.0 m and ± 30 cm for vegetation $\geq$ 2.0 m). From a total of 354 points (occupied and unoccupied combined), percent cover was calculated (in percent classes) of tall grass $\geq$ 0.3 m. (TGC), short grass < 0.3 m. (SGC), tall shrubs $\geq$ 3.0 m. (TSC), medium shrubs < 3.0 and $\geq$ 1.0 m. (MSC), short shrubs < 1.0 m. (SSC), crop (CC), bare ground (BC), mesquite (MC), and huisache (HC). Percent classes were as follows; 0 to 10% = 1, 11 to 20% = 2, 21 to 30% = 3 and so on, all the way to 91 to 100% = 10. I also calculated mean height of tall grass (TGH), short grass (SGH), tall shrubs (TSH), medium shrubs (MSH), short shrubs (SSH), mesquite (MH),

and huisache (HH). To compare occupied habitats with a sample of habitat available at random, systematic 10 to 20 km apart random points (n = 64) were sampled within each site and measured for each of the same habitat variables. Each habitat point was plotted using a Universal Transverse Mercator System (UTMS), NAD27-Mexico, with a Global Positions System (GPS) unit.

Feather collections for stable isotope analyses were obtained using a walk-in trap baited with a protected live mouse. Birds were aged according to moult sequence based on presence or absence of retained juvenile feathers, as well as feathers P6 characteristics as described in Pyle (1997). Protocol for capturing and handling shrikes was approved by University of Saskatchewan Animal Care Committee according to Canadian Council for Animal Care protocols. Field work and capture permits were obtained from the Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT).

4.2.2. STABLE ISOTOPE ANALYSIS

Feather samples were cleaned in 2:1 chloroform:methanol solution overnight, drained and air dried under a fumehood also overnight. Feather vanes were then cut and 350μg ±10μg weighed into 4.0 x 3.2 mm silver capsules for online hydrogen isotope analysis by continuous-flow isotope-ratio mass spectrometry (CFIRMS). Analytical measurements of hydrogen stable isotope (δD) followed a "comparative equilibration" technique described by Wassenaar and Hobson (2003). Isotopic values were expressed in delta notation in parts per thousand (‰) as the non-exchangeable hydrogen portion of samples normalized on the VSMOW-SLAP (Vienna Standard Mean Ocean Water-Standard Light Antarctic Precipitation) standard scale. Feather samples were analyzed at the stable-isotope facility of the National Water Research Centre in Saskatoon, Canada.

To categorized birds into northern migrants or Mexican residents, I compared the observed feather δD values (δD_f) of the innermost tertial feather S9 with the expected δD_f for North American raptors for northeastern Mexico derived from Lott and Smith (2006). Resident birds that grew their feather in northeastern Mexico were expected to have δD_f of -35‰ and more enriched, whereas birds that grew their feathers at more northern latitudes than the sampling area (considered migrants) were expected to have δD_f values of < -35‰.

4.2.3. STATISTICAL ANALYSIS

The distribution of each variable was evaluated for normality using Kolmogorov-Smirnov test (Sokal and Rohlf 1995) and rank transformed in an attempt to achieve normality using SPSS (2005). Next, I tested for between-group (i.e. resident vs. migrant) differences using multiple variables simultaneously with the multi-response permutation procedure (MRPP; Mielke and Berry 1982, Zimmerman et al. 1985) in PC-ORD (McCune and Mefford 1999). MRPP is a nonparametric multivariate method that does not require assumptions of multivariate normality and homogeneity of variances. The strategy of using MRPP was to establish whether there were significant differences in habitat structure between occupied vs. unoccupied, resident vs. migrant sites, and age classes. MRPP compares the observed between-group average Euclidean distances with the average distances that would have resulted from all other possible combinations of the data under the null hypothesis. I then ran a Monte Carlo test (10,000 permutations) using the Indicator Species Analysis (ISA) tool (Dufrene and Legendre 1997) in PC-ORD, to evaluate which habitat features were the main contributors to the separation in

the groups (i.e. presence/absence, migrant/resident). This method combines information on the concentration of species abundance in a particular group and the faithfulness of occurrence of an individual in a particular group (Dufrene and Legendre 1997).

As an alternative approach, I used an information-theoretic approach (Burnham and Anderson 1998), using the habitat contributors selected through ISA, to evaluate habitat structure of sites with and without shrikes and of those occupied by residents vs. migrants. I chose this method, because of its potential to provide a more meaningful insight for conservation, planning and for future research (Anderson et al. 2000). The Akaike's Information Criterion (AIC) model selection approach relies on comparing a set of models representing multiple competing hypotheses about ecological processes (Hobbs and Hilborn 2006). This method allows one to select the best model (or set of models) from an *a priori* variables, rank and scale the models, and include model selection uncertainty into estimates of precision (Burnham and Anderson 2001). This approach is based on the principle of parsimony, compromising model fit and precision. Hypotheses with lower AIC values are most parsimonious. With the data at hand, competing models were corrected for small sample size (AIC_c) (Burnham and Anderson 1998). Generally, models differing by less than 2 AIC_c units exhibit strong support relative to competing models. Differences in AIC_c units of 2-4, 4-7, and more than 7 exhibit some, little, and no support relative to other models, respectively (Burnham and Anderson 1998). Maximized log-likelihood was obtained from binary logistic regression in SPSS version 14 and AIC_c computations were calculated using Excel. I calculated ΔAIC_c which is the difference between a particular model and the model with the lowest AIC_c value, and normalized Akaike weights, so we had a relative measure of how well a

model fits the data, compared with competing models (Burnham and Anderson 1998, (Anderson et al. 2000).

4.3. RESULTS

Shrikes were present in 7 out of the 64 randomly sampled sites, and so these 7 sites were included within the "occupied" group. In total, 297 sites were occupied by shrikes and 57 were not. For those birds that I measured δD values in feathers, 43 sites were occupied by resident shrikes, and 103 were occupied by migrants. Age did not play a role in habitat selection (MRPP, T = -0.42, P = 0.28, n = 146), so individuals of different ages were lumped in further analyses. I found a significant difference between the habitat structure of sites occupied by shrikes and those that were not (MRPP, T = -8.04, $P < 0.001$, n = 354), as well as between those sites occupied by residents and those occupied by migrants (MRPP, T = -2.95, P = 0.01, n = 146)

The habitat features that were identified as indicating presence/absence of shrikes in northeastern Mexico were percent cover of tall shrubs (ISA; P = 0.003), percent cover of mesquite (ISA; P = 0.01), percent cover of huisache (ISA; P = 0.01), average height of tall shrubs (ISA; P = 0.01), and average height of huisache (ISA; P = 0.01). On average, the unoccupied habitat contained a greater mean height of tall shrubs (mean difference of > 2.0 m; Table 4.1) and huisache (mean difference of > 0.5 m), and a greater percent cover class of tall shrubs, mesquite and huisache. Percent cover of bare ground was the only habitat feature indicating differences between those sites occupied by residents vs. migrants (ISA; P = 0.002). Migrant shrikes occupied sites where bare ground was more predominant.

Table 4.5. Mean height of tall shrubs and huisache between those sites occupied vs. unoccupied by Loggerhead Shrikes in northeastern Mexico in winter. In both cases, vegetation of unoccupied sites had a greater mean height than occupied sites.

Variables	Occupied		Unoccupied	
	Mean	SD	Mean	SD
Tall shrub height	5.08	0.27	7.22	0.16
Huisache height	2.93	1.11	3.52	1.52

The model with the lowest AIC_c and greatest empirical support (64%) in the candidate model set was the additive model of percent cover of tall shrubs, mesquite, and huisache (TSC + MC + HC; Table 4.2). The second-best model, TSC*TSH + MC received 21% of the support ($\Delta AIC_c = 2.23$). All models with a TSC effect had lower AIC_c than the models without it.

4.4. DISCUSSION

Wintering sites occupied by Loggerhead Shrike sites in northeastern Mexico were structurally different from unoccupied sites. An important difference was that, on average, occupied habitats contained shorter tall shrubs and huisache plants and fewer tall shrubs, mesquite and huisache. Similarly, habitats occupied by resident shrikes were structurally different from those occupied by migrants. On average, resident shrikes occupied sites that had taller mesquite vegetation. Based on that differential habitat occupancy differences were found, I surmised that habitat availability may be a likely limiting factor for both resident and migratory shrike populations in northeastern Mexico.

It is no secret that loss and degradation of the North American natural grassland is a major factor affecting the decline in avian populations in both breeding and wintering grounds (reviewed in Newton 2004); including shrikes (Lymn and Temple 1991, Cade and Woods 1997). Presence of other raptors within the wintering territories of wintering shrikes has also been suggested as a factor restricting shrike's use of habitats (Kim et al. 2003). Such interspecific competition may well displace resident and migrant shrikes to marginal habitats, potentially enhancing competitions for food (Sherry et al. 2005). Since several authors have suggested that nonmigratory populations seize year-round territories

Table 4.6. Selection results for models explaining variation in winter habitat occupancy by Loggerhead Shrike in northeastern Mexico. Models include the best model (lowest AIC_c value) and candidate models with more than 2 units from the best model. Number of parameters (*K*) and Akaike weights for each model are provided. The seven models examined include, TSC = tall shrub cover, MC = mesquite cover, HC = huisache cover, TSH = tall shrub height, HH = huisache height. The additive effect of percent cover of tall shrub, mesquite, and huisache received the most support occupied Loggerhead Shrike in northeastern Mexico in winter.

Model	*K*	AIC	Δ AIC_c[a]	Akaike Weight[b]
TSC + MC + HC	5	285.9	0.00	0.64
TSC*TSH + MC	4	288.2	2.23	0.21
TSC*TSH + HC*HH	4	289.9	3.93	0.09
MC + HC*HH	4	292.2	6.20	0.03
TSH + HT + MC	5	292.4	6.44	0.03
MC + HC	4	301.2	15.3	0.00
MC*HC	3	307.9	21.9	0.00

[a] Difference between AIC_c of the current model and the minimum observed value.
[b] Normalized Akaike weight (Burham and Anderson 1998).

(Miller 1931, Temple 1995) and can even increase the size of their territory in winter in search of prey (Blumton 1989, Hunter et al. 1995), I suggest that migrant populations are potentially more greatly limited by habitat availability than residents.

Undisputedly, the coast of the Gulf of Mexico has been affected by habitat loss and degradation due to effects of development, agriculture, livestock, deforestation, and introduction of exotic species since the early 1900s (reviewed in Barrow et al. 2005). The conversion of the native Tamaulipan thornscrub vegetation into rowcrop plantations (e.g., rice, sorghum and citrus) and the encroachment of woody vegetation from overgrazing and fire suppression have created a shift in avian communities attracting grain-eating and scrub-dependent species, respectively (Kridelbaugh 1981, Lymn and Temple 1991, Lloyd et al. 1998, Kirkpatrick et al. 2002). Interestingly, populations of members of the family Icteridae have steadily increased over the same period (~ 60 years) that shrike populations sharing the same wintering areas have steadily decreased (Brooks and Temple 1990).

The absence of data on historical and current land-use in relationship to Loggerhead Shrike abundances in northeastern Mexico makes it difficult to generalize the effects of habitat loss to agriculture and woody vegetation encroachment occurring there. Northern Mexico has been undergoing desertification from overgrazing for quite some time (Estrada Berg et al. 1999) and a drought since 1992 (Macías-Duarte et al. 2004); both which are suggested to directly affect bird and prey abundance in arid places (Newton 1998, Jamus et al. 2003, respectively). Moreover, management practices of lease hunting, a popular enterprise of the Tamaulipan thornscrub areas, that advocate the removal of thornscrub in strips, has been suggested to increase edge habitat for game

species (Barrow et al. 2005), but reduce overall avian diversity (Vega and Rappole 1994). Historically, northern Mexico did not experience the same level of urbanization and habitat alteration that occurred in the United States (Enriquez 2001). Initially, the presence of Apache Indians in the late 1800s in northern Mexico and the subsequent Mexican revolution delayed water development and livestock before 1940 (Enríquez 2001). Furthermore, the habitat devastation rendered by red fire ants to some of U.S. Gulf States which boosted the use of pesticides did not enter the desert areas of Texas (Lynm and Temple 1991), and presumably Mexico.

Questionably, I may have had a number of road-biases in my study design. Habitats along roadways may change at a different rate than off-road habitats (Keller and Scallan 1999), and may not accurately reflect the overall habitat and land-use of the general area (Best et al. 1995). Consequently, roads have been shown to attract a different bird community under- and over-estimating of some bird species (Rotenberry and Knick 1995, Sutter et al. 2000), because they usually over represent the abundance of exposed soil (road) and pastureland (mowed or burnt). However, with that said, although I used roads to search for shrikes, not all shrikes were detected right alongside roads, so habitat descriptions on those observations did not account for roads considerably or at all. In other instances, narrow vehicle trails in areas with no ground cover (e.g. in deserted areas), did not appear to break the landscape, and therefore unlikely to have biased those plots.

To the best of my knowledge, this is the first time that structure and variability of habitat used by wintering Loggerhead Shrikes have been investigated in Mexico. From these results, I concluded that habitats occupied by Loggerhead Shrikes were structurally

different from those that were not. Similarly, I found differences in habitat structure between those habitats that were occupied by resident and those that were occupied by migrant shrikes. Based on that differences were found, I surmised that habitat availability may be a limiting factor, potentially enhancing competitions for food and habitat, for both resident and wintering shrike populations in northeastern Mexico. I recommend that future studies should investigate reproductive rates and density-dependent responses, such as competition for habitat and food, to resident populations during the summer. Since, in theory, as habitat gets smaller, the competition for such constraints increases as density of individuals increases, reducing reproduction (Sherry and Holmes 1995). I also encourage the assessment of over-winter survival rates between resident and migrants and a more detail habitat assessments to identify source and sink habitats. My results on habitat used by Loggerhead shrikes during winter in Mexico will establish important baseline data for succeeding wintering studies of shrikes and other grassland birds. Moreover, these data may also be used to compare habitat features in other parts of the Loggerhead Shrike's wintering range where they are less common or habitat limiting.

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CHAPTER 5: SUMMARY AND SYNTHESIS

Establishing avian migratory connectivity, the linking of breeding, stopover, and wintering grounds of individuals and populations, allows a better understanding of where in the annual cycle limitations to populations may be occurring. In North America, a major obstacle in creating such migratory links has been the difficulty of tracking individuals during migration and onto their wintering areas (Webster et al. 2002). Traditional extrinsic markers, such as leg bands, have not proven useful, because for the vast majority of species, they have not provided sample sizes large enough to have sufficient statistical power for useful inference (reviewed in Hobson 2003). Fortunately, intrinsic markers, such as the relative abundance of naturally occurring stable isotopes of elements common to foodwebs (C, N, O, H, S) and population genetics have recently assisted researchers in making connections. Also, a major benefit of these techniques is that they do not rely on recaptures of marked individuals or constant tracking (Hobson 2003, Rubenstein and Hobson 2004).

The western Loggerhead Shrike, *Lanius ludovicianus excubitorides* Swainson, is a threatened species in Canada, and its decline is attributed to habitat loss on both the breeding and wintering grounds (Johns et al. 1994). While population trends and habitat availability is reasonably well known on the breeding grounds, little is known about such factors on the wintering grounds. For this research, I relied on stable-hydrogen isotope analysis of feathers and claws and microsatellite DNA analyses of feather bases to provide spatial information of the origins of individuals captured on the wintering grounds. This approach was adopted to: 1) elucidate migratory connectivity of

Loggerhead Shrikes wintering in Mexico, and 2) explore habitat structure used by resident and migratory Loggerhead Shrikes wintering in northeastern Mexico.

Creating migratory connectivity using stable isotope measurements of avian tissues depends, in part, on choosing the tissue which most accurately reflects the location where that tissue was grown. In Chapter 2, I examined patterns of stable-hydrogen isotope distributions in feathers of Loggerhead Shrikes wintering in Mexico, with the intention of linking breeding and wintering grounds of migratory *L. l. excubitorides*. I also investigated potential movements of Mexican winter resident individuals. For the latter, I developed a shrike-specific deuterium base map for Mexico, using shrike museum specimens of known summer provenance,. The stable-isotope approach is based on the fact that naturally occurring deuterium (^{2}H, measured as δD) abundance in growing-season precipitation are closely related to latitude and can be correlated with deuterium concentrations in feathers (Hobson and Wassenaar, 1997). Stable-hydrogen isotope analyses of inner secondary feather (S9) of all Loggerhead Shrikes examined in Mexico during winter indicated that northeastern (States of Coahuila, Nuevo León, and Tamaulipas) and south-central (States of Aguascalientes, Jalisco, and Michoacán) sites in Mexico consisted of 63.8% and 73.7% of migrant individuals from northern breeding grounds. Also, 28.1% of the shrikes captured at the north-central Mexico site (States of Chihuahua and Durango) were migrants. These results suggested a leapfrog migration pattern, where birds from the northernmost breeding ranges from northern U.S. and Canada migrated to the southernmost sampling region (Aguascalientes, Jalisco and Michoacán) to winter. Additionally, isotopic evidence suggested dispersal of birds moving into the Chihuahuan desert from southwestern U.S.

and northeastern Mexico to winter. This finding is interesting because presently it is not know the extent to which resident populations disperse or migrate. Several authors have suggested that nonmigratory shrikes seize year-round territories (Miller 1931, Brooks and Temple 1990), while others have reported that they move from year-to-year, possibly in response to changes in food supply (Kridelbaugh 1983, Yosef 1996) or weather (Miller 1931, Hunter et al. 1995). Moreover, it has been suggested that migratory shrikes may display a step-migration pattern, where they may use more than one wintering site and migrate between them (Safriel 1995). However, unequivocally, those birds sampled in the Chihuahuan desert (States of Chihuahua and Durango) with more positive δD values than expected for that area, whether they were residents or migrants, used areas around the Gulf of Mexico as molting grounds before moving to the Chihuahuan desert to winter.

Population-specific DNA markers can be used successfully to identify breeding population affinity of individuals during all stages of the annual cycle (Webster et al. 2002). In theory, when genetic variation is structured geographically on the breeding grounds, breeding provenance of individuals sampled during migration or on wintering sites can be identified based on similarity of allele frequency (Haig et al. 1997, Webster et al. 2002). In Chapter 3, I used DNA microsatellite markers and a newly developed Bayesian clustering analysis (Pritchard et al. 2000) to; 1) classify individuals from prairie Canada into a number of genetic clusters probabilistically or jointly to two or more clusters (Pritchard et al. 2000, Rosenberg et al. 2002), and 2) used genetic clusters based on membership of assignment, *Q*, derived from known origin individuals as training samples to classify individuals of unknown origin to population(s) (e.g., Beaumont et al. 2001). Posterior probability of Bayesian structure analysis of

microsatellite DNA using no *a priori* information revealed that Loggerhead Shrikes from Alberta to southwestern Manitoba form a single genetic cluster. Results of assignment tests based on *Q* indicated that sampled areas of north-central (Sates of Chihuahua and Durango) and northeastern (States of Coahuila, Nuevo León and Tamaulipas) Mexico contained a great proportion of wintering migrant shrikes of all sampled areas in Mexico; consisting of 18.6% and 20.3% of all captured shrikes within these regions, respectively. Additionally, the relatively low proportion of migratory individuals (8.3%) found in south-central Mexico (Aguascalientes, Jalisco, Michoacán), provided preliminary evidence of a chain migration pattern, suggesting that shrikes from the northernmost breeding ranges of northern U.S. and Canada winter in more northern areas of Mexico than birds from more southern breeding ranges.

Habitat structure and variability for both migrants and resident Loggerhead Shrikes have not been studied well on the wintering grounds (Johns et al. 1994, Yosef 1996, Hobson and Wassenaar 2001). Thus, it has been difficult to adequately evaluate the relative contribution of changes in wintering areas to the decline of Loggerhead Shrike populations. In Chapter 4, I used multiple response permutation procedures to compare features of utilized Loggerhead Shrike habitats with those of unoccupied, random sites in the same area, as well as those features of habitats utilized by residents versus migrants wintering in northeastern Mexico. The intention of such analyses was to assess differential habitat occupancy between groups. I also used indicator species analyses and Akaike model selection procedures to identify those habitat features most likely contributing to the distinction between occupied vs. unoccupied and resident vs. migrants. To separate migrants from residents on the wintering grounds, I used stable-

hydrogen isotope analyses of feathers to categorize those birds that grew their feathers locally from those that grew their feathers farther north (Hobson and Wassenaar 2001, Pérez and Hobson 2006). Habitat structure analyses indicated that occupied shrike habitat was structurally different from unoccupied habitats. An important difference between occupied versus unoccupied habitats was that, on average, occupied habitat contained shorter tall shrubs and huisache and fewer tall shrubs, mesquite and huisache. Similarly, there was a difference in habitat structure between those habitats that were occupied by resident shrikes and those that were occupied by migrants. Resident shrikes occupied sites that had less percentage of bare ground. Since differential habitat occupancy between presence vs. absence and resident vs. migrant was different, I surmise that habitat availability may be a likely limiting factor for both resident and migratory shrike populations in northeastern Mexico. It is no secret that loss and degradation of the North American natural grassland is a major factor affecting the decline in avian populations in both breeding and wintering grounds (reviewed in Newton 2004); including shrikes (Lymn and Temple 1991, Cade and Woods 1997). Presence of other raptors within the wintering territories of wintering shrikes has been suggested as a factor restricting shrike use of habitats (Kim et al. 2003). Such interspecific competition may well displace resident and migrant shrikes to marginal habitats, potentially enhancing competitions for food (Sherry et al. 2005). Since several authors have suggested that nonmigratory populations seize year-round territories (Miller 1931, Temple 1995) and can even increase the size of their territory in winter (Blumton 1989, Collins 1996), I hypothesize that migrant populations are potentially more greatly limited by habitat availability than residents.

Originally, the intention of this study was to combine the use of stable isotope and genetic techniques to create migratory connectivity (Webster et al. 2002); however, with the data at hand, it was not possible to use them in concert for this species. Currently, only two studies (Clegg et al. 2003, Kelly et al. 2006) have combined genetic and stable isotope techniques for such a purpose and these had partially disparate results. Clegg et al. (2003) found that for Wilson's Warblers (*Wilsonia pusilla*) the combination of genetics and isotopes only revealed general associations (low resolution) between different geographical regions; whereas , in a study of Swainson's Thrush (*Catharus ustulatus*) Kelly et al. (2006) found that they could predict the site-specific origin of thrushes with 76-80% accuracy. The fact that I was not able to use these two techniques in concert was not too surprising given the two very different foundations of the techniques. Stable isotope analyses in feathers only track the movements of individuals within previous season or year, whereas genetics tracks the movement of genes in evolutionary times. Besides, unlike the species of the two above mentioned studies, Loggerhead Shrikes have overlapping breeding and wintering ranges and mixing of residents and migrants in their wintering grounds. In retrospect, I recommend that if these two techniques were to be used in concert for a Loggerhead Shrike study, a more complete sampling of breeding populations across the entire species range, may improve the assignment of unknown individuals to their breeding affiliation; assuming that individuals of the same summering areas are more morphologically, behaviorally, and genetically alike (Miller 1931).

Stable isotope analyses suggested that shrikes from northernmost breeding areas used leapfrog migration to the southernmost wintering area of this study, while genetics

suggested that they used chain migration to the northernmost areas of this study to winter. The discrepancy between these results is of great interest in learning how to use these techniques in concert in future shrike studies, and hence raised various possible explanations. First, granting that stable isotope values suggested that the southernmost sampling region of Mexico (Aguascalientes, Jalisco, Michoacán) was occupied by long-distance migrants, but genetics did not assigned them back to prairie Canada clusters, I hypothesize that shrikes wintering in those regions could well be long-distance migrants, but from areas west of the Rocky mountains (e.g., *L. l. gambeli* or *L. l. nevadensis*; Miller 1931). Second, based on stable isotope values, virtually no shrikes sampled in north-central Mexico (Chihuahua and Durango) were long-distance migrants while genetics revealed the opposite, I hypothesize that those individuals could well be nonmigratory *L. l. excubitorides* from more southern portions of their range (see Miller 1931), so they would have enriched deuterium values but would be *L. l. excubitorides* nonetheless. Ultimately, attempting to couple stable isotope and genetics techniques for this species will not reach its complete potential, until a complete sampling of all breeding populations across the entire range of the Loggerhead Shrikes to obtain all putative expected isotopic and genetic templates, With that said, I recommend three future studies: 1) an investigation of clinal variation in genetic structure of breeding populations across the species range. A more complete breeding genetic affiliation of populations would reduce uncertainty when using assignment tests to train individuals to breeding populations; 2) an investigation of clinal moulting strategy using stable isotopes. Physiological demands of long-distance migration may promote differences among shrike populations at different latitudes, and results in several ecological and

morphological effects (Clegg et al. 2003), such as suspended moulting strategy in northern populations of Loggerhead Shrikes to perhaps compensate for shorter breeding season (Pérez and Hobson 2006); and 3) begin an examination into regional effects (e.g. land use) over demographic processes for Loggerhead Shrikes in Mexico during the reproductive period. Such investigations may enhance the understanding of potential limitations from habitat changes for residents that could be extrapolated to migrants.

The ability to assign wintering *L. l. excubitorides* back to their breeding grounds is still limited. Nonetheless, stable-hydrogen isotope and microsatellite DNA analyses have provided information on origins of long-distance migrant Loggerhead Shrikes that far exceed that gained from 50 years of banding. Overall, these results have provided new evidence that a significant proportion of *Lanius ludovicianus*, most likely *L. l. excubitorides*, from the northern and central parts of their breeding range winter in northern and south-central Mexico. Furthermore, if characteristics of wintering grounds in Mexico are indeed affecting breeding ground productivity and survival of migratory populations, then protecting wintering habitats may present the greatest conservation benefit in terms of maintaining stable breeding populations, while ramifications of conserving those habitats for other wintering populations may contribute to maintaining overall population's genetic diversity.

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APPENDIX 1: ISOTOPIC EVALUATION OF INTERRUPTED MOLT IN NORTHERN BREEDING POPULATIONS OF THE LOGGERHEAD SHRIKE

A.1. INTRODUCTION

Molt, the seasonal replacement of feathers, is one of the most energy-demanding processes in the annual life cycle of birds (Young 1991, Marks 1993, Howell et al. 1999). For birds that migrate between breeding and wintering grounds, the timing and extent of molt represents an evolutionary trade-off between competing demands for successful reproduction and preparation for migratory flight (Howell et al. 1999, 2003, Leu and Thompson 2002). However, despite its importance as a life history trait, molt receives comparatively little attention (Young 1991, Thompson and Leu 1994, Leu and Thompson 2002). This paucity of information is partly due to the fact that the establishment of molting patterns relies heavily upon museum specimens (Howell et al. 1999) and is inherently biased to those areas where birds are accessible for capture and study, typically on the breeding grounds or at migration stopover sites in Holarctic areas (Thompson and Leu 1994). As a result, information on molt is often incomplete with respect to extent, timing, and location of molt among age and sex classes, and across populations (Young 1991, Leu and Thompson 2002, Siikamäki et al. 1994). Molt is influenced by food availability, age, and sex and can differ within species (Thompson and Leu 1994, Hemborg 1999, Leu and Thompson 2002). In North America, the first and adult prebasic molt for most passerines occurs after the breeding season (whether they breed or not) and usually precedes autumn migration, but may also take place during migration or on the wintering grounds (Pyle 1997, Leu and Thompson 2002). Some species begin molting shortly after the breeding season, suspend or interrupt molt during fall migration, and

complete it on the wintering grounds (Pyle 1997, Leu and Thompson 2002).

Shrikes (Laniidae) are a predominantly Old World family that includes species and populations exhibiting diverse molt strategies (Lefranc and Worfolk 1997). Sedentary shrikes tend to undertake a complete molt soon after breeding, while some migratory populations undergo a postbreeding molt in the vicinity of their breeding grounds prior to fall migration (Miller 1928, Yosef 1996). Others may not start molt until after migration (Yosef 1996) and those that begin their molt on the breeding grounds may either continue it during fall migration or suspend molt during migration and resume molting again at their wintering areas (Palmer 1898, Miller 1931, Lefranc and Worfolk 1997). In North America, the Loggerhead Shrike (*Lanius ludovicianus*) occurs as 11 subspecies and is widespread, being found roughly from coast to coast and from southern Canada to the Isthmus of Tehuantepec in Mexico (Miller 1931). Northernmost populations are migratory and winter in the southern U.S. and Mexico (Fig. A.1). Information on the molt of this species, especially for these migratory populations, is relatively poor and several possible strategies have been reported or assumed (Yosef 1996).

Previously, Hobson and Wassenaar (2001) investigated migratory connectivity between breeding and wintering grounds of Loggerhead Shrikes using stable hydrogen isotope analysis of feathers. This approach is based on the fact that a robust latitudinal gradient in growing-season averaged deuterium exists in precipitation in North America and this pattern is reflected in local foodwebs across the continent (Hobson and Wassenaar 1997). Thus, feathers reflect this spatial pattern in deuterium concentrations, making it possible to infer latitudinal locations of molt from isotopic analysis of feathers.

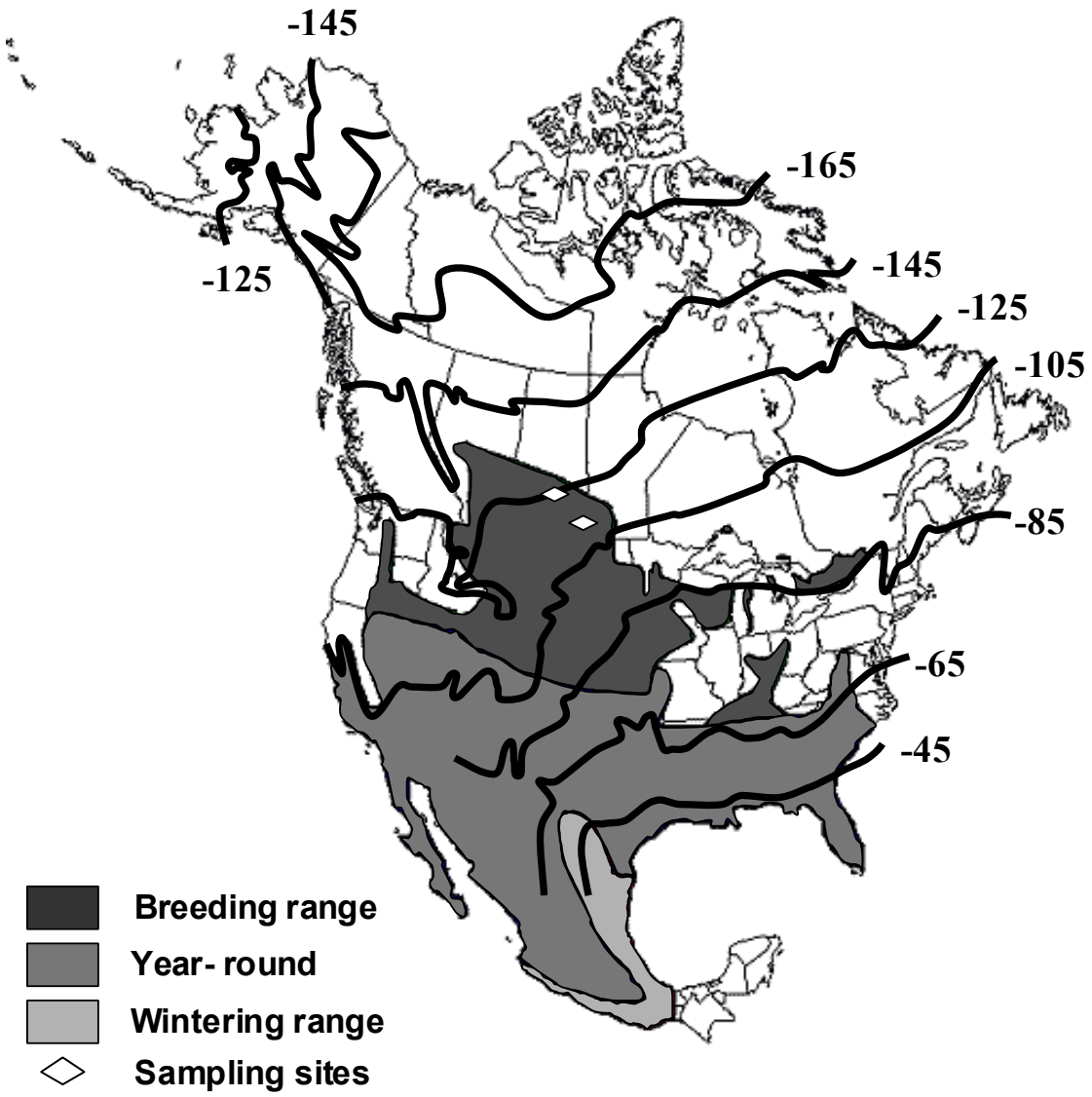

Figure A.1. Breeding, wintering and year-round distribution of the Loggerhead Shrike in North America, and the deuterium (δD) contour lines based on a geographical information system-based model of δD values of feathers for the average growing-season (Meehan et al., 2004). We used an isotopic discrimination factor between δD of precipitation (δDp) and δD of feathers (δDf) of -25‰. Diamonds represent the sampling locations in central Saskatchewan and in southern regions of the Saskatchewan-Manitoba border.

This approach has significant advantages over mark-recapture methods, since it does not depend on initial capture and marking of individuals. Rather, identification of molt location is possible for each bird captured (Hobson 1999). Using this technique, Hobson and Wassenaar (2001) were able to identify the relative use of wintering grounds in the southern United States and northern Mexico by populations of northern migratory shrikes. A key assumption of that study was that the outer tail (rectrix) feather that they analyzed was indeed grown on or close to the breeding grounds prior to fall migration. That assumption was based on reports by Miller (1928), but it was not clear to what extent this was true of northern populations. Northern birds may be expected to differ in molt strategy from more southerly birds (Yosef 1996, Pyle 1997). In this study, I investigated molt in northernmost migratory Loggerhead Shrikes by isotopically examining feathers along a sequence of primary, secondary, tertial and tail feathers. I reasoned that δD values of feathers could be used to assign approximate latitude, making it possible to identify which feathers were grown on the breeding grounds, during fall migration, and on the wintering grounds (Norris et al. 2004).

A.2. METHODS

A.2.1. FEATHER COLLECTION

I obtained feather samples from the northernmost population of Loggerhead Shrikes by opportunistically detecting and capturing individuals along highways and grid roads in central and southeastern Saskatchewan and southwestern Manitoba. Birds were captured using a walk-in trap baited with a (protected) live mouse. Sampling took place from 24 June to 6 July 2004. All birds were sexed according to presence or absence of a brood patch and cloacal protuberance. I attempted to age birds to second year (SY) and

after-second-year (ASY) based on criteria outlined in Pyle (1997) for this species. However, these techniques were abandoned, since I did not feel we could confidently classify birds in this northern population using these methods. Indeed, as noted by Pyle (1997), during some months within my study period Loggerhead Shrikes can be aged with only 5%–25% accuracy. All birds were marked with a U.S. Fish and Wildlife Service aluminum band. I clipped approximately 1.0 × 0.5 cm sections of the proximal web from remiges and rectrices to minimize effects on flight (these sections overlapped adjacent feathers during flight). I sampled primaries P1, P3, P6, and P9, secondaries S1, S3, and S6, tertial S9, and rectrices R1, R3, and R6. Each feather piece was placed in a separate, labeled paper envelope.

I sampled 27 adult birds and tried to obtain all 11 feather samples from each individual. However, not all eleven samples were always taken from each bird, because occasionally feathers were missing or being replaced. However, in all other cases, the previous year's feather was sampled. Eighteen birds were captured in central Saskatchewan and nine within the putative hybrid zone of SE Saskatchewan and SW Manitoba.

A.2.2. STABLE-ISOTOPE ANALYSIS

Feather samples were cleaned in 2:1 chloroform:methanol solution overnight, drained, and air dried under a fumehood for at least 3 hr. Feather vane subsamples were then cut and 350μg ± 10 μg weighed into 4.0 × 3.2 mm silver capsules for online hydrogen isotope analysis by continuous-flow isotope-ratio mass spectrometry (CFIRMS). Stable hydrogen isotope analytical measurements (δD) followed the "comparative equilibration" technique described by Wassenaar and Hobson (2003).

Isotopic values were expressed in delta notation in parts per thousand (‰) as the nonexchangeable hydrogen portion of samples normalized on the Vienna Standard Mean Ocean Water–Standard Light Antarctic Precipitation (VSMOW-SLAP) standard scale. Feather samples were analyzed at the stable-isotope facility of the National Water Research Centre in Saskatoon, Canada.

A.2.3. STATISTICAL ANALYSIS

I depicted our feather isotope data graphically using frequency distributions to examine for potential bimodal patterns corresponding to feather growth on breeding and nonbreeding (i.e. *en route* and wintering) areas. By inspection of the feather δD contour map for North America (Fig. 1), I estimated a cutoff of –90‰ between breeding and nonbreeding areas. Since the original data was non-normal (Fig. A.2), I opted to group feathers (P1s, P3s, etc.) and analyze them independently to see if molting differed between the sexes. For this I used chi-square and Fisher's exact tests (in 2 × 2 contingency tables, with the smallest expected frequency less than 5; Siegel 1956). I also used chi-square and Fisher's exact tests to test for differences in frequencies of molt within primaries, secondaries (including tertial S9), and rectrices. The Bonferroni correction (Sokal and Rohlf 1995) was used to reduce possible Type I error and alpha was subsequently set at 0.008 for primaries and secondaries and 0.016 for rectrices. Independent sample *t*-tests were used for parametric data to test whether molting patterns differed between sexes and to test differences in feather isotope values between sampling areas. I was interested in determining the degree to which P1 showed isotopic concordance with the breeding ground signature expected for feathers. Therefore, for the

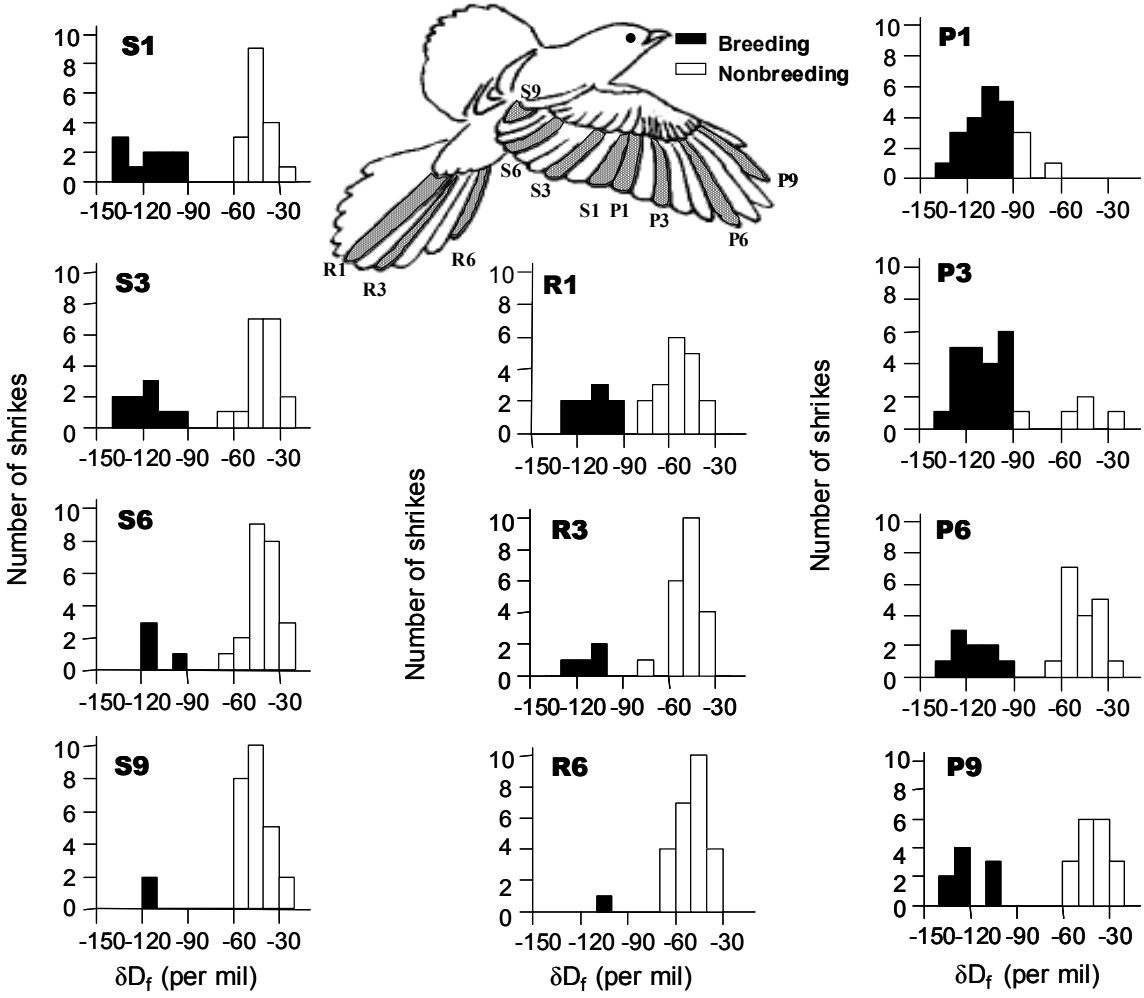

Figure A.2. In general, deuterium values of primary (P1, P3, P6, P9), secondary (S1, S3, S6, S9) and rectrix (R1, R3, R6) feathers of summering Loggerhead Shrikes sampled in Saskatchewan and Manitoba, Canada, revealed a bimodal distribution, indicating that flight-feather molt occurs either on the breeding or at nonbreeding areas. Shrike sketch was based on Harris and Franklin (2000).

two collection sites, I compared our observed P1 δD values with expected feather deuterium values (δD_f) derived from the growing-season, altitudinally corrected continental pattern of Meehan et al. (2004) and corrected for isotopic discrimination between feather and precipitation δD (δD_p) by 25‰ (Wassenaar and Hobson 2000). Statistical analyses were performed using SPSS version 12.0 for windows (SPSS 2004). All results are expressed as means ± SD.

A.3. RESULTS

Feathers from individuals showed considerable variation in isotopic structure (Table 1), revealing that they were not all grown on the breeding grounds (Fig. 1). After assigning feather origins based on their range of δD_f values, we found no effect of sex on where feathers were grown (breeding or nonbreeding areas) across feathers (all $P > 0.07$), so I combined male and female feathers within type for all further analyses. Within primary feathers, we found no difference in distribution of assigned origin between P1 vs.P3 and P6 vs. P9 feathers (Fisher's exact test, $P = 0.71$ and $\chi^2_1 < 0.001$, $P > 0.99$, respectively). Comparisons of origin for all other primary feather combinations were significant (all $P < 0.001$). Comparisons of origin for all secondary feather combinations were nonsignificant after the Bonferroni correction (all $P > 0.02$). Within rectrices, with the exception of R1 vs. R6 (Fisher's exact test, $P = 0.01$) all comparisons were nonsignificant (R1 vs. R3, $\chi^2_1 = 2.1$, $P = 0.15$; R3 vs. R6, Fisher's exact test, $P > 0.19$). There was difference in δD_f between the two sampling areas ($t_{21} = 3.7$, $P < 0.001$). The expected average δD_f value in central Saskatchewan was –125‰, whereas the mean observed average was –112‰ (95% CI = –119‰ to –103‰). In the SE Saskatchewan and SW Manitoba area, the expected average δD_f value was –112‰, whereas the mean

observed average was –91‰ (95% CI = –100‰ to –82‰; Table A.1). Subsequent primaries showed increasing likelihood of being grown south of breeding sites.

In general, feather groups (primaries, secondaries, tertial and rectrices) showed a bimodal distribution, indicating they were grown either on the breeding grounds or on nonbreeding areas (Fig. A.1, A.2). Thus, in this population, molt appears to be initiated on the breeding grounds and then suspended pending arrival on the wintering (or more southern molting) grounds. The main segregation among individuals occurred with the degree of primary molt prior to migration (Table A.2).

A.4. DISCUSSION

Our isotopic analysis of feathers from northern summering Loggerhead Shrikes indicated that, in adults, flight-feather molt was initiated on the breeding grounds starting with P1, but was rarely completed prior to fall migration. Instead, following some partial primary replacement, molt appeared to be largely suspended until birds reached either their wintering grounds or southern regions that overlap with the known winter range of this species. On average, the most positive δD_f values were found in feather S9. It is possible that S9 indicates a molting area prior to reaching the ultimate wintering grounds along the Gulf States of the U.S. and northern Mexico, as in, for example, Lazuli Buntings (*Passerina amoena*; Young 1991), and Baird's Sparrows (*Ammodramus bairdii*; Voelker 2004). Unfortunately, the lack of suitable precipitation δD contours for Mexico precluded accurately assigning birds to that region. The suspension of molt in our shrikes was clearly not total and some individuals continued replacing feathers during their autumn movements to the wintering grounds as evidenced by intermediate δD_f values. However, most individuals replaced the majority of their flight feathers below the –65‰

Table A.7 Summary of deuterium (δD) values of flight feathers from Loggerhead Shrikes sampled in prairie Canada (Fig. A.1). For all feathers, Loggerhead Shrikes from central Saskatchewan (A) were more depleted in deuterium than feathers of shrikes from SE Saskatchewan–SW Manitoba (B), indicating that, on average, shrikes from site A molted their feathers at more northerly latitudes than conspecifics from site B. P1 refers to the innermost primary feather, P9 is the outermost secondary feather, S1 is the outermost secondary feather, S6 is the innermost secondary feather, S9 is the innermost tertial feather, R1 is the innermost rectrix, R6 is the outermost rectrix (Fig. A.2) and *n* is the sample size.

Feather		*n*	Mean ± SD (‰)	95% CI (‰)	Range (‰)
P1					
	A	14	–111.5 ± 13.1	–119.0 to –103.9	–130.1 to –90.0
	B	9	–91.1 ± 12.4	–100.7 to –81.6	–106.6 to –64.4
P3					
	A	17	–105.5 ± 28.0	–119.9 to –91.2	–131.9 to –25.7
	B	9	–85.5 ± 23.7	–103.8 to –67.3	–108.9 to –41.4
P6					
	A	18	–73.4 ± 38.0	–92.2 to –54.4	–133.1 to –24.3
	B	9	–63.2 ± 29.4	–85.8 to –40.7	–108.6 to –36.5
P9					
	A	18	–70.1 ± 41.2	–90.6 to –49.6	–133.2 to –28.7
	B	9	–60.0 ± 34.6	–86.6 to –33.4	–108.4 to –26.2
S1					
	A	18	–74.4 ± 39.1	–93.9 to –55.0	–134.6 to –30.5
	B	9	–61.2 ± 32.0	–85.9 to –36.6	–109.7 to –29.1
S3					

	A	18	–69.7 ± 40.0	–89.6 to –49.8	–132.1 to –23.9
	B	9	–60.3 ± 34.0	–86.5 to –34.2	–112.0 to –32.4
S6					
	A	18	–53.2 ± 30.2	–68.3 to –38.2	–119.6 to –22.9
	B	9	–47.3 ± 19.8	–62.5 to –32.1	–93.2 to –25.2
S9					
	A	18	–51.3 ± 24.1	–63.3 to –39.3	–113.5 to –26.5
	B	9	–45.6 ± 5.5	–49.9 to –41.4	–54.1 to –36.5
R1					
	A	18	–80.7 ± 31.3	–96.3 to –65.2	–125.4 to –37.5
	B	9	–57.6 ± 16.2	–70.0 to –45.1	–95.5 to –40.9
R3					
	A	18	–61.0 ± 27.7	–74.7 to –47.2	–127.9 to –35.4
	B	7	–53.4 ± 24.1	–75.7 to –31.1	–106.0 to –31.2
R6					
	A	18	–53.9 ± 16.5	–62.1 to –45.6	–107.3 to –33.4
	B	8	–46.5 ± 8.5	–53.6 to –39.4	–54.7 to –30.0

Table A.8. Distribution of feather molt for each individual Loggerhead Shrike sampled according to the isotopic criteria: breeding grounds (less than –90‰), or nonbreeding areas (greater than –90‰). Feathers P1, P3, P6, and P9 refer to primary feathers 1, 3, 6, and 9. Feathers S1, S3, and S6 refer to secondary feathers 1, 3, 6. Feather S9 refers to the innermost tertial feather. Feathers R1, R3, and R6 refer to rectrices 1, 3, and 6 (Fig. A.2). Adult Loggerhead Shrikes initiated their flight-feather molt with P1 on the breeding grounds, but molt was not completed prior to fall migration. Following some partial primary replacement, molt was completed on the wintering or more southerly molting grounds.

	Location of molt	
Band-number	Breeding	Nonbreeding
Saskatchewan-Manitoba		
1891-50014	P1, P3	P6, P9, S1, S3, S6, S9, R1, R3, R6
1891-50015	P1	P3, P6, P9, S1, S3, S6, S9, R1, R6
1891-50016	P1, P3, R1	P6, P9, S1, S3, S6, S9
1891-50017		P1, P3, P6, P9, S1, S3, S6, S9, R1, R3, R9
1891-50018	P3	P1, P6, P9, S1, S3, S6, S9, R1, R3, R6
1891-50019	P1, P3, P6, P9, S1, S3, S6, R3	S9, R1, R6
1891-50020	P1, P3, P6, P9, S1, S3	S6, S9, R1, R3, R6
1891-50021	P3	P1, P6, P9, S1, S3, S6, S9, R1, R3, R6
1891-50022	P1, P3, P6, P9, S1, S3	S6, S9, R1, R3, R6
Central Saskatchewan		
1731-01101	P1, P3, S1	P6, P9, S3, S6, S9, R1, R3, R6
1731-01102	P1, R1	P3, P6, P9, S1, S3, S6, S9, R3, R6
1731-01103	P1, P3, P6, P9, S1, S3	S6, S9, R1, R3, R6
1731-01104	P1	P3, P6, P9, S1, S3, S6, S9, R1, R3, R6

1731-01105		P6, P9, S1, S3, S6, S9, R1, R3, R6
1731-01106	P1, P3, R1, R6	P6, P9, S1, S3, S9, R3
1731-01107	P1, P3	P6, P9, S1, S3, S6, S9, R1, R3, R6
1731-01108	P1, R1, R3	P3, P6, P9, S1, S3, S6, S9, R6
1731-01109	P1, P3, P6, P9, S1, S3, S6	S9, R1, R3, R6
1731-01110	P1, P3, P6, P9, S1, S3, R3	S6, S9, R1, R6
1731-01111	P3, P6, P9, S1, S3, S6, S9, R3	R1, R6
1731-01112	P3, P6, P9, S1, S3, S6	S9, R1, R3, R6
1731-01113	P1, P3, R1	P6, P9, S1, S3, S6, S9, R3, R6
1731-01114	P1, P3, R1	P6, P9, S1, S3, S6, S9, R3, R6
1731-01115	P1, P3, S9, R1	P6, P9, S1, S3, S6, R3, R6
1731-01116	P1, P3, R1	P6, P9, S1, S3, S6, S9, R3, R6
1731-01117	P3, P6, P9, S1, S3	S6, S9, R1, R3, R6
1731-01118	P1, P3, R1	P6, P9, S1, S3, S6, S9, R3, R6

δD_f contour line.

Our isotopic evidence strongly suggests that the innermost primary flight feathers of migratory northern-latitude adult shrikes were consistently replaced before fall migration, commencing with P1 and continuing outwardly (Palmer 1898, Miller 1928, Yosef 1996). Miller (1928) reported that as the third primary is lost, the center rectrices drop and feather replacement continues outwardly, a pattern also supported by our data. Our analysis also supports the suggestion that molting of the inner tail feathers usually precedes secondary feather replacement and begins with R1, continuing outward (Miller 1928). However, rectrices can often be replaced adventitiously and therefore may not be the most reliable flight feather tract to use in molt studies. According to Miller (1928), secondary molt starts as P4 is dropped at one or two points simultaneously, with either tertial S8 or secondary S1, or both. Yosef (1996) also suggested that molting of secondary feathers starts at two points, but instead with S1 and S6. Our results show that the secondary molting succession began with outermost S1 and continued inward to S6. Both Palmer (1898) and Miller (1928) stated that secondary replacement culminates toward the center of the wing with either S5 or S6, which agrees with our results. Both Miller (1928) and Pyle (1997) suggested that S9, in both preformative and prebasic molt, is usually replaced prior to fall migration. Pyle (1997) stated that in ~93% of birds, the first prebasic (preformative) molt included 1–3 tertials. Interestingly, our data suggest that feather S9 was most consistently replaced last, at southernmost molting latitudes. Since this result is incongruous with previous molting accounts, we hypothesize that shrikes may adopt a previously undocumented prealternate molt, which may include tertials on the wintering grounds (P. Pyle, Point Reyes Bird Observatory, pers. comm.).

Alternatively, the molt sequence of tertials could be S7–S8–S9 or S8–S7–S9, centering at S7 or S8, which could explain the replacement of S9 at southernmost molting latitudes during the prebasic molt (P. Pyle, pers. comm.).

The expected δD values for P1 from birds breeding at two sites in Saskatchewan and Manitoba were more depleted than those measured. There are several possible explanations for this. First, since we had no control over dispersal, our sample could have included birds that moved into our area having grown their feathers farther south the previous year (Hobson et al. 2004). This would result in feathers being more deuterium-enriched than expected. Secondly, there is evidence that raptorial birds have more positive discrimination factors between growing-season average precipitation δD values and feather δD values (Lott and Smith 2006). This would result in feathers having higher δD values than those expected from the altitude-corrected map of Meehan et al. (2004). In addition, since we sampled the base region of feather vanes, representing the last portion to be grown, this growth may have occurred during the initial stages of migration. Finally, any given feather-growing season can be displaced isotopically from the long-term average pattern previously found for North America (Hobson and Wassenaar 1997, Meehan et al. 2004, Hobson 2005). Currently, we have no way to evaluate these possibilities. Nonetheless, this phenomenon does not alter our interpretation of relative locations of molt sites for the birds examined, since the bimodal nature of the results clearly identifies two primary areas of molt that correspond to northern and southern latitudes.

Individual variation in molt may be related to contrasting time and energy budgets of individuals based on breeding success and general phenology (Norris et al. 2004).

Competing energetic demands of molt, the need for premigratory conditioning (Marks 1993), and brood rearing will, in part, be influenced by food availability (Young 1991, Leu and Thompson 2002). It is also possible that there is a selection pressure on shrikes to reach their wintering grounds as soon as possible, since they are known to be territorial at wintering sites (Temple 1995) and birds returning earlier can presumably increase their chances of securing better winter territories. Should shrikes suspend molt to compensate for relatively short breeding seasons at northern latitudes in favor of reaching wintering sites earlier? I predict that more southerly breeding populations will show less tendency to interrupt molt following breeding. Hemborg et al. (2001) found that male Pied Flycatchers (*Ficedula hypoleuca*) from northern latitudes overlapped breeding and postnuptial molt to compensate for short breeding seasons. Howell et al. (1999) observed that in three different species of gulls, residents and short-distance migrants began their prebasic molt months earlier than their long-distance migratory counterparts. Perhaps previously unaccounted-for intraspecific differences among various populations of shrikes from different breeding latitudes may explain the overall variability encountered among descriptions of molt in this species (Miller 1931, Yosef 1996). These are important factors to consider, since populations from the same locality will tend to be more alike with respect to molt than birds from widely separate localities within the range of the species (Miller 1931).

Unquestionably, if accurate age information were available for our sample, a much more powerful interpretation of molting patterns in this species would be possible. Miller (1928) wrote the most thorough systematic review of molting patterns in Loggerhead Shrikes and similarly was only able to age birds to after-hatch-year (AHY)

during the latter half of the breeding season. An alternative interpretation of our data is that the bimodal distribution of the δD_f values is in fact an effect of a bimodal age distribution. Pyle (1997) stated that during the preformative molt, ~50% of shrikes show an eccentric molt pattern that can include the outer 4–6 primaries (i.e., containing P6 and P9) and the inner 3–5 secondaries (i.e., containing S6). Therefore, it could be argued that the bimodal distribution is the result of SY birds with retained juvenile inner primaries and outer secondaries (P1, P3, S1, S3), thus having a breeding ground isotopic signal, and outer primaries and inner secondaries representing those feathers subsequently grown on the wintering grounds. Although possible, our results emphatically show that most birds grew their feathers on both the breeding and wintering grounds and it is unlikely that our sample consisted of only SY birds.

It is clear that shrikes, including those from northern populations, begin their molt on the breeding grounds (Palmer 1898, Miller 1928, 1931, Yosef 1996, Pyle 1997), but the extension of molt following fall migration has not previously been documented. More studies are needed to examine how this strategy may change with latitude of the breeding population. We also encourage long-term isotopic studies on marked birds of known age and reproductive success. Identifying which feather(s) are most consistently replaced at specific latitudes during migration may in turn indicate the use of staging areas, which can have important conservation implications (Leu and Thompson 2002).

Our results have implications for the previous isotopic study of Hobson and Wassenaar (2001), which used outer rectrices to indicate the breeding area origins of shrikes on the wintering grounds. The reduced likelihood that rectrices are molted on the breeding grounds, at least in northern populations, suggests that these authors provided

very conservative estimates of the proportion of northern birds in various southern wintering populations. Thus, future studies should use P1 as a good indicator of breeding location and S9 as an indicator of wintering location.

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