

**POPULATION CONNECTIVITY: COMBINING METHODS
FOR ESTIMATING AVIAN DISPERSAL AND
MIGRATORY LINKAGES**

DISSERTATION

**Presented in Partial Fulfillment of the Requirements for the Degree of Doctor of
Philosophy in the Graduate School of The Ohio State University**

By

Siri B. Ibargüen

The Ohio State University

2004

Dissertation Committee:

Dr. Thomas A. Waite, Advisor

Dr. H. Lisle Gibbs

Dr. Douglas A. Nelson

Dr. Mark S. Woodrey

Approved by

**Advisor
Evolution, Ecology, & Organismal
Biology**

ABSTRACT

We use a variety of methods to study population connectivity. In Chapter 1, we use stable isotope ratios in feathers to make Bayesian inferences about the migratory connectivity between breeding and wintering grounds of Henslow's sparrows. We use hydrogen and carbon stable isotope ratios (δH and δC). We compare the δH and δC of feathers from wintering sparrows to five breeding region δH and δC to estimate the probability that each individual wintering sparrow originated from each of the five regions. Breeding bird abundances are used as prior probabilities of breeding region origin. We conclude that there are no clear linkages between specific breeding regions and wintering sites.

In Chapter 2, we use three methods to estimate dispersal in Henslow's sparrows. 1) δH in feathers are used to determine whether an individual breeding bird has a δH signature characteristic of the breeding site. 2) Song structure is used as the signature of an individual's previous breeding-ground origin. 3) Genetic markers are used to evaluate population structure. Genetic structure is evaluated using three estimates. *Fst* estimates and private alleles are used to calculate the number of migrants per generation (Nm) between sites. Private alleles are evaluated to determine if they are truly private. A Bayesian clustering method is used to infer the number of populations. All methods revealed high rates of dispersal.

In Chapter 3, three methods for estimating dispersal are compared: deltaH in feathers, genetic population structure, and spatial autocorrelation (SAC). We compare the dispersal estimates of five migratory species.

With the SAC analysis, we find no clear evidence for dispersal as a major synchronizing agent. However, new statistical methods may allow for the parsing out the effect of dispersal. One species had historically high dispersal (limited genetic structure) but currently low dispersal (high r^2 for the deltaH correlations). Another species had a deltaH r^2 value indicating low current dispersal. Three other species are all found to have high dispersal, both historically and currently. Comparing dispersal estimates may allow researchers to evaluate how dispersal rates have changed over time, as well as how well estimation methods agree.

Dedicated to Bo and Leah

ACKNOWLEDGMENTS

Many people made this work possible. I am most grateful to Tom Waite for helping me through the design, analysis and writing of this dissertation. Without his support, guidance, patience, flexibility, humor and friendship I would not have been able to complete this research. Thanks also to the rest of my dissertation committee members: Mark Woodrey, for introducing me to Henslow's sparrows, organizing the wintering-ground fieldwork, for doing all the sampling in North Carolina, and keeping me excited about the research. Lisle Gibbs, for helping me with the genetic analysis and making me think more deeply about the meaning behind the analysis. Doug Nelson, for helping me with the acoustic analysis and for programming the custom Signal macro for me. I thank the people who helped with the fieldwork and/or logistics. In the wintering grounds: Michelle Davis, Lori Yates, David Cimprich, Colleen Dwyer, Ryan Heise, Stefan Woltmann, Mollie Cashner, Rob Smith, Jen Owen, Chris Szell, Sarah Mabey, Leah Bray, Daniel Hudson, Chris Melder, Kenneth Moore, David Plair, Stacy Peterson, Ken Hackman, Tacy Anderson, Katherin Barrow, Laura Blacken, Melissa Davis, Laura Edgar, Jason Edwards, Jonathan Faulkner, Amy Foster, Heather Foster, Jones Goodman, Jay Jordan, Drew Lang, Alicia LeBlanc, Rachael McClure, Andy Middleton, William Ogle, Bethany Porter, Justin Rives, Melissa Robinson, Ryan Sartain, Stacy Slone, William Stianche, Erin White, Todd Engstrom, Douglas McNair, Keith Ouchley, Jeff Buler, Mike

Baker, Melissa Powell, Rebecca Finzer, Nellwyn McInnis, Melissa Andre, Lauren Miller, Jill Coutmoutso, Molly Embree, Giff Beaton, Tylan Dean, Jim Johnson, Beau Gregory, Bob Ford and his Ornithology class from the University of Tennessee. In the breeding grounds: Daniel Brauning, Greg Forcey, Chris Grainer, Evan Blumer, Danny Ingold, Don Sheroan, Jeff Jones, Andrew Leonard, Daniel Moss, Joe Robb, Teresa Lewis, Jason Lewis, Karen Owens, Jim Keir, Ken Rosenthal, David Sample, David Mehlman, Robert Hamilton, Barbara Van Slyke, Tom Van Slyke, Craig Davis, John Wright, Paula Wright, Robin Krebbs, Steve Joule, Alicia Bacci, Maiken Winter, Robert Grosholz, Elizabeth Vaughn, Jarling Ho, Allison Reid, Paul Doherty, Bo Bunnell, Kristin Field, and Scott Hull. Thanks also to the folks who helped with the genetic and bioacoustic analyses: Jose Diaz, Patricia Parker, Sandra Gaunt, Jill Soha, Sarah Corey, and Kelly Ketchum. I thank the thousands of U.S. and Canadian participants of the North American Breeding Bird Survey, including USGS and CWS researchers and managers. Finally, I thank Andy Royle and William Link for the valuable discussions on Bayesian analysis and relative abundances. Funding was provided by grants from Ohio Department of Natural Resources, Division of Wildlife; Columbus Zoo, and Mississippi Natural Heritage Fund.

VITA

EDUCATION

- 1988-1991 Undergraduate at Santa Monica College
- 1992-1994 Undergraduate at University of California, Los Angeles
- June 1994 B.S. (with honors) in biology, with an emphasis in ecology, behavior, & evolution from University of California, Los Angeles
- 1996-1997 Ph.D. student at Michigan Technological University
- 1997-1999 M.S. student at The Ohio State University
- June 1999 M.S. in Evolution, Ecology, & Organismal Biology at The Ohio State University
- 2001-2004 Ph.D. candidate at The Ohio State University

PROFESSIONAL EXPERIENCE

- 6/94-8/94 Field Assistant for Martin Cody, University of California, Los Angeles
- 11/94-6/95 Field Assistant for David Gubernick, University of Wisconsin
- 3/96-5/96 Field Assistant for Gwen Bachman, University of Oklahoma
- 6/96-8/96 Biological science technician, Forest Service, Hiawatha National Forest

AWARDS

- 1996 Michigan Technological University Fellowship
- 1996 King-Chavez-Parks Fellowship, Michigan Technological University
- 1997 Dean's Graduate Enrichment Fellowship/Associateship, The Ohio State University

PUBLICATIONS

Vucetich, J.A., Waite, T.A., Qvarnemark, L., Ibargüen, S. 2000. Population variability and extinction risk. *Conservation Biology* 14: 1704-1714.

FIELDS OF STUDY

MAJOR FIELD

Evolution, Ecology & Organismal Biology

TABLE OF CONTENTS

	<u>Page</u>
Abstract.....	ii
Dedication.....	iv
Acknowledgments.....	v
Vita	vii
List of Tables	xi
List of Figures.....	xv
Introduction.....	1
Chapter 1: Linking breeding and wintering grounds using stable isotope data: a Bayesian approach.....	15
Summary.....	15
Introduction.....	17
Methods.....	22
Results.....	29
Discussion.....	40
Acknowledgments.....	47
References.....	49
Chapter 2: Estimating songbird dispersal using hydrogen isotope ratios, meme flow and gene flow	52
Summary.....	52
Introduction.....	54
Methods.....	63

	<u>Page</u>
Results.....	73
Discussion.....	78
Acknowledgments.....	84
References.....	86
Chapter 3: How do dispersal estimates compare? A review and prospectus ..	91
Summary.....	91
Introduction.....	94
Methods.....	104
Results.....	111
Discussion.....	117
Acknowledgments.....	123
References.....	124
Appendix A Tables of additional genetic and bioacoustic information for Henslow’s sparrows	129
Bibliography	135

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1.1 Synopses of avian studies that used natural stable isotope ratios.	19
1.2 Sampling locations, years, and stable isotope ratio statistics for breeding and wintering Henslow’s sparrows.	23
1.3 Results of multiple regression analysis of how the stable isotope ratios of hydrogen (δH) and carbon (δC) in Henslow’s sparrow feathers varied along latitude and longitude.	30
1.4 Parameters used to calculate the univariate and bivariate normal distributions of the stable isotope data for each breeding region, and the relative abundance of Henslow’s sparrows within each breeding region.	30
2.1 Estimated proportions of immigrants per generation versus meme mutations per generation in four songbird species.....	67

<u>Table</u>	<u>Page</u>
2.2 Estimated number of truly private alleles among putative private alleles (PPA) with 95% certainty in each population.	71
2.3 Example of the computation procedure for estimating the number of putatively private alleles (PPA) with 95% certainty, using the North Carolina population, with 4 PPAs.....	72
2.4. Three sets of parameters and assumptions used in Bayesian analyses of genetic structure, using the software package <i>Structure</i>	73
2.5 Estimated proportions of dispersers in four sampling sites across the breeding range of the Henslow’s sparrow, based on stable deuterium/hydrogen (δH) feather ratios.	74
2.6 Estimated proportions of dispersers in four sampling sites across the breeding range of the Henslow’s sparrow, based on song structure.....	75
2.7. Pairwise Nm calculated from F_{st} estimates (above diagonal) and Nm estimates based on private alleles (below diagonal) for four Henslow’s sparrow populations.....	77

<u>Table</u>	<u>Page</u>
2.8 Advantages, potential applications, and challenges of using genetic, stable isotope, and song data in estimating dispersal.	85
3.1 Decision criteria for the most influential agent of spatial autocorrelation.	102
3.2 Four possible dispersal rate patterns using long-term (i.e., historical) and short-term (i.e., current) dispersal estimates.	104
3.3 Sample sizes and locations where Henslow’s sparrow blood and feathers were collected.	107
3.4 Dispersal estimates and patterns of five North American passerines.	113
3.5 Outcomes of spatial autocorrelation analysis of three North American passerines.	117
A.1 Primers screened for microsatellite analysis.	129
A.2 PCR protocols for the five primer sets used in the analysis.	130
A.3 The F_{IS} (P -values) of each locus across seven Henslow’s sparrow breeding sites.	131

<u>Table</u>	<u>Page</u>
A.4 The observed and expected heterozygosities (H_o and H_e respectively) and calculations of null allele frequencies (of loci determined to have null alleles) for seven Henslow's sparrow breeding sites.....	132
A.5 Estimated proportion of breeding dispersers in four sampling sites across the breeding range of the Henslow's sparrow, based on song structure, but without the third song component.	133
A.6 Pairwise F_{st} estimates calculated with the correction for null alleles	134

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1.1 Hypothetical distributions of stable isotope ratios of feathers from two regions of the breeding ground..	20
1.2 Breeding regions and sampling sites of Henslow's sparrows.....	24
1.3 Proportions of individual Henslow's sparrows on the breeding grounds correctly assigned to their breeding region..	31
1.4 Pie charts showing the proportions of Henslow's sparrows sampled at a wintering site that were assigned to each of four breeding regions, using the highest probability criterion and δH data.	34
1.5 Pie charts showing the proportions of Henslow's sparrows sampled at a wintering site that were assigned to each of two breeding regions, using the ≥ 0.5 probability criterion and δH data.	35

<u>Figure</u>	<u>Page</u>
1.6 Pie charts showing the proportions of Henslow’s sparrows sampled at a wintering site that were assigned to each of four breeding regions, using the highest probability criterion, and δH and δC data.....	36
1.7 Pie charts showing the proportions of Henslow’s sparrows sampled at a wintering site that were assigned to each of three breeding regions, using the ≥ 0.5 probability criterion, and δH and δC data.....	37
1.8 Panels showing the results of the Bayesian analysis for all Henslow’s sparrows sampled at a wintering site, using flat prior probabilities (0.2).....	38
1.9 Panels showing the results of the Bayesian analysis for all Henslow’s sparrows sampled at a wintering site, using relative abundances.....	39
2.1 Stable deuterium/hydrogen isotope contour lines and sites where rectrices and songs (numbered sites) and additional genetic data (lettered sites) were collected from Henslow's sparrows.....	56

<u>Figure</u>	<u>Page</u>
2.2 Sonograms of two representative Henslow's sparrow songs	66
2.3 Discriminant functions of Henslow's sparrow songs from four populations	76
3.1 Contour map of feather isotope ratios.....	110
3.2 Maps for A) Wilson's warbler, B) black-throated blue warbler, C) red-winged blackbird.	113

INTRODUCTION

COMBINING METHODS FOR THE STUDY OF AVIAN POPULATION CONNECTIVITY

Tracking the movements of individuals is a compelling but difficult task, especially for organisms such as small migratory songbirds. Traditional tracking techniques are inadequate: most songbirds are too small to carry a radio transmitter for long distances and massive banding efforts have provided precious few returns. For example, of the 689,019 nongame birds banded in 2001, only 8,057 (1%) were recovered (Gustafson & Hildenbrand 1999). Consequently, little is known about individual bird movements. This ignorance is worrisome considering the unfavorable conservation status of many species.

Here, we investigate two kinds of population connectivity, migratory linkages between breeding and wintering grounds, and dispersal between populations. In the first chapter, we evaluate the linkages between the breeding and wintering grounds of Henslow's sparrows (*Ammodramus henslowii*) using two data sets in a Bayesian analysis.

In the second chapter, we evaluate the dispersal rates of Henslow's sparrows using three dispersal estimates. In the third chapter, we compare three methods for estimating dispersal in five migratory bird species.

CHAPTER 1: LINKING BREEDING AND WINTERING GROUNDS USING STABLE ISOTOPE

DATA: A BAYESIAN APPROACH

Determining connectivity (Webster *et al.* 2002) between breeding and wintering grounds is important for understanding population dynamics in migratory birds. Some studies have found correlations between conditions on the wintering grounds and fitness components on the breeding grounds (e.g., Marra, Hobson & Holmes 1998; Gill *et al.* 2001). Thus, if a breeding population has strong linkages to certain wintering sites, then coordinated management schemes between these two regions may decrease the populations' vulnerability to extinction. Here we use natural stable isotope ratios in feathers along with North American Breeding Bird Survey census data in a Bayesian analysis of migratory connectivity in Henslow's sparrows.

Researchers have recently begun using natural isotope ratios in studies of bird migration (e.g., Chamberlain *et al.* 1997; Hobson & Wassenaar 1997, Marra, Hobson & Holmes 1998; Hobson *et al.* 2001). Chemical elements usually occur as two stable isotopes, with one of the isotopes being far more abundant than the other (Hoefs 1980). Depending on the element, the ratio of the two isotopes can vary geographically due to properties of the parent rock, climate, altitude, latitude, and distance from oceanic, meteoric, or paleo waters (Van der Merwe *et al.* 1990, Körner *et al.* 1991, Schaffener & Swart 1991, Chamberlain *et al.* 1997, Hobson & Wassenaar 1997). Isotope ratios in an

organism's tissues correlate with environmental ratios, thus tissue isotope ratios can also vary geographically. This covariation provides a natural marker that can be used to identify the region where the organism was during tissues growth (Kelly & Finch 1998). Stable isotope ratio data have also been used in conjunction with other data to determine migration linkages between breeding and wintering grounds (Clegg et al. 2003).

Past studies using stable isotope ratios have been valuable in revealing the potential of these natural markers in migration studies. One important concern, however, is that ignoring spatial patterns of bird abundance in breeding and wintering grounds could lead to spurious predictions (Royle & Rubenstein, unpublished ms.). If regional abundance is not considered, then a wintering bird may be assigned to a breeding region of low abundance, simply because it has that region's stable isotope signature, and not because most of the birds with that signature breed in that region. The assignment of a wintering individual to a breeding region should be made probabilistically and should take into account variation in abundance among regions of the breeding range. Thus, the assignment of a wintering individual to a region of the breeding range is an intrinsically Bayesian problem.

Here, we evaluate linkages between the breeding and wintering grounds of Henslow's sparrows (*Ammodramus henslowii*). This short-distance migrant is in decline throughout its breeding range and hence is of special conservation concern (Sauer et al. 2001). It is an obligate member of grassland ecosystems, which have been diminishing throughout North America due to industrial agriculture, suburban sprawl, and fire suppression (Sample 1989; Herkert 1991, 1994; Swengel 1996). Although prescribed fire can be used to maintain this species' wintering habitat in the southeastern United

States (Plentovich et al. 1999, Tucker et al. 2003), much of this potential habitat is currently unsuitable owing to fire-suppression practices. Operating in a vacuum of knowledge about the linkages between breeding and wintering populations limits our ability to devise appropriate habitat-management plans. (By September 2000, only 10 of 2,761 banded Henslow's sparrows had been recovered [Gustafson and Hildenbrand 1999].) For instance, managers have no way of knowing which portion of the wintering range to target to compensate for declines in particular breeding populations. By determining linkages between breeding and wintering populations, biologists could prioritize habitat-management needs.

Linkages between Henslow's sparrow breeding and wintering grounds are evaluated using feather stable isotope ratios of hydrogen and carbon and relative abundance data taken from the North American Breeding Bird Survey (BBS) website. The BBS is an annual survey of North American breeding birds taken from roadside routes across most of the United States and Canada (USGS Patuxent Wildlife Research Center 2003a). The Henslow's sparrow breeding range is calculated using routes where this species was detected between 1993 and 2002. The breeding range is divided into five regions and relative abundances of Henslow's sparrows are estimated using the BBS data. These relative abundances are used as the prior probabilities of the breeding-ground origins of wintering birds. To evaluate the effect of relative abundances as prior distributions, another analysis is done using flat prior probabilities, where each wintering individual is assumed to have an equal probability of originating from any of the breeding regions.

Feathers gathered from birds in the breeding ground are used to estimate hydrogen and carbon isotopic characteristics of each breeding region. Environmental hydrogen isotope ratios vary geographically across North America (Hobson & Wassenaar 1997), thus the feather isotope ratios of this element have been used in various studies of migratory linkages (e.g. Hobson & Wassenaar 1997, Chamberlain et al. 1997, Wassenaar & Hobson 2000a, Hobson et al. 2001, Meehan et al. 2001, Kelly et al. 2001). Carbon isotope ratios have been used previously in diet and trophic level studies (e.g. Forero & Hobson 2003, McCutchan et al. 2003). This element has also been used in migratory linkages studies, both to distinguish the type of habitat birds originated from (e.g. Marra et al. 1998, Wassenaar & Hobson 2000a) and to distinguish the region of origin (e.g. Chamberlain et al. 1997, Graves et al. 2002, Rubenstein et al. 2002, Royle & Rubenstein, unpublished ms.). Studies on the spatial patterns of feather carbon isotope ratios across breeding ranges have produced mixed results. Whereas some studies found little or no correlation between δC and latitude (e.g. Wassenaar & Hobson 2001), others have found breeding ground δC to be correlated with altitude (Graves et al. 2002) and latitude (Rubenstein et al. 2002). To compare the effect of using two rather than just one isotope on migratory linkage estimates, we use the isotope ratio distributions of feather δH alone and along with δC . Posterior probabilities of the breeding-ground origins of wintering birds are calculated using the isotope and BBS data sets.

CHAPTER 2: ESTIMATING SONGBIRD DISPERSAL USING HYDROGEN ISOTOPE RATIOS, MEME FLOW AND GENE FLOW

The rate and pattern of dispersal among populations can have a great effect on the populations' evolution and ecology. In populations of conservation concern,

dispersal rate can be an important factor in conservation planning. Because of its importance, dispersal has been studied in many species using various techniques. Unfortunately, little is known about which individuals disperse, precisely when they disperse, and how far they disperse. This makes estimating dispersal rate a daunting task. If traditional tracking techniques (e.g., radio telemetry and mark-recapture) are used, then large numbers of individuals need to be studied over long periods in order to maximize the chances of detecting and tracking a dispersal event. Instead of studying dispersal by using artificial markers such as bands and tags, we use natural markers that identify an individual as a new immigrant into a focal population. The first two natural markers, stable isotope ratios in feathers and song structure, both indicate whether an individual originated from the focal population. We use these two markers along with genetic markers, which estimate genetically effective dispersal, to estimate dispersal (and gene flow) in the Henslow's sparrow.

Using stable isotope ratios to estimate dispersal is a recent application of this natural marker (Hobson et al. 2001). Because δH in feathers correlates with environmental δH where the feather was grown (Hobson & Wassenaar 1997), feathers grown during the previous breeding season will have a δH representative of the previous breeding-ground region. If an individual has immigrated a long distance into a new breeding region, its feathers will have a δH representative of its previous breeding region. Because δH varies predictably along a gradient in North America, this isotope ratio can be used as a signature of an individual's previous breeding region.

If a male's song structure is unusual in comparison to other songs in the breeding area, then he may be an immigrant to that area. Learned song can be considered a cultural trait or meme ("an entity that is capable of being transmitted from one brain to another," Dawkins 1976). If a species' song structure varies geographically and is learned, then a male's song structure may indicate where he learned the song. Another source of unusual songs is through song mutation during learning (Lynch 1996). Hence, the rate of song mutation must be taken into account when estimating the proportion of dispersers into a population with song structure.

We compare these two dispersal estimates with three methods of evaluating genetic population structure. The first method uses F_{st} estimates, the second uses private alleles (alleles found only in a single population), and the third uses a Bayesian clustering approach. The first two methods are used to estimate the number of effective migrants per generation among populations. The third method uses a Bayesian approach to infer the number of true populations and to identify immigrants.

Genetic structure among populations arises when they become genetically isolated. Over a long enough period, allele frequencies at neutral loci may diverge among populations, due to genetic drift and/or natural selection (Moritz 1994). In Wright's (1931) classical island model, F_{st} is inversely proportional to Nm ($F_{st} = 1/[4Nm+1]$; Slatkin 1985, but see Whitlock & McCauley 1998 for a critique). Private alleles are also related to Nm among populations (Slatkin 1985). Computer simulations have shown the average frequency of private alleles is

inversely and roughly linearly related to Nm (Slatkin 1981, 1985; Barton & Slatkin 1986). We also use a novel method to evaluate the probability that an allele is truly private and not simply appearing so due to insufficient sampling. Estimates of Nm using F_{st} and private alleles make a number of assumptions (Whitlock & McCauley 1998), including the assumption that the populations are at evolutionary equilibrium.

The computer program *Structure* (Pritchard, Stephens & Donnelly 2000) uses a Bayesian clustering method to evaluate genetic structure among sampling sites. The program uses individuals' genotypes to determine the number of clusters (i.e., populations) in a data set. The probability that the data set represents K populations is calculated and can be used to infer the most probable number of populations. Each individual is also assigned a probability of being from each cluster. These probabilities can be used to determine if an individual is an immigrant.

The genetic analyses, using microsatellite data, estimate population structure in Henslow's sparrows. These estimates are then compared with dispersal rates estimated using stable isotope ratio and meme flow. In the stable isotope analysis, the δH in an individual's flight feathers is compared to the expected δH of the sampling site to judge whether the bird is an immigrant. The proportion of immigrants into a site is then calculated. In the meme flow analysis, an individual's song structure is compared to the song structure of other individuals from the same sampling site to judge whether the bird is an immigrant. Again, the proportion of immigrants into a site is then calculated.

CHAPTER 3: HOW DO DISPERSAL ESTIMATES COMPARE? A REVIEW AND PROSPECTUS

A variety of techniques have been used to estimate dispersal patterns, such as mark-recapture programs (e.g., Mennechez, Schtickzelle & Baguette 2003), radio-tracking (e.g., Blouin-Demers & Weatherhead 2002), and genetic population structure (e.g., Gibbs, Dawson & Hobson 2000). Recently, two other techniques have shown promise as indicators of dispersal: stable isotope ratios in tissues (Hobson et al. 2001) and spatial autocorrelation of population densities (Koenig 1999). These various methods may produce different, even contradictory, dispersal estimates. The various methods may also estimate dispersal at different time scales. Finally, one method may estimate the numbers of individuals dispersing from/to an area, while another method estimates the average amount of effective gene flow among populations over time. The variation in dispersal estimates is worrisome, for an accurate and appropriate dispersal estimate may be critical for the design of a species' conservation management plan. We compare three methods for estimating dispersal.

These three methods, using genetic markers, stable isotope ratios (using δH), and spatial autocorrelation, are compared across five avian species: red-winged blackbird (*Agelaius phoeniceus*), Bicknell's thrush (*Catharus bicknelli*), black-throated blue warbler (*Dendroica caerulescens*), Wilson's warbler (*Wilsonia pusilla*) and Henslow's sparrow. For all species except the Henslow's sparrow, published genetic and stable isotope data are used. Analysis on the genetic and stable isotope data for the Henslow's sparrow is presented, as are all of the spatial autocorrelation analyses. The use of genetic markers in dispersal estimates is described above. More on the use of δH in feathers and spatial autocorrelation as dispersal estimates is presented below.

The relationship between the δH in feathers and sampling location has been suggested as a potential indicator of the amount of dispersal in a population. In a study of Bicknell's thrushes (*Catharus bicknelli*; Hobson et al. 2001), the relationship between feather δH and breeding ground precipitation δH was weaker than the relationships found in previous studies (Chamberlain et al. 1997, Hobson & Wassenaar 1997), which the authors proposed could be due to inter-year dispersal. If a bird disperses to a new breeding site (after winter), its feather δH will correlate with the δH of its previous breeding site (because it undergoes molt at the end of the breeding season) rather than site of capture. Here we use Pearson's r^2 values of correlations between feather δH and local precipitation or latitude as estimates of dispersal among populations.

Spatial autocorrelation (SAC), the synchrony of populations over time and space, is thought to be the result of any combination of three processes: 1) predators moving from areas of low to high prey density, 2) density-dependent dispersal, and 3) density-independent factors affecting populations across a region, also known as the Moran effect (Koenig 1999, Swanson & Johnson 1999). Here we focus on the latter two processes.

Distinguishing between dispersal and the Moran effect as the main synchronizing agent among populations has produced much discussion in the biological literature (e.g., Ripa 2000). It has been proposed that density-dependent dispersal will produce SAC patterns that decay more quickly over distance than SAC patterns produced by the Moran effect (Ranta et al. 1995). However, the Moran effect may always be present, while dispersal may simply be an additional synchronizing agent (Ripa 2000).

Here we determine areas of SAC in three species (red-winged blackbirds, black-throated blue warbler, and Wilson's warbler) using census data from the North American

Breeding Bird Survey (BBS). Not enough BBS data were available for SAC analysis of the other two species. To determine the main synchronizing agent for these three species we use a set of criteria developed to help distinguish between the two candidates (Swanson & Johnson 1999). The method uses two criteria. The first evaluates the density-dependent pattern among the synchronizing populations. Populations that are synchronized mostly due to the Moran effect will have homogeneous density-dependent structures. Populations that are heterogeneous in their density-dependent structure will be synchronized because of additional agents (Swanson & Johnson 1999). The second criterion evaluates the synchrony-distance relationship among populations. Populations with a significant relationship between synchrony and interpopulation distance are likely to be synchronized because of density-dependent dispersal.

Because one of the dispersal estimates (gene flow) provides an estimate of the effective gene flow over time, it can be used to estimate long-term dispersal, while estimates using δH in feathers estimate inter-year dispersal. Hence, it is possible to compare historical dispersal rates with current dispersal rates among populations. By evaluating the historical patterns of dispersal among populations, we may be able to determine if a species' dispersal rate has changed over time. If so, it may be due to anthropogenic causes and may have conservation implications.

The studies presented here use a comprehensive combination of old and new methods in an attempt to better understand population connectivity between breeding and wintering grounds and between breeding populations. Comparing the outcomes from different methods of estimating dispersal allows us to evaluate how well their estimates agree. Using a Bayesian approach to integrate information from different sources allows

researchers to maximize the strengths of the various data sets. By approaching migration connectivity and dispersal in different ways, I hope our work helps initiate new discussions and brings more innovation into these research fields.

REFERENCES

- Barton, N.H. & Slatkin, M. (1986) A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity* **56**, 409-415.
- Blouin-Demers, G. and Weatherhead, P.J. (2002) Implications of movement patterns for gene flow in black rat snakes (*Elaphe obsoleta*). *Canadian Journal of Zoology* **80**, 1162-1172.
- Chamberlain, C.P., Blum, J.D., Holmes, R.T., Fen, X., Sherry, T.W. & Graves, G.R. (1997) The use of isotope tracers for identifying populations of migratory birds. *Oecologia*, **109**, 132-141.
- Clegg, S.M., Kelly, J.F., Kimura, M., & Smith, T.B. (2003) Combining genetic markers and stable isotopes to reveal population connectivity and migration patterns in a Neotropical migrant, Wilson's warbler (*Wilsonia pusilla*). *Molecular Ecology* **12**, 819-830.
- Dawkins, R. (1976) *The Selfish Gene*. Oxford University Press, New York.
- Forero, M.G. & Hobson, K.A. (2003) Using stable isotopes of nitrogen and carbon to study seabird ecology: Applications in the Mediterranean seabird community. *Scientia Marina*, **67**, 23-32.
- Gibbs, H.L., Dawson, R.J.G. & Hobson, K.A. (2000) Limited differentiation in microsatellite DNA variation among northern populations of the yellow warbler: evidence for male-biased gene flow? *Molecular Ecology*, **9**, 2137-2147.
- Gill, J.A., Norris, K., Potts, P.M., Gunnarsson, T.G., Atkinson P.W. & Sutherland W.J. (2001) The buffer effect and large-scale population regulation in migratory birds. *Nature* **412**, 436-438.
- Graves, G.R., Romanek, C.S. & Navarro, A.R. (2002) Stable isotope signature of philopatry and dispersal in a migratory songbird. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 8097-8100.
- Gustafson, M.E. & Hildebrand, J. (1999) Bird Banding Laboratory Homepage, ver 12/02/99. [Online]. Available: <http://www.pwrc.usgs.gov/bbl/> [Access date: 10/22/03]
- Herkert, J.R. (1991) Prairie birds in Illinois: populations response to two centuries of habitat change. *Illinois Natural History Survey Bulletin*, **34**, 393-399.
- Herkert, J.R. (1994) Status and habitat selection of the Henslow's Sparrow in Illinois. *Wilson Bulletin*, **106**, 35-45.
- Hobson, K.A. & Wassenaar, L.I. (1997) Linking breeding and wintering grounds of neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. *Oecologia*, **109**, 142-148.

- Hobson, K.A., McFarland, K.P., Wassenaar, L.I., Rimmer, C.C. & Goetz, J.E. (2001) Linking breeding and wintering grounds of Bicknell's Thrushes using stable isotope analyses of feathers. *Auk*, **118**,16-23.
- Hoefs, J. (1980) *Stable isotope geochemistry*. Springer-Verlag, Berlin.
- Kelly, J.F. & Finch, D.M. (1998) Tracking migrant songbirds with stable isotopes. *Trends in Ecology and Evolution*, **13**, 48-49.
- Kelly, J.F., Atudorei, V., Sharp, Z.D. & Finch, D.M. (2001) Insights into Wilson's warbler migration from analyses of hydrogen stable-isotope ratios. *Oecologia*, **130**, 216-221.
- Koenig, W.D. (1999) Spatial Autocorrelation of ecological phenomena. *Trends in Ecology and Evolution* **14**, 22-26.
- Körner, C.H., Farquhar, G.D. & Wong, S.C. (1991) Carbon isotope discrimination by plants follows latitudinal and altitudinal trends. *Oecologia*, **88**, 30-40.
- Lynch, A. (1996) The population memetics of birdsong. *Ecology and Evolution of Acoustic Communication in Birds* (eds D.E. Kroodsma & E.H. Miller), pp. 181-197. Cornell University Press, Ithaca, NY.
- Marra, P.P., Hobson, K.A. & Holmes, R.T. (1998) Linking winter and summer events in a migratory bird by using stable-isotopes. *Science*, **292**, 1884-1886.
- McCutchan, J.H., Jr., Lewis, W.M., Jr., Kendall, C. & McGrath, C.C. (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, **102**, 378-390.
- Meehan, T.D., Lott, C.A., Sharp, Z.D., Smith, R.B., Rosenfield, R.N, Stewart, A.C. & Murphy, R.K. (2001) Using hydrogen isotope geochemistry to estimate the natal latitudes of immature Cooper's hawks migrating through the Florida Keys. *Condor*, **103**, 11-20.
- Mennechez, G., Schtickzelle, N., and Baguette, M. (2003) Metapopulation dynamics of the bog fritillary butterfly: Comparison of demographic parameters and dispersal between a continuous and a highly fragmented landscape. *Landscape Ecology* **18**, 279, 291.
- Moritz, C. (1994) Defining 'evolutionarily significant units' for conservation. *Trends in Ecology and Evolution*, **9**, 373-375.
- Plentovich, S., Holler, N.R. & Hill, G.E. (1999) Habitat requirements for Henslow's sparrows wintering in silvicultural lands of the Gulf Coastal Plain. *Auk*, **116**, 109-115.
- Pritchard, J.K, Stephens, M. & Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945-959.
- Ranta, E., Kaitala, V., Lindström, J., & Lindén, H. (1995) Synchrony in population dynamics. *Proceedings of the Royal Society of London B* **262**, 113-118.
- Ripa, J. (2000) Analysing the Moran effect and dispersal: their significance and interaction in synchronous population dynamics. *Oikos* **89**, 175-187.
- Rubenstein, D.R., Chamberlain, C.P., Holmes, R.T., Ayres, M.P., Waldbauer, J.R., Graves, G.R. & Tuross, N.C. (2002) Linking breeding and wintering ranges of a migratory songbirds using stable isotopes. *Science*, **295**, 1061-1065.
- Sample, D.W. (1989) *Grassland birds in southern Wisconsin: habitat preference, population trends, and response to land use changes*. MS thesis, University of Wisconsin, Madison, U.S.A.

- Sauer, J.R., Hines, J.E. & Fallon, J. (2001) The North American Breeding Bird Survey, results and analysis 1966-2000. Version 2001.2, USGS Patuxent Wildlife Research Center, Laurel, MD. <http://www.mbr-pwrc.sgs.gov/bbs/bbs.html>
- Schaffner, F.C. & Swart, P.K. (1991) Influence of diet and environmental water on the carbon and oxygen isotopic signatures of seabird eggshell carbonate. *Bulletin of Marine Science*, **48**, 23-38.
- Slatkin, M. (1985b) Rare alleles as indicators of gene flow. *Evolution*, **39**, 53-65.
- Slatkin, M. (1981) Estimating levels of gene flow in natural populations. *Genetics*, **99**, 323-335.
- Swanson, B.J. & Johnson, D.R. (1999). Distinguishing causes of intraspecific synchrony in population dynamics. *Oikos* **86**, 265-274.
- Swengel, S.C. (1996) Management responses of three species of declining sparrows in tallgrass prairie. *Bird Conservation International*, **6**, 241-253.
- Tucker, J.W. & Robinson, W.D. (2003) Influence of season and frequency of fire on Henslow's Sparrows (*Ammodramus henslowii*) wintering on Gulf Coast pitcher plant bogs. *Auk*, **120**, 96-106.
- USGS Patuxent Wildlife Research Center (2003a) North American Breeding Bird Survey About BBS, 1, December 2003. (<http://www.mp2-pwrc.usgs.gov/bbs/about/index.html>).
- Van der Merwe, N.J., Lee-Thorpe, J.A., Thackeray, J.F., Hall-Margin, A., Kruger, F.J., Coetzee, H., Bell, R.H.V. & Lindeque, M. (1990) Source-area determination of elephant ivory by isotopic analysis. *Nature*, **346**, 744-746.
- Wassenaar, L.I. & Hobson, K.A. (2000a) Evaluating the use of stable-carbon ($\delta^{13}\text{C}$) and H (δD) isotope ratios in feathers to infer breeding origins of Red-winged Blackbirds. *Ecological Applications*, **10**, 911-916.
- Wassenaar, L.I. & Hobson, K.A. (2000a) Evaluating the use of stable-carbon ($\delta^{13}\text{C}$) and H (δD) isotope ratios in feathers to infer breeding origins of Red-winged Blackbirds. *Ecological Applications*, **10**, 911-916.
- Wassenaar, L.I. & Hobson, K.A. (2001) A stable-isotope approach to delineate geographical catchment areas of avian migration monitoring stations in North America. *Environmental Science and Technology* **35**, 1845-1850.
- Webster, M.S., Marra, P.P., Haig, S.M, Bensch, S. & Holmes, R.T. (2002) Links between worlds: unraveling migratory connectivity. *Trends in Ecology and Evolution* **17**, 76-83.
- Whitlock, M.C & McCauley, D.E. (1998) Indirect measures of gene flow and migration: $F_{st} \neq 1/(4Nm+1)$. *Heredity* **82**, 117-125.
- Wright, S. (1931) Evolution in Mendelian populations. *Genetics* **16**, 97-159.

CHAPTER 1

LINKING BREEDING AND WINTERING GROUNDS

USING STABLE ISOTOPE DATA: A BAYESIAN

APPROACH

SUMMARY

1. We combine stable isotope and abundance data to make Bayesian inferences about linkages between the breeding and wintering grounds of Henslow's sparrows (*Ammodramus henslowii*).
2. Stable isotope ratios in flight feathers correlate with environmental stable isotope ratios where feathers are grown. In this species, feathers are molted and regenerated in late summer. Thus the stable isotope ratio of a feather from a wintering bird provides a signature of the location where that individual spent the preceding breeding season. We explore the use of hydrogen stable isotope ratios with and without carbon stable isotope ratios (δC) as signatures of an individual's breeding region.
3. Relative abundances in five breeding-ground regions are calculated using Breeding Bird Survey (BBS) data.

4. It is unclear whether the inclusion of δC data in the analysis improved our Bayesian inferences. The δC data may have helped distinguish some breeding regions or they could have led to spurious breeding-region assignments. We hope additional δC data from across North America will be gathered in other studies to resolve this question.
5. In our analysis, the stable isotope ratio data may be weak and overwhelmed by the prior probabilities. This weakness may be due to the large overlap in distributions of stable isotope ratios in the breeding regions.
6. According to the Bayesian analyses, Henslow's sparrows at wintering sites typically comprised combinations of individuals from various parts of the breeding range. No evidence for any clear linkages between breeding and wintering regions emerged. Thus, coordination of grassland bird management between these two ranges can be more generalized rather than between specific areas.
7. By using the Bayesian approach in investigations of migratory linkages between breeding and wintering grounds, researchers can take advantage of information from various sources to calculate the probabilities of origin from several breeding sites for individual wintering birds.

INTRODUCTION

Establishing linkages between the breeding and wintering grounds of individuals has been a compelling task for conservation biologists. Knowledge about any linkages between these two regions could help managers coordinate conservation efforts for species of concern. Determining linkages for small migratory birds is especially challenging. Radio and global positioning system transmitters are often too bulky for small birds and banding efforts have produced far too few data to reveal such linkages. Consequently, our knowledge of such linkages in small birds has been almost nonexistent, until recently, when researchers began to address this problem with a promising new method. This method uses natural stable isotope ratios in avian tissues to determine linkages between breeding and wintering grounds (e.g., Chamberlain *et al.* 1997, Hobson & Wassenaar 1997, Marra, Hobson & Holmes 1998, Hobson *et al.* 2001; see Table 1.1 for synopses).

Briefly, this method takes advantage of the facts that: 1) stable isotope ratios of some elements vary geographically in the environment; 2) these elements are incorporated into growing feathers (or other tissues); and 3) the resulting stable isotope ratios in feathers correlate with the environmental ratios where the feathers were grown. Thus, stable isotope ratios of naturally occurring elements in the environment are natural markers of the region where an organism's tissues were grown (Kelly & Finch 1998). In birds breeding in the north-temperate zone, the annual molt typically occurs during late summer, before migration to the wintering grounds. Thus, the stable isotope ratio of a feather from a wintering bird provides a signature of the location where that individual spent the preceding breeding season.

In stable isotope studies of avian migration, two methodological considerations are important. First, the stable isotope ratios characterizing a region need to be determined by measuring the feathers' ratios. To ensure the feathers were grown in the region, feathers should be taken from adults known to be long-term residents, fledglings, or nestlings (Hobson *et al.* 2001, Meehan *et al.* 2001, Lott, Meehan & Heath 2003). Otherwise, the researcher runs the risk of collecting feathers from immigrants and thereby confounding inferences about the breeding-ground origin of wintering birds. Second, how an element's stable isotope ratio varies geographically needs to be understood. Natural stable isotope ratios can be affected by altitude or marine systems (e.g., hydrogen in Hobson *et al.* 2000, Lott, Meehan & Heath 2003; carbon in Graves, Romanek & Navarro 2002). Therefore, distant sites may have similar isotope ratios due to similarities in their altitude or proximity to marine systems. Recently, researchers have begun incorporating these considerations into their study designs (e.g., Meehan *et al.* 2001, Lott, Meehan & Heath 2003).

Another important consideration, which has been overlooked until now (see also Royle & Rubenstein, unpublished ms.), is that the "assignment" of a wintering individual to a region of the breeding range is an intrinsically Bayesian problem. Assignments should be made probabilistically and should take into account variation in abundance among regions of the breeding range. Consider the following illustration. The stable isotope ratios of birds from different regions of the breeding range will have characteristic distributions and these distributions may be overlapping. In the

Study	Organisms	Stable Isotope	Tissue	Contribution
Hobson & Wassenaar 1997	Neotropic-Nearctic migratory songbirds	Hydrogen	Feathers	Demonstrated positive correlations between feather δH^1 and growing season precipitation ratios where breeding birds were sampled. Wintering birds in Guatemala had feather δH consistent with the δH of the species' breeding ranges.
Chamberlain <i>et al.</i> 1997	Neotropic-Nearctic migratory songbirds	Hydrogen Carbon Strontium	Feathers Bone	Demonstrated positive correlations between feather δH and latitude. Feather δC^2 found to vary with breeding ground latitude, with the exception of an Alberta, Canada site. Bone δSr^3 also showed a geographic pattern in the breeding grounds.
Marra <i>et al.</i> 1998	Neotropic-Nearctic migratory songbirds	Carbon	Blood	Linked wintering habitat quality with breeding arrival and condition.
Hobson <i>et al.</i> 2001	Neotropic-Nearctic migratory songbirds	Hydrogen	Feather	Proposed weaker relationship between feather δH and breeding ground precipitation δH could be due to inter-year dispersal or variation in δH due to altitude or proximity to marine systems.
Graves <i>et al.</i> 2002	Neotropic-Nearctic migratory songbirds	Carbon Nitrogen	Feather	Found age and altitudinal effects on δC . Found age and temporal effects on δN^5
Lott <i>et al.</i> 2003	Raptors	Hydrogen Sulfur	Feather	Used nestling and fledglings to demonstrate how raptors that forage on marine-based food have δH that do not correlate with the precipitation δH of the area where the birds were collected. Birds that foraged on marine prey had higher δS^4 than birds that foraged on inland prey.
Clegg <i>et al.</i> 2003	Neotropic-Nearctic migratory songbirds	Hydrogen	Feather	Used δH data (which distinguished northern and southern breeding origins) along with microsatellite data (which distinguished eastern and western breeding origins) to determine that this species migrates in a leapfrog fashion.

¹hydrogen:deuterium ratios ²carbon12:carbon13 ratios ³strontium86:strontium88 ratios

⁴sulfur34:sulfur32 ratios ⁵nitrogen15:nitrogen14 ratios

Table 1.1. Synopses of avian studies that used natural stable isotope ratios.

hypothetical example shown in Figure 1.1, because population B is so much larger than population A, most individuals appearing to belong to population A actually belong to population B. If relative abundance were ignored, then many wintering individuals originating from populations B but with stable isotope ratios falling within the distribution for population A would be incorrectly assigned. By taking into account relative abundance, wintering individuals should be more reliably assigned to the correct region of the breeding range.

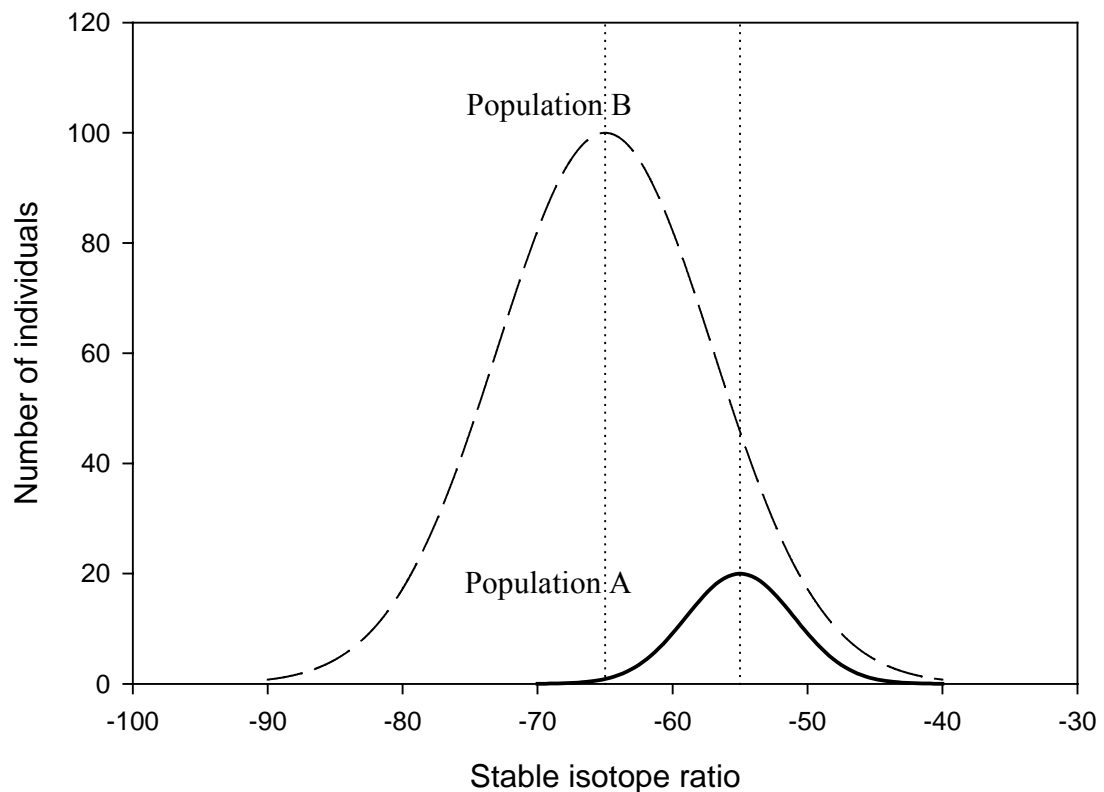


Figure 1.1. Hypothetical distributions of stable isotope ratios of feathers from two regions of the breeding ground.

Taking relative abundance into account, the probability that a wintering individual originated from a particular region of the breeding range can be calculated using Bayes' theorem:

$$\Pr(H_i | data) = \frac{\Pr(H_i) \Pr(data | H_i)}{\sum_j^N \Pr(H_j) \Pr(data | H_j)} \quad \text{Equation 1}$$

where $\Pr(H_i | data)$ = the posterior probability that the wintering individual originated from breeding region i , given the stable isotope ratio of its feather; $\Pr(H_i)$ = the prior probability that the wintering individual originated from breeding region i ; $\Pr(data | H_i)$ = the probability that the wintering individual would have the observed stable isotope ratio given the likelihood function of breeding region i .

This approach allows for comparison of the probabilities of multiple hypotheses, rather than the “reject/accept” outcome of a single null hypothesis based on the frequentist approach (Anderson 1998). In studies of migratory linkages, the hypotheses are the breeding-ground origins of a wintering bird given its stable isotope signature.

Here we combine stable isotope and abundance data to make Bayesian inferences about linkages between the breeding and wintering grounds of Henslow's sparrow (*Ammodramus henslowii*), a North American grassland bird. This short-distance migrant has been in decline throughout its breeding range (Sauer, Hines & Fallon 2001) and thus is a species of special conservation concern. By determining any linkages between breeding and wintering ranges, grassland managers in both ranges could coordinate conservation plans for this species.

METHODS

FIELD WORK

Henslow's sparrows were captured from 12 breeding-ground sites and five wintering-ground sites (Table 1.2). Some sites were visited only in 1999 or 2000; other sites were visited both years. On the breeding grounds, birds were caught using mist nets (30 mm mesh, 6 m long) and a playback of Henslow's sparrow songs. The songs were taken from the Borror Lab of Bioacoustics collection at The Ohio State University. Singing males were lured into the mist nets with the playbacks. On the wintering grounds, investigators flushed Henslow's sparrows into mist nets while walking abreast through a grassland site. Once caught, each sparrow was banded with a USFWS (United States Fish and Wildlife Service) band and morphometric measurements were made; approximately 20 μ l of blood were collected from the brachial vein for a genetic study (Chapter 2). For the stable isotope analysis, three tail feathers and four or five contour feathers were collected.

STABLE ISOTOPE ANALYSIS

Feather Preparation – Rectrices were soaked for 1 h, rinsed twice in a 2:1 chloroform:ethanol solution, and air dried. For analysis of deuterium:hydrogen ratio (δ H), 350 μ g (\pm 10 μ g) of finely cut feather were enclosed in 4 mm x 3.2 mm silver capsules. For analysis of carbon12:carbon13 ratio (δ C), 1 mg (\pm 0.1 mg) of finely cut feather was enclosed in 4 mm x 6 mm tin capsules. These samples were then sent to the Stable Isotope Hydrology and Ecology Laboratory at the National Water Research Institute in Saskatoon, Saskatchewan, Canada for analysis.

Site (code)	Years sampled	Breeding Region	Location	Feather δH (‰)			Feather δC (‰)		
				N	Mean	SD	N	Mean	SD
Breeding Ground Sampling Sites									
Niawathe Prairie, Missouri (A)	1999, 2000	Southwest	37°41'N, 93°45'W	19	-52.6	8.5	21	-18.8	2.7
Buena Vista Grasslands, Wisconsin (B)	1999, 2000	Northwest	44°20'N, 89°50'W	10	-64.6	11.6	10	-20.1	2.6
Shawnee National Forest, Illinois (C)	1999	Midwest	37°30'N, 88°40'W	4	-45.4	6.9	6	-17.4	1.8
Big Oaks National Wildlife Refuge, Indiana (D)	1999	Midwest	38°44'N, 85°22'W	9	-53.7	9.4	9	-19.9	1.3
Wright Patterson Air Force Base, Ohio (E)	2000	Midwest	39°45'N, 84°11'W	3	-60.0	4.6	3	-20.4	3.8
Tri-Valley Wildlife Area, Ohio (F)	2000	Midwest	40°04'N, 81°58'W	10	-56.1	5.5	10	-21.9	1.2
Woodbury Wildlife Area, Ohio (G)	2000	Midwest	40°20'N, 82°00'W	9	-64.3	8.3	10	-22.1	2.0
Crown City Wildlife Area, Ohio (H)	2000	Midwest	38°35'N, 82°16'W	10	-58.7	8.9	10	-21.7	1.4
Egypt Valley Wildlife Area, Ohio (I)	2000	Midwest	40°05'N, 81°10'W	10	-68.7	12.7	10	-22.3	1.9
Fort Drum, New York (J)	1999, 2000	Northeast	44°07'N, 75°18'W	16	-81.1	21.3	17	-23.6	0.7
Clarion County, Pennsylvania (K)	1999	Northeast	41°05'N, 79°26'W	3	-63.0	6.1	4	-22.0	2.4
Voice of America Tower, North Carolina (L)	1999, 2000	Southeast	35°37'N, 77°29'W	15	-52.8	16.4	18	-19.2	2.7
Wintering Ground Sampling Sites									
Fort Polk, Louisiana (M)	1999, 2000		31°00'N, 93°12'W	20	-69.2	24.9	20	-17.6	3.3
Mississippi Sandhill Crane National Wildlife Refuge, Mississippi (N)	1999, 2000		30°28'N, 88°37'W	19	-66.4	15.1	19	-19.2	3.7
Apalachicola National Forest, Florida (O)	1999, 2000		30°00'N, 85°00'W	20	-62.4	11.3	20	20.1	6.4
McIntosh & Glynn counties, Georgia (P)	2000		31°23'N, 81°31'W	12	-61.8	8.5	12	-21.1	3
Three Lakes Conservation Area, Florida (Q)	2000		28°07'N, 81°15'W	4	-55.8	5.7	4	-18.1	3.9

Table 1.2. Sampling locations, years, and stable isotope statistics for breeding and wintering Henslow's sparrows. Codes following the names of sampling sites correspond to those found on the map in Figure 1.2.

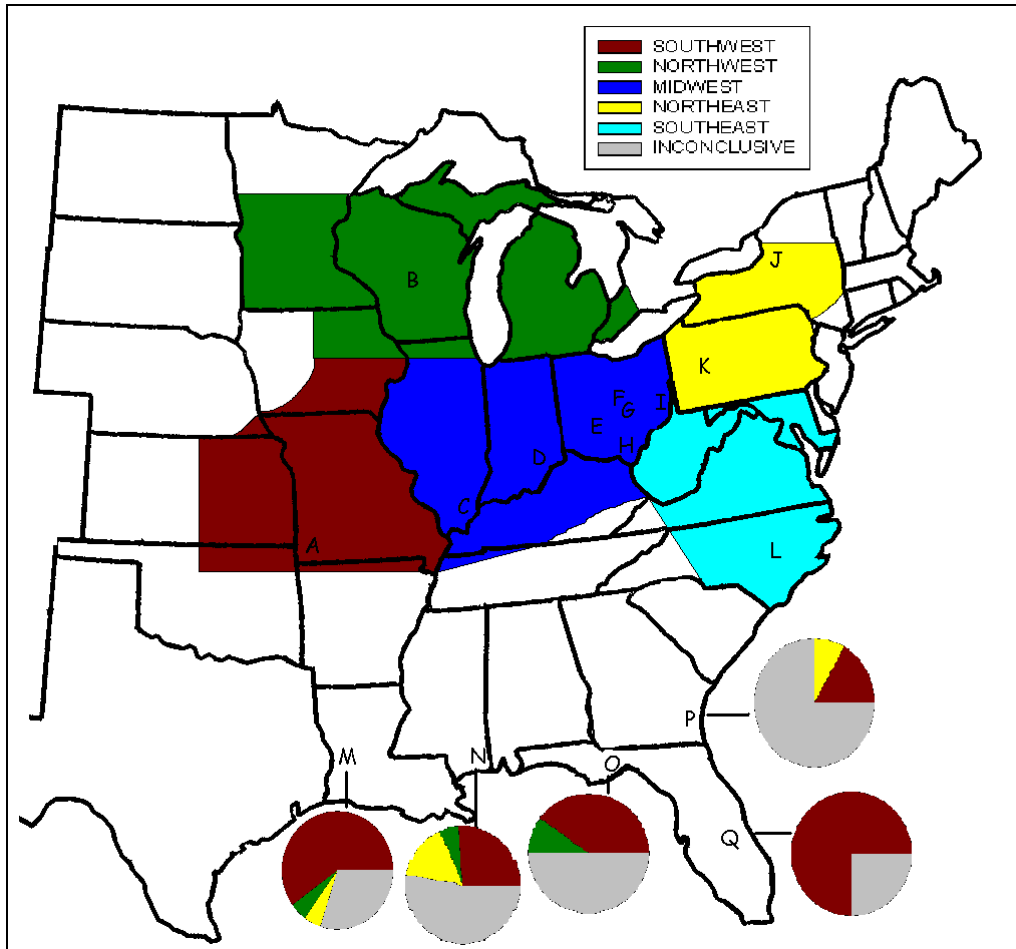


Figure 1.2. Breeding regions and sampling sites of Henslow’s sparrows. Range limits based on data from the North American Breeding Bird Survey (1993 to 2002). Sampling-site codes correspond to the names and locations reported in Table 1.1. Pie charts are the same as in Figure 1.7.

Stable Isotope Ratio Estimates – Because hydrogen molecules in feather keratin can be exchanged with ambient hydrogen (Hobson & Wassenaar 1997, Chamberlain *et al.* 1997), the δH feather samples were allowed to equilibrate with steam of known isotopic composition. This procedure ensured a known δH for each sample's exchangeable hydrogen. Samples were then combusted, waters produced from the combustion were reduced to H_2 , and isotope ratios of these gas samples were measured with a Micromass OptimaTM mass spectrometer. Because carbon molecules in feather keratin are fixed, the feathers for the δC analysis did not undergo steam equilibration. These samples were combusted and the resulting CO_2 was measured with the same mass spectrometer. (A more detailed description can be found in Wassenaar & Hobson 2000.) The δC values are reported in parts per thousand (‰) deviation from the PDB carbon standard (a Cretaceous belemnite from the Peedee Formation of South Carolina). The δH values are reported in parts per thousand deviation from Vienna Standard Mean Ocean Water Standard (VSMOW) and normalized to the VSMOW/SLAP (Standard Light Antarctic Precipitation) scale.

If a sample's isotope ratio fell well outside the range of other samples, a repeated measure was made, provided enough feather sample remained (δH : 11, δC : 20 repeated measures). If the repeats were similar, then an average of the two ratios was used (δH : 9, δC : 16 means). If the second isotope ratio fell within the range of other feather samples, the second estimate was used (δH : 2, δC : 4 second estimates). Isotope ratio values that fell outside three standard deviations from the overall mean for that element were considered outliers and not used in the analysis (δH : 3, δC : 0 outliers).

To evaluate any effect of year on stable isotope ratios, the ratios from breeding ground sites sampled in both 1999 and 2000 were compared using *t*-tests, assuming equal variances. Multiple linear regression was used to evaluate latitudinal and longitudinal variation in δH and δC of sparrows on the breeding grounds. Both procedures used SPSS routines (2001).

RELATIVE ABUNDANCE DATA ANALYSIS

Relative abundances are calculated using Breeding Bird Survey (BBS) data. The BBS is an annual survey of North American breeding birds taken from roadside routes across most of the United States and southern Canada (USGS Patuxent Wildlife Research Center 2003a). The breeding range of the Henslow's sparrows was divided into five regions (Fig. 1.2) and the relative abundance of Henslow's sparrows in these regions was estimated using BBS data collected 1993 to 2002. First, the current breeding range was operationally defined as encompassing BBS routes where Henslow's sparrows were detected in any of the 10 years (data downloaded from USGS Patuxent Wildlife Research Center 2003b). Margins of the breeding range were placed 100 km beyond peripheral survey routes where Henslow's sparrows were detected. Second, to calculate the number of detections per unit effort for each region, the total number of Henslow's sparrows detected over the 10 years was divided by the number of active routes (including routes with no detections) for those years (data downloaded from USGS Patuxent Wildlife Research Center 2001). Finally, the relative abundance for each region was calculated by dividing the detections per unit effort by the largest regional value. These estimates were

used as prior probabilities in the Bayesian analysis. By assumption, a wintering individual's probability of originating from a particular breeding region is unaffected by the distance between the wintering sites and the breeding region.

BAYESIAN ANALYSIS

Each sampling site was assigned to one of five regions of the breeding range (Table 1.2). For each breeding region, likelihood functions were calculated using the isotope ratios from feathers collected at the sampling sites on the breeding range. The estimated distributional properties (i.e., mean and variance) of isotope ratios of feathers at sampling sites within a region are assumed to represent the distributional properties for that breeding region. To compare the effect of using the δC data, analyses were done using the δH data with and without the δC data. Hence, likelihood functions were calculated using δH data with and without δC data. When the δH data were used alone, each breeding region's likelihood function was based on a univariate normal distribution:

$$F(x) = \frac{e^{-1/2\left(\frac{x-\mu}{\sigma}\right)^2}}{\sigma\sqrt{2\pi}} \quad \text{Equation 2}$$

where x = measured δH for a wintering individual's feathers, μ = mean δH for the breeding region, and σ = standard deviation of δH for the breeding region.

By contrast, when the δH data were used along with δC data, each breeding region's likelihood function was based on a bivariate normal distribution:

$$F(x_H, x_C) = \frac{1}{2\pi\sigma_H\sigma_C\sqrt{1-\rho_{HC}^2}} \exp\left\{-\frac{1}{2(1-\rho_{HC}^2)}\left[\left(\frac{x_H - \mu_H}{\sigma_H}\right)^2 - 2\rho_{HC}\left(\frac{x_H - \mu_H}{\sigma_H}\right)\left(\frac{x_C - \mu_C}{\sigma_C}\right) + \left(\frac{x_C - \mu_C}{\sigma_C}\right)^2\right]\right\}$$

Equation 3

where $x_H = \delta H$ of a wintering individual's feathers, $x_C = \delta C$ of a wintering individual's feathers, $\mu_H =$ mean δH for the breeding region, $\mu_C =$ mean δC for the breeding region, $\sigma_H =$ standard deviation of δH for the breeding region, $\sigma_C =$ standard deviation δC for the breeding region, and $\rho_{HC} =$ coefficient of correlation between δH and δC for the breeding region.

These density functions used the overall mean and standard deviations of all the sampling sites assigned to a given breeding region. The isotope ratio signature for each wintering individual was used to calculate that individual's likelihood of originating from each breeding region ($\Pr(\text{data}|H_i)$ in Equation 1).

Before using these density functions to assign wintering individuals to a breeding region, we asked whether they could be used to assign individuals of known breeding origin to the correct breeding region. Specifically, to determine whether a particular individual would be assigned to the correct breeding region, that individual's δH value (or δH and δC values) was withheld to estimate the mean and variance for that region, and then the probability that the individual belonged to each of the breeding regions was calculated. The individual was assigned to the region with the highest posterior probability. The proportion of individuals assigned to the correct region was determined.

For each wintering individual, Bayesian inference was used to assign the individual to a breeding ground origin. Each wintering individual was assigned to a

breeding region using two criteria. Under the first criterion, it was assigned to the breeding region with the highest posterior probability. Under the second criterion, it was assigned to a breeding region only if its posterior probability was at least 0.5. If the highest posterior probability was <0.5 , then the individual's breeding origin was classified as "inconclusive."

Finally, the "strength" of the stable isotope data was evaluated in the following way. One analysis was done using flat prior probabilities (0.2 for each breeding region). A second analysis was done using the regions' relative abundances as the prior probabilities. If the posterior probabilities produced by both analyses tend to "track" the prior probabilities, then the isotope data are uninformative and thus overwhelmed by the prior probabilities.

RESULTS

To evaluate the effect of year on the stable isotope ratios of breeding birds, *t*-tests for both δH and δC were performed for four sites, which were sampled in 1999 and 2000. Of these eight tests, none were significantly different after the Benjamini-Hochberg (1995) correction for multiplicity; however, two tests were nominally significant (North Carolina, $p=0.013$ and New York, $p=0.034$). Multiple regression analysis revealed both δH and δC varied with latitude and longitude on the breeding range (Table 1.3). Latitude and longitude explained approximately 36% of the variance in δH and 25% in δC . Latitude and longitude were better predictors of δH than δC (compare unstandardized coefficients in Table 1.3).

Isotope	R ²	F(df)	P	Unstandardized Coefficients (s.e.)			Standardized Coefficients	
				Constant	Latitude	Longitude	β_{latitude}	$\beta_{\text{longitude}}$
δH	0.361	50.729 (1, 123)	0.000	-1.769 (23.216)	-2.800 (0.407)	0.625 (0.173)	-0.503	0.265
δC	0.247	38.939 (1, 133)	0.000	-22.467 (4.047)	-0.362 (0.071)	0.194 (0.030)	-0.355	0.447

Table 1.3. Results of multiple regression analysis of how the stable isotope ratios of hydrogen (δH) and carbon (δC) in Henslow's sparrow feathers varied along latitude and longitude.

Breeding Region	δH		δC		δH and δC coefficient of correlation	Relative abundance
	Mean	Standard deviation	Mean	Standard deviation		
Southwest	-52.62	8.48	-18.80	2.67	0.09	1
Northwest	-64.60	11.64	-20.08	2.65	0.29	0.45
Midwest	-59.26	9.97	-21.11	2.27	0.49	0.64
Northeast	-78.24	20.67	-23.33	1.33	-0.11	0.25
Southeast	-52.86	16.44	-19.19	2.69	0.14	0.07

Table 1.4. Parameters used to calculate the univariate and bivariate normal distributions of the stable isotope data for each breeding region, and the relative abundance of Henslow's sparrows within each breeding region. The univariate distributions were calculated using only the δH data, whereas the bivariate distributions were calculated using both the δH and δC data. The relative abundances were calculated using data from the North American Breeding Bird Survey.

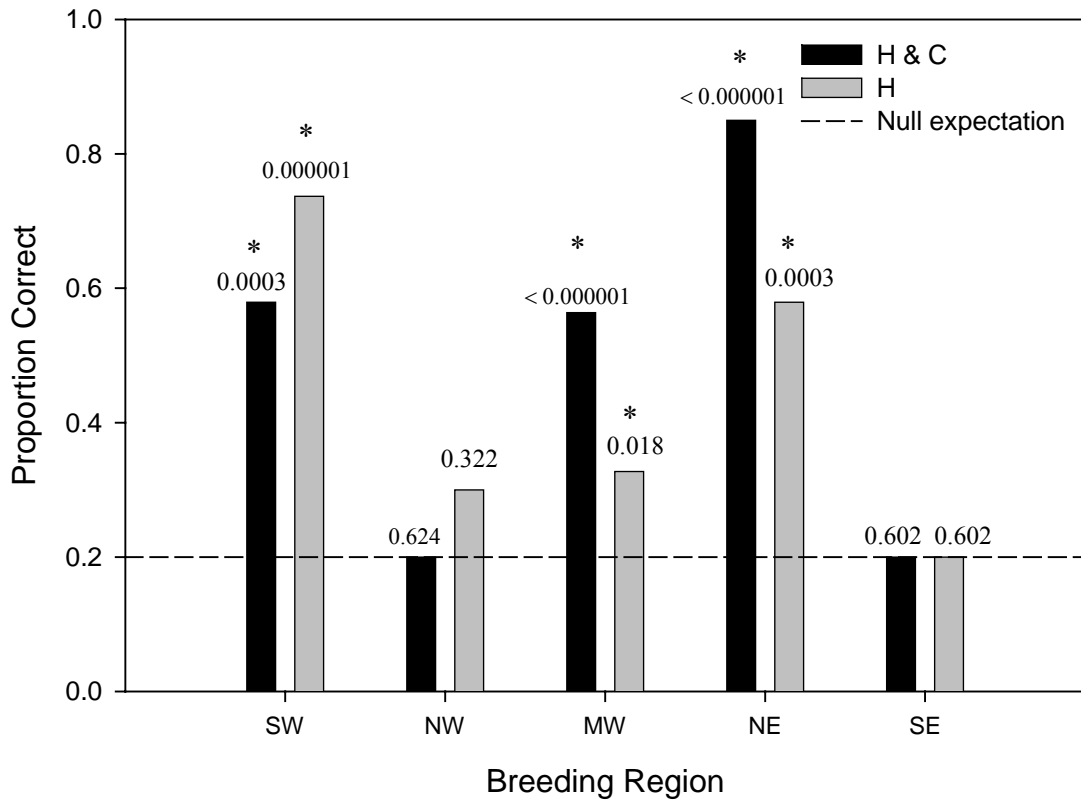


Figure 1.3. Proportions of individual Henslow’s sparrows on the breeding grounds correctly assigned to their breeding region. One analysis uses breeding region distributions based on hydrogen stable isotope ratios (gray bars) and the other analysis uses distributions based on hydrogen and carbon stable isotope ratios (black bars). The null expectation of the proportion of breeding assignments to each region is 0.2 (dotted lines). *P*-values are above each bar. Proportions with asterisks are significantly different from 0.2 after the Benjamini-Hochberg [1995] correction for multiplicity (binomial test).

The statistics used to generate the univariate and bivariate normal distributions (probability density functions) are reported in Table 1.4. The evaluation of accuracy-of-assignment of known individuals to breeding region, based on these density functions, produced mixed results (Fig. 1.3). In the southwest and northwest breeding regions, a larger proportion of breeding birds were correctly assigned to their breeding regions using δH only. In the midwest and northeast regions, a larger proportion of individuals were correctly assigned using both δH and δC . In the southeast region, the proportion of individuals correctly assigned using δH only was identical to the proportion using both δH and δC . The proportion of individuals correctly assigned was greater than expected by chance (binomial test; Fig. 1.3) in the southwestern, midwestern, and northeastern regions.

The BBS data indicate considerable variation in abundance of Henslow's sparrows among the five breeding regions (Table 1.4). These relative abundances were used as the prior probabilities for calculating the breeding-origin probabilities of each wintering sparrow. The posterior probabilities of breeding origins were calculated using univariate (Figs. 1.4 and 1.5) and bivariate (Figs. 1.6 and 1.7) density functions. Figures 1.4 and 1.6 show the proportions of individuals assigned to the breeding regions, based on the criterion that a wintering sparrow is assigned to the breeding region with the highest posterior probability. Figures 1.5 and 1.7 show the proportions of individuals assigned to the breeding regions, based on the criterion that a wintering sparrow is assigned to the breeding origin with a posterior probability ≥ 0.5 . The breeding origin of any wintering individual whose highest posterior probability is < 0.5 is classified as inconclusive.

Both univariate and bivariate analyses revealed a mixture of breeding region origins for sparrows captured at each wintering site. All wintering sites in all analyses contained some individuals that were assigned to the southwestern breeding region. However, at no wintering site in any analysis was even a single sparrow assigned to the southeastern breeding region. More individuals were assigned to the inconclusive category in the analysis using δH only than in the analysis using both δH and δC . For four wintering sites (Louisiana, Mississippi, Northern Florida and Georgia), the univariate analysis assigned sparrows to fewer breeding regions than the bivariate analysis (Figs. 1.5 and 1.7). In the bivariate analysis, Louisiana and Mississippi include wintering sparrows assigned to the northwestern region (Fig 1.7). No sparrows were assigned to this breeding region in the univariate analysis (Fig. 1.5). Finally, the Northern Florida and Georgia sites contain sparrows assigned to the northeastern region in the bivariate analysis (Fig. 1.7), but none in the univariate analysis (Fig. 1.5).

Figures 1.8 and 1.9 demonstrate the contribution of the stable isotope data to the breeding-origin assignments of wintering birds. Figure 1.8 displays the results of the Bayesian analysis where the prior probabilities (open circles) are flat (i.e., each wintering bird has an equal prior probability [0.2] of originating from each of the five breeding regions). The gray diamonds represent each wintering bird's posterior probabilities for each breeding region. The black triangles represent the mean posterior probabilities for each breeding region. All mean posterior probabilities fell within 0.16 of the flat prior probabilities. Figure 1.9 displays the results for the analysis where the prior probabilities

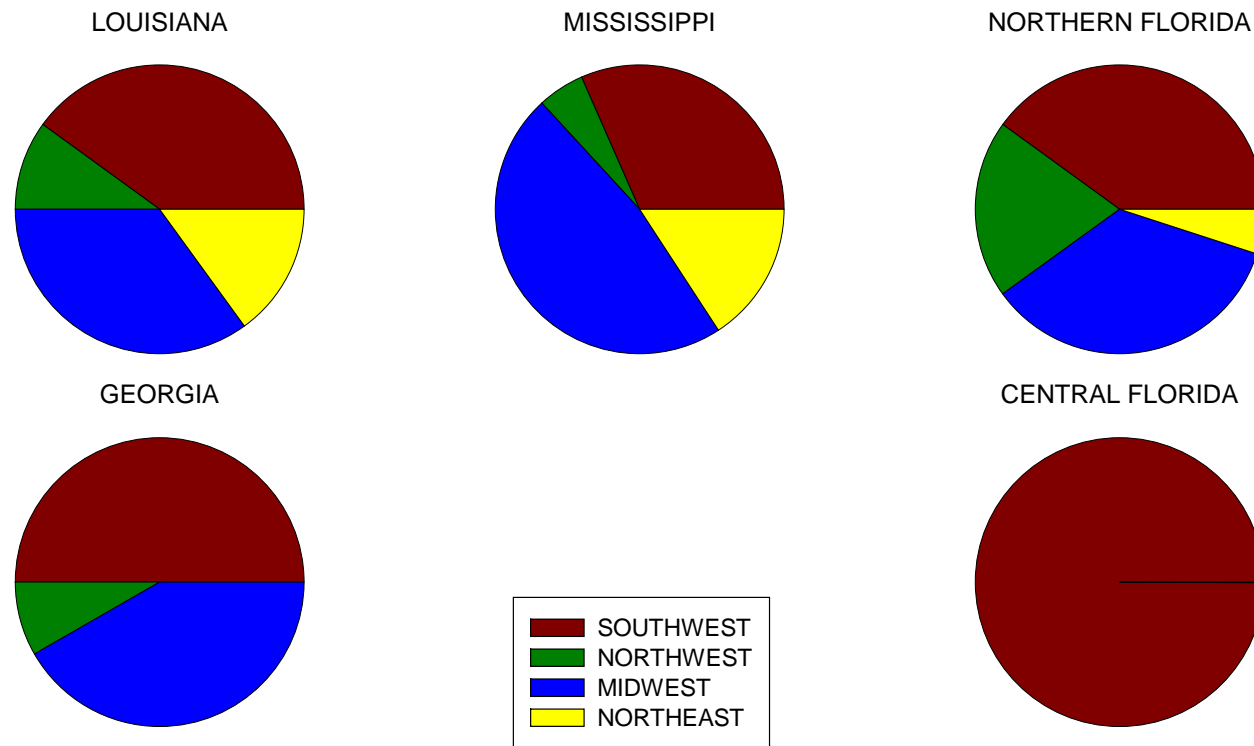


Figure 1.4. Pie charts showing the proportions of Henslow's sparrows sampled at a wintering site that were assigned to each of four breeding regions, using the highest probability criterion and δH data. (No wintering individuals were assigned to the southeastern breeding region.) Assignments were made by Bayesian inference, where each individual was assigned to the breeding region with the highest posterior probability. The prior probabilities were the relative abundances of Henslow's sparrows in each of the five breeding regions.

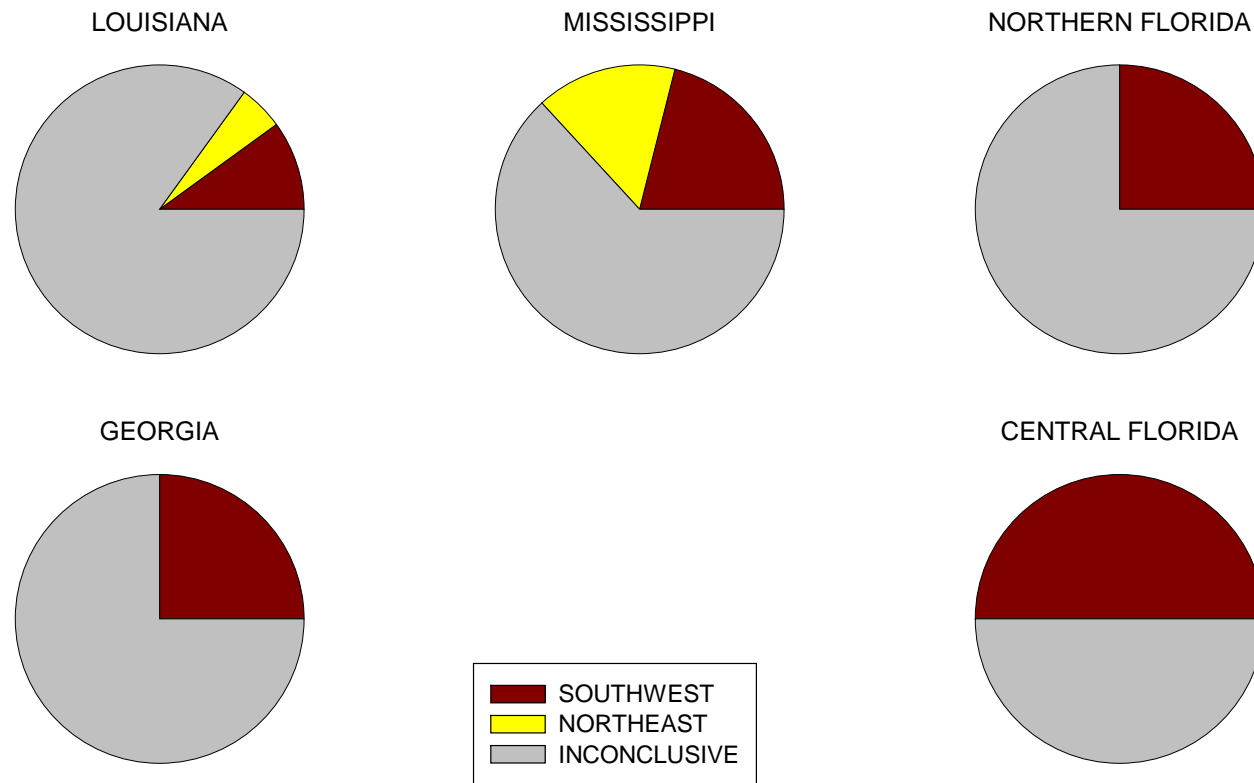


Figure 1.5. Pie charts showing the proportions of Henslow's sparrows sampled at a wintering site that were assigned to each of two breeding regions, using the ≥ 0.5 probability criterion and δH data. (No wintering individuals were assigned to the southeastern, northwestern, or midwestern breeding regions.) Assignments were made by Bayesian inference, where each individual was assigned to the breeding region with a posterior probability ≥ 0.5 . Individuals with no posterior probabilities ≥ 0.5 were assigned to the inconclusive category. The prior probabilities were the relative abundances of Henslow's sparrows in each of the five breeding regions.

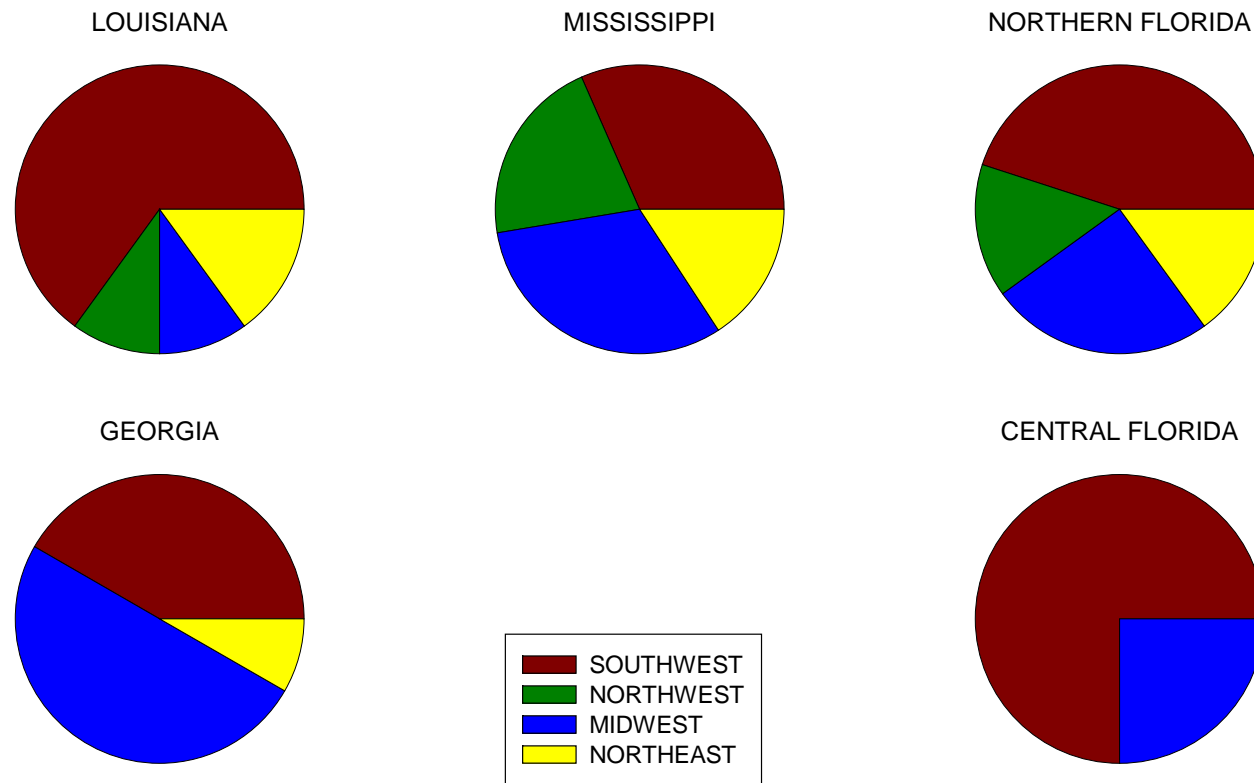


Figure 1.6. Pie charts showing the proportions of Henslow's sparrows sampled at a wintering site that were assigned to each of four breeding regions, using the highest probability criterion, and δH and δC data. (No wintering individuals were assigned to the southeastern breeding region.) Assignments were made by Bayesian inference, where each individual was assigned to the breeding region with the highest posterior probability. The prior probabilities were the relative abundances of Henslow's sparrows in each of the five breeding regions.

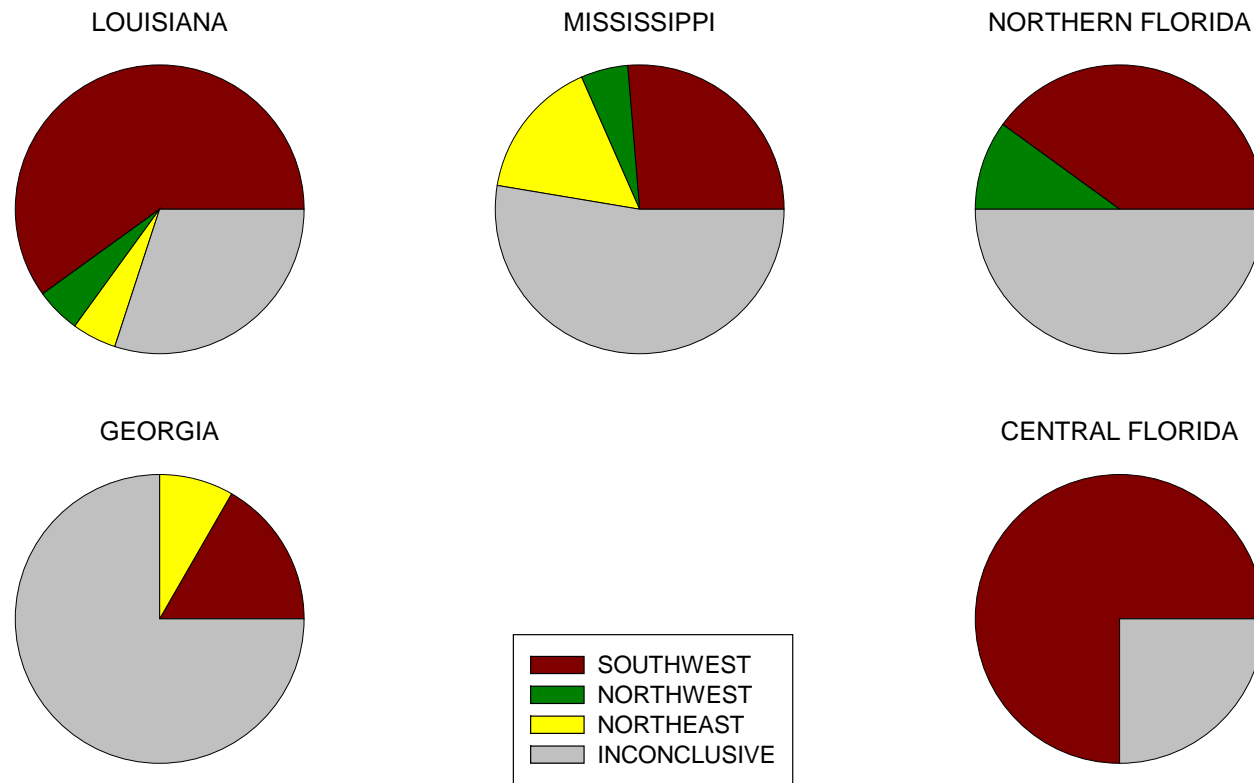


Figure 1.7. Pie charts showing the proportions of Henslow's sparrows sampled at a wintering site that were assigned to each of three breeding regions, using the ≥ 0.5 probability criterion, and δH and δC data. (No wintering individuals were assigned to the southeastern or midwestern breeding regions.) Assignments were made by Bayesian inference, where each individual was assigned to the breeding region with a posterior probability ≥ 0.5 . Individuals with no posterior probabilities ≥ 0.5 were assigned to the inconclusive category. The prior probabilities were the relative abundances of Henslow's sparrows in each of the five breeding regions

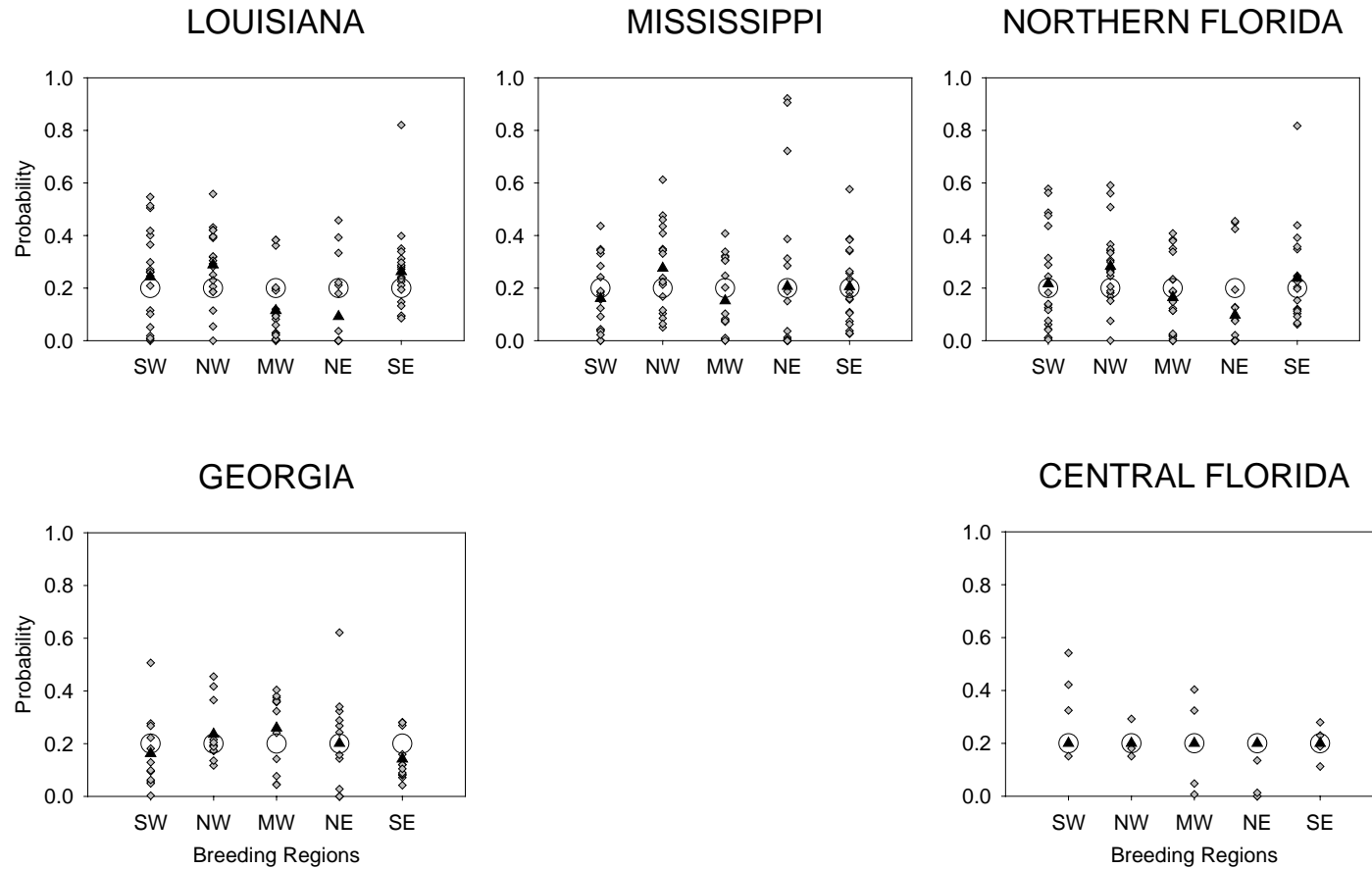


Figure 1.8. Panels showing the results of the Bayesian analysis for all Henslow's sparrows sampled at a wintering site, using flat prior probabilities (0.2). Gray diamonds represent posterior probabilities for all individuals and all breeding regions. Black triangles represent the mean posterior probability for each breeding region.

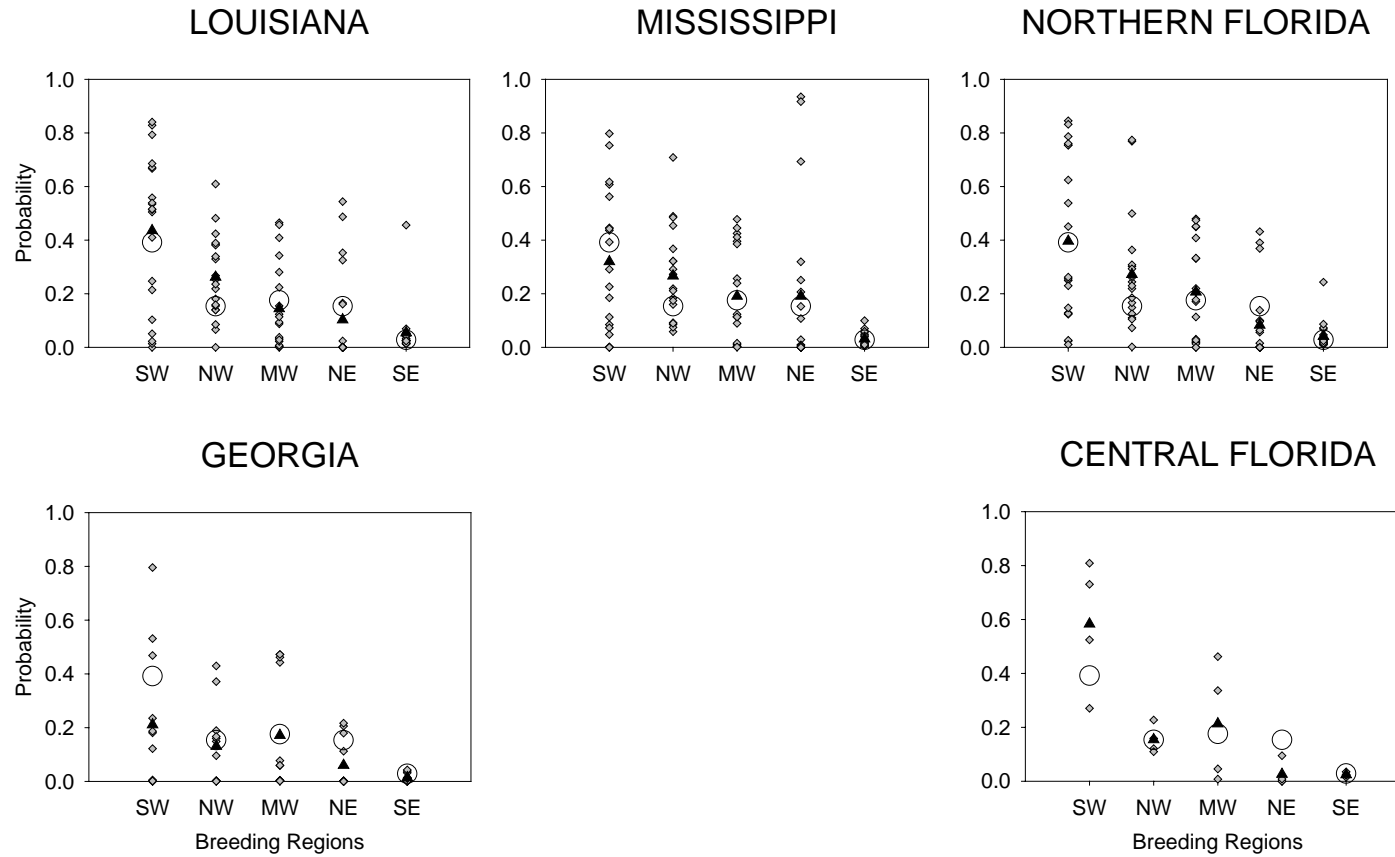


Figure 1.9. Panels showing the results of the Bayesian analysis for all Henslow's sparrows sampled at a wintering site, using relative abundances. The relative abundances of Henslow's sparrows in each of the five breeding regions were used as prior probabilities. Gray diamonds represent posterior probabilities for all individuals and all breeding regions. Black triangles represent the mean posterior probability for each breeding region.

are the relative abundances of Henslow's sparrows. Again, the gray diamonds represent individual posterior probabilities and black triangles represent mean posterior probabilities. These mean probabilities fell within 0.18 of the BBS prior probabilities. The 'tracking' of the mean posterior probabilities to the prior probabilities indicates that the stable isotope data made a weak contribution to the Bayesian inference.

DISCUSSION

According to the Bayesian analyses, wintering sites typically have Henslow's sparrows from various parts of the breeding range (Figs. 1.4 – 1.7). However, some patterns did emerge. First, the western portion of the wintering range included sparrows from more breeding regions than did the eastern portion of the wintering range (Figs. 1.4 – 1.7). This pattern may be attributable to the smaller sample sizes of the eastern sites (Table 1.2) and the higher abundance of Henslow's sparrows in the western part of the breeding range (Table 1.4). A second pattern of note is that only the two most western wintering sites contained any sparrows that were assigned to the northwestern breeding region (Fig. 1.7). This pattern may be attributable to an implicit assumption of the prior distributions, specifically that a wintering sparrow's probability of originating from a breeding region was independent of distance between the wintering site and the breeding region. If this assumption is wrong, this would explain why none of the sparrows captured at eastern wintering sites were assigned to the western part of the breeding range. However, it would not explain why only sites in the western part of the wintering range would contain sparrows originating from the northeastern breeding region (Fig. 1.5). Thus, there is a weak tendency for sparrows in the western part of the wintering range to originate from

the western part of the breeding range (Fig. 1.7), but there is also apparently a tendency for sparrows in the eastern part of the breeding range to overwinter in the western part of the wintering range. Overall, the analyses suggest that various parts of the wintering range receive migrants from multiple regions of the breeding range. No evidence for any clear linkages between breeding and wintering regions emerged.

Migrating individuals may not be flying longer distances directly to a permanent wintering site (e.g., from the northeastern breeding range to a western wintering site rather than to a closer eastern wintering site). Wintering birds may be moving around the wintering grounds in search of appropriate habitat. Thus, the weak linkages between breeding and wintering grounds may be due to movements of wintering birds across the wintering grounds.

Whether our Bayesian inferences were improved by including δC data remains unclear. When we included these data in the preliminary analysis (Fig. 1.3), the proportions of sparrows on the breeding grounds assigned to the correct breeding site increased within some regions but decreased within other regions. The δC variation over space found in this study (Table 1.3) and in another study (Chamberlain *et al.* 1997) could indicate that the δC data have some value. However, if the δC values are only characteristic of local sites and do not vary predictably across geographic space, then this additional data may only introduce more noise into the analysis. The addition of δC into the analysis decreased the number of inconclusive assignments (compare Figs. 1.5 and 2.7). We do not know if the δC data helped distinguish some breeding regions or if the additional data led to spurious breeding region assignments. We hope additional δC data from across North America will be gathered in other studies to resolve this question.

Our inferences about the origins of wintering sparrows could have been compromised by any between-year variation in stable isotope ratios at breeding sites. Although we assumed no significant between-year variation, we did find nominally significant between-year differences in δH for the breeding sites in North Carolina and New York. Such differences could be attributable to immigration into breeding sites by individuals with feather δH characteristic of the environmental δH at a previous breeding site (high immigration rates were found in these two sites, Chapter 2). For this reason, workers have recently improved their sampling methods, collecting feathers from nestling and fledglings rather than adults to ensure that the feathers were grown at the sampling site (e.g., Meehan *et al.* 2001, Lott, Meehan & Heath 2003). Unfortunately, it was not feasible for us to collect feathers from nestlings and fledglings throughout the breeding range.

Our inferences could have been hardly influenced by the stable isotope data. To evaluate this possibility, we asked whether the mean posterior probabilities of breeding-region origin closely track both the flat (Fig. 1.8) and relative abundance prior probabilities (Fig. 1.9). These tracking patterns indicate that the stable isotope ratio data may be weak and overwhelmed by the prior probabilities. This weakness may be due to the large overlap in distributions of stable isotope ratio (see means and standard deviations reported in Table 1.4). This overlap may be caused by the mainly east-west, rather than north-south, oriented breeding range of Henslow's sparrows. The δH distributions tend to vary in latitude more than longitude (Table 1.3). Additionally, latitude and longitude explained less of the variance in δC . The overwhelming strength of the prior probabilities makes it important to interpret our inferences cautiously.

Despite these apparent weaknesses of the stable isotope data, sparrows from the southwestern, midwestern, and northeastern breeding regions were correctly assigned to breeding regions more frequently than expected by chance (Fig. 1.3). This result suggests that the stable isotope data might have improved our ability to assign wintering sparrows to breeding regions. Keeping these strengths and weaknesses of the isotope data in mind, we tentatively suggest that the results of the bivariate analysis with the inconclusive category (Fig. 1.7) are the best estimates of breeding origin of wintering sparrows. The breeding-origin assignments made with this analysis are based on posterior probabilities ≥ 0.5 , far greater than the probability due to chance (0.2). Based on this analysis, a large proportion of wintering Henslow's sparrows originates from the southwestern breeding region, with smaller proportions originating from the northwestern and northeastern breeding regions (Fig. 1.2).

USING BAYESIAN INFERENCE TO ASSESS MIGRATORY LINKAGES

Over the last decade, stable isotope ratios have been used to determine linkages between breeding and wintering grounds of various bird species (e.g. Hobson & Wassenaar 1997, Chamberlain *et al.* 1997, Marra Hobson & Holmes 1998). These pioneering studies have been invaluable in the development of the technique, and recent improvements (e.g. Wassenaar & Hobson 2000) have made the technique more affordable and popular among avian ecologists. However, until now (see also Royle & Rubenstein, unpublished ms.), Bayesian methods have not been used for data analysis. Consequently, most attempts to establish linkages between breeding and wintering grounds have failed to take advantage of prior information (e.g., population densities on the breeding grounds).

Studies that have taken other information into account, such as microsatellite data (Clegg *et al.* 2003), have not used the information to generate prior probability distributions for Bayesian analysis. Our results highlight the importance of incorporating relative abundance across the breeding range into prior probabilities. If breeding-origin assignments are based on stable isotope ratios only, regions of the breeding range with large populations may be underrepresented (as illustrated in Fig. 1.1). By using relative breeding bird abundances as prior probabilities, more accurate assignments can be made.

The use of Bayesian analysis to establish migratory linkages should be used with a couple of potential pitfalls in mind. First, there is a risk of distorting the posterior probabilities by using poorly designed prior probabilities. Hence, researchers need to take care when developing prior probability distributions. Second, if the current data (including stable isotope data) used to produce the likelihood function are weak, they may be overwhelmed by the prior probabilities. If so, then the current data contribute little to the posterior probabilities and may be altogether irrelevant (Anderson 1998). The strength of the current data can be evaluated by comparing outcomes based on more than one set of prior probabilities, preferably prior probabilities that are drastically different (Ellison 1996). If the comparisons produce similar posterior probabilities, then the likelihood functions are strong enough to dominate the prior distributions. If the results are drastically different and the posterior probabilities are very much like the prior probabilities, then the current data may be of little or no use. By careful design and analysis, these weaknesses can be avoided (in the case of prior probabilities) or at least clearly evaluated (in the case of likelihood functions).

Our analysis highlights some important concerns about the use of stable isotopes to link breeding and wintering grounds. First, stable isotope signatures may not be of much use when the distributions of isotope ratios for different regions are overlapping. Second, to ensure the estimated isotope ratios truly represent the sampled breeding region, it is important to sample birds known to have grown their feathers in the breeding region where they were caught. Third, the chemical elements used in the analysis to characterize the isotopic signature of a breeding region could either improve the distinction between breeding sites or introduce noise (and even bias) into the analysis. However, the Bayesian approach has one huge advantage over the classical statistical approach. Even though a given study may produce limited or perhaps even misleading results, that information may be used to improve the estimate of prior probabilities in future studies of migration linkages. By using all of the information available, a Bayesian approach to determining linkages between breeding and wintering grounds takes advantage of past studies and contributes to future ones.

In a future comparison, we will divide the breeding range in two ways. First, we will use cluster analysis on the isotope ratio characteristics of the breeding sites to maximize the amount of differentiation among sites. Second, we will divide the breeding range into two regions, a northern and southern region, to maximize the amount of information provided by the North American δH gradient. The results using these two methods will be compared with the current linkage results using the five breeding regions.

IMPLICATIONS FOR HENSLOW'S SPARROW CONSERVATION AND MANAGEMENT

Regarding linkages between the breeding and wintering grounds of Henslow's sparrows, this study indicates diffuse migration of breeding birds across the wintering range (Fig. 1.7). According to our results to date, the southwestern breeding region appears to be an important origin for many wintering sites, while the southeastern breeding region contributes little to the wintering sites sampled in this study. Because there are no clear linkages between parts of the breeding and wintering grounds (e.g., sparrows from eastern breeding regions do not necessarily spend the winter in eastern wintering regions), grassland bird managers in these two ranges do not need to coordinate management plans of specific breeding and wintering sites. Instead, more generalized coordination of breeding and wintering grounds should be sufficient. Large dispersal and high gene flow estimates of breeding Henslow's sparrows (Chapter 2) also suggest that the breeding populations are not isolated and thus require management at a range-wide level rather than at a local level.

Our inferences could be refined by the addition of new data sets. For example, as the technologies advance, miniaturized global positioning devices (Reynolds & Riley 2002, von Hünenbein *et al.* 2000) or satellite Doppler tracking transmitters (Argos 2001) could be placed on breeding individuals. These individuals could be tracked to their wintering grounds; feathers could be analyzed for trace elements or pollen grains that are characteristic of the breeding region; genetic and morphometric markers could be used to identify breeding populations. Finally, an intelligent design data analysis (Amin, Bramer & Emslie 2003) could be used to produce decision rules to determine population origins.

By using the Bayesian approach in investigations of migratory linkages between breeding and wintering grounds, researchers can take advantage of information from various sources to calculate the probabilities of origin from several breeding sites for individual wintering birds. Data sources could include those used here (feather δH and δC and BBS data) as well as various others (e.g., mark-recapture, genetic, behavioral, and morphometric data). These additional data could be incorporated into prior probabilities and thus increase the accuracy of posterior probability estimates. The determination of migratory linkage is intrinsically a Bayesian question, where the breeding origin of individual birds calls for the calculation of probabilities from several possible origins. We promote increased reliance on this approach in studies attempting to determine individuals' origins or to establish linkages between breeding and wintering populations.

ACKNOWLEDGMENTS

Many people made this work possible. We thank the people who helped with the fieldwork and/or logistics. In the wintering grounds: Michelle Davis, Lori Yates, David Cimprich, Colleen Dwyer, Ryan Heise, Stefan Woltmann, Mollie Cashner, Rob Smith, Jen Owen, Chris Szell, Sarah Mabey, Leah Bray, Daniel Hudson, Chris Melder, Kenneth Moore, David Plair, Stacy Peterson, Ken Hackman, Tacy Anderson, Katherin Barrow, Laura Blacken, Melissa Davis, Laura Edgar, Jason Edwards, Jonathan Faulkner, Amy Foster, Heather Foster, Jones Goodman, Jay Jordan, Drew Lang, Alicia LeBlanc, Rachael McClure, Andy Middleton, William Ogle, Bethany Porter, Justin Rives, Melissa Robinson, Ryan Sartain, Stacy Slone, William Stianche, Erin White, Todd Engstrom, Douglas McNair, Keith Ouchley, Jeff Buler, Mike Baker, Melissa Powell, Rebecca

Finzer, Nellwyn McInnis, Melissa Andre, Lauren Miller, Jill Coutmoutso, Molly Embree, Giff Beaton, Tylan Dean, Jim Johnson, Beau Gregory, Bob Ford and his Ornithology class from the University of Tennessee. In the breeding grounds: Daniel Brauning, Greg Forcey, Chris Grainer, Evan Blumer, Danny Ingold, Don Sheroan, Jeff Jones, Andrew Leonard, Daniel Moss, Joe Robb, Teresa Lewis, Jason Lewis, Karen Owens, Jim Keir, Ken Rosenthal, David Sample, David Mehlman, Robert Hamilton, Barbara Van Slyke, Tom Van Slyke, Craig Davis, John Wright, Paula Wright, Robin Krebbs, Steve Joule, Alicia Bacci, Maiken Winter, Robert Grosholz, Elizabeth Vaughn, Jarling Ho, Allison Reid, Paul Doherty, Bo Bunnell, Kristin Field, and Scott Hull. Finally, we thank the thousands of U.S. and Canadian participants of the North American Breeding Bird Survey, including USGS and CWS researchers and managers. Finally, we thank Andy Royle and William Link for the valuable discussions on Bayesian analysis and relative abundances. Funding was provided by grants from Ohio Department of Natural Resources, Division of Wildlife; Columbus Zoo, and Mississippi Natural Heritage Fund.

REFERENCES

- Alisauskas, R.T. & Hobson, K.A. (1993) Determination of lesser snow goose diets and winter distribution using stable isotope analysis. *Journal of Wildlife Management*, **57**, 49-54.
- Alisauskas, R.T., Klass, E.E., Hobson, K.A. & Ankney, C.D. (1998) Stable carbon isotopes support use of adventitious color to discern winter origins of lesser snow geese. *Journal of Field Ornithology*, **69**, 262-268.
- Amin, R., Bramer, M.A. & Emslie, R. (2003) Intelligent data analysis for conservation: experiments with rhino horn fingerprint identification. *Applications and Innovations in Intelligent Systems X* (eds A. Macintosh, R. Ellis & R. Coenen), pp. 207-222. Springer-Verlag, New York.
- Anderson, J.L. (1998) Embracing uncertainty: The interface of Bayesian statistics and cognitive psychology. *Conservation Ecology* [online], **2**, 2. (<http://www.consecol.org/vol2/iss1/art2>).
- Argos website (2001) http://www.argosinc.com/system_overview.htm.
- Benjamini, Y. & Hochberg, Y. (1995) Controlling the false discovery rate – a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B – Statistical Methodology* **57**, 289-300.
- Chamberlain, C.P., Blum, J.D., Holmes, R.T., Fen, X., Sherry, T.W. & Graves, G.R. (1997) The use of isotope tracers for identifying populations of migratory birds. *Oecologia*, **109**, 132-141.
- Clegg, S.M., Kelly, J.F., Kimura, M. & Smith, T.B. (2003) Combining genetic markers and stable isotopes to reveal population connectivity and migration patterns in a Neotropical migrant, Wilson's warbler (*Wilsonia pusilla*). *Molecular Ecology*, **12**, 819-830.
- Ellison, A.M. (1996) An introduction to Bayesian inference for ecological research and environmental decision-making. *Ecological Applications*, **6**, 1036-1046.
- Forero, M.G. & Hobson, K.A. (2003) Using stable isotopes of nitrogen and carbon to study seabird ecology: Applications in the Mediterranean seabird community. *Scientia Marina*, **67**, 23-32.
- Graves, G.R., Romanek, C.S. & Navarro, A.R. (2002) Stable isotope signature of philopatry and dispersal in a migratory songbird. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 8097-8100.
- Gustafson, M.E. & Hildebrand, J. (1999) Bird Banding Laboratory Homepage, ver 12/02/99. [Online]. Available: <http://www.pwrc.usgs.gov/bbl/> [Access date: 10/22/03]
- Herkert, J.R. (1991) Prairie birds in Illinois: populations response to two centuries of habitat change. *Illinois Natural History Survey Bulletin*, **34**, 393-399.
- Herkert, J.R. (1994) Status and habitat selection of the Henslow's Sparrow in Illinois. *Wilson Bulletin*, **106**, 35-45.
- Hobson, K.A. (1999) Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia*, **120**, 314-326.
- Hobson, K.A. & Wassenaar, L.I. (1997) Linking breeding and wintering grounds of neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. *Oecologia*, **109**, 142-148.

- Hobson, K.A., Brua, R.B., Hohman, W.L. & Wassenaar, L.I. (2000) Low frequency of “double molt” of remiges in ruddy ducks revealed by stable isotopes: implications for tracking migratory waterfowl. *Auk*, **117**, 129-135.
- Hobson, K.A., McFarland, K.P., Wassenaar, L.I., Rimmer, C.C. & Goetz, J.E. (2001) Linking breeding and wintering grounds of Bicknell’s Thrushes using stable isotope analyses of feathers. *Auk*, **118**, 16-23.
- Hoefs, J. (1980) *Stable isotope geochemistry*. Springer-Verlag, Berlin.
- Kelly, J.F. & Finch, D.M. (1998) Tracking migrant songbirds with stable isotopes. *Trends in Ecology and Evolution*, **13**, 48-49.
- Kelly, J.F., Atudorei, V., Sharp, Z.D. & Finch, D.M. (2001) Insights into Wilson’s warbler migration from analyses of hydrogen stable-isotope ratios. *Oecologia*, **130**, 216-221.
- Körner, C.H., Farquhar, G.D. & Wong, S.C. (1991) Carbon isotope discrimination by plants follows latitudinal and altitudinal trends. *Oecologia*, **88**, 30-40.
- Lott, C.A., Meehan, T.D. & Heath J.A. (2003) Estimating the latitudinal origins of migratory birds using hydrogen and sulfur stable isotopes in feathers: influence of marine prey base. *Oecologia*, **134**, 505-510.
- Marra, P.P., Hobson, K.A. & Holmes, R.T. (1998) Linking winter and summer events in a migratory bird by using stable-isotopes. *Science*, **292**, 1884-1886.
- McCutchan, J.H., Jr., Lewis, W.M., Jr., Kendall, C. & McGrath, C.C. (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, **102**, 378-390.
- Meehan, T.D., Lott, C.A., Sharp, Z.D., Smith, R.B., Rosenfield, R.N, Stewart, A.C. & Murphy, R.K. (2001) Using hydrogen isotope geochemistry to estimate the natal latitudes of immature Cooper’s hawks migrating through the Florida Keys. *Condor*, **103**, 11-20.
- North American Breeding Bird Survey website (2003)USGS-<http://www.mp2-pwrc.usgs.gov/bbs/about/index.html>.
- Plentovich, S., Holler, N.R. & Hill, G.E. (1999) Habitat requirements for Henslow’s sparrows wintering in silvicultural lands of the Gulf Coastal Plain. *Auk*, **116**, 109-115.
- Reynolds, D.R. & Riley J.R. (2002) Remote-sensing, telemetric and computer-based technologies for investigating insect movement: a survey of existing and potential techniques. *Computers and Electronics in Agriculture* **35**, 271-307.
- Rubenstein, D.R., Chamberlain, C.P., Holmes, R.T., Ayres, M.P., Waldbauer, J.R., Graves, G.R. & Tuross, N.C. (2002) Linking breeding and wintering ranges of a migratory songbirds using stable isotopes. *Science*, **295**, 1061-1065.
- Sample, D.W. (1989) Grassland birds in southern Wisconsin: habitat preference, population trends, and response to land use changes. *MS thesis, University of Wisconsin, Madison, U.S.A.*
- Sauer, J.R., Hines, J.E. & Fallon, J. (2001) The North American Breeding Bird Survey, results and analysis 1966-2000. Version 2001.2, USGS Patuxent Wildlife Research Center, Laurel, MD. <http://www.mbr-pwrc.usgs.gov/bbs/bbs.html>
- Schaffner, F.C. & Swart, P.K. (1991) Influence of diet and environmental water on the carbon and oxygen isotopic signatures of seabird eggshell carbonate. *Bulletin of Marine Science*, **48**, 23-38.
- SPSS for Windows (2001) Rel. 11.0.1. Chicago: SPSS Inc.

- Swengel, S.C. (1996) Management responses of three species of declining sparrows in tallgrass prairie. *Bird Conservation International*, **6**, 241-253.
- Tucker, J.W. & Robinson, W.D. (2003) Influence of season and frequency of fire on Henslow's Sparrows (*Ammodramus henslowii*) wintering on Gulf Coast pitcher plant bogs. *Auk*, **120**, 96-106.
- USGS Patuxent Wildlife Research Center (2001) North American Breeding Bird Survey ftp data set, version 2001.0. <ftp://pwrctpr.er.usgs.gov/mp/bbs/datafiles/>.
- USGS Patuxent Wildlife Research Center (2003a) North American Breeding Bird Survey About BBS, 1, December 2003. (<http://www.mp2-pwrc.usgs.gov/bbs/about/index.html>).
- USGS Patuxent Wildlife Research Center (2003b) North American Breeding Bird Survey Internet data set, 26, November 2003. (<http://www.mp2-pwrc.usgs.gov/bbs/retrieval/>).
- Van der Merwe, N.J., Lee-Thorpe, J.A., Thackeray, J.F., Hall-Margin, A., Kruger, F.J., Coetzee, H., Bell, R.H.V. & Lindeque, M. (1990) Source-area determination of elephant ivory by isotopic analysis. *Nature*, **346**, 744-746.
- Von Hünenbein, K., Hamann, H.J., Rüter, E. & Wiltshcko, W. (2000) A GPS-based system for recording the flight paths of birds. *Naturwissenschaften* **87**, 278-279.
- Wassenaar, L.I. & Hobson, K.A. (2000) Improved method for determining the stable-hydrogen isotopic composition (δD) of complex organic materials of environmental interest. *Environmental Science and Technology* **34**, 2354-2360.
- Wassenaar, L.I. & Hobson, K.A. (2001) A stable-isotope approach to delineate geographical catchment areas of avian migration monitoring stations in North America. *Environmental Science and Technology*, **35**, 1845-1850.

CHAPTER 2

ESTIMATING SONGBIRD DISPERSAL USING STABLE ISOTOPE RATIOS, MEME FLOW AND GENE FLOW

SUMMARY

1. We estimate dispersal rates in Henslow's sparrows (*Ammodramus henslowii*) using three methods: deuterium/hydrogen stable isotope ratios (δH) in feathers, meme flow as estimated from geographic variation in song, and genetic population structure based on microsatellite markers.
2. δH in flight feathers correlates with environmental δH where feathers are grown. In this species, feathers are molted and regenerated in late summer. Because precipitation δH varies along a geographic gradient, feather δH provides a signature of an individual's previous breeding site or natal origin. The proportion of sparrows with feather δH values falling outside the expected range is taken as an estimate of immigration.
3. The meme flow method takes advantage of geographic variation in a species' song characteristics, which is thought to be a byproduct of cultural evolution.

Unusual songs in a local population may arise from immigration or from mutation during song learning. Thus, the proportion of males with locally distinctive songs not attributable to mutation is taken as an estimate of immigration.

4. We use three methods for evaluating genetic structure (Wright's classical F_{st} -based method, a private-alleles method, and a Bayesian clustering method). Based on the first two methods, we estimate the number of migrants per generation (Nm) among populations.
5. We introduce a statistical method for evaluating whether a putatively private allele is truly private and not simply a byproduct of insufficient sampling.
6. All methods revealed high rates of dispersal among Henslow's sparrow populations.
7. We promote further evaluation of the δH and meme flow methods for estimating contemporary (between-year) dispersal. These methods do not require extensive mark/resighting efforts as with bird banding. They may prove to be useful in various fields of biology, especially in conservation biology, where dispersal estimates are important for assessing risk associated with inbreeding and for designing reserves.

INTRODUCTION

The dispersal of organisms is a phenomenon of fundamental importance in various fields of biology including evolutionary biology, ecology, conservation biology and agronomy. In evolutionary biology, gene flow and natural selection affect how populations differentiate (Erich & Raven 1969). Generally, dispersal rates reflect the amount of gene flow among populations. High gene flow may cause demes, even those separated by great distances, to be part of a large panmictic population. Low gene flow may isolate even neighboring populations. In ecology, interactions between predators and prey (Weisser 2001) and between parasites and hosts (Boulinier, McCoy & Sorci 2001) can affect rates of dispersal for both host and parasite. The rate of dispersal may covary with rate of succession of a species' habitat, with species that select ephemeral habitats having higher dispersal rates (Wiens 2001). In conservation biology, dispersal must be taken into account in reserve design (e.g., Acosta 2002). The amount of dispersal between suitable habitat patches in a reserve may be affected by the landscape surrounding the patches (Wiens 2001). Low dispersal may contribute to high rates of local extinction and low rates of recolonization. High dispersal may synchronize subpopulations, thereby exposing an entire metapopulation to stochastic effects, predation, or disease (Macdonald & Johnson 2001). Finally, in agronomy, dispersal of agricultural pests and biological control species (Alomar, Goula & Albajes 2002), and dispersal of genetically modified crops (Warwick, Beckie & Small 1999) can have great economic consequences.

Despite the undeniable importance of dispersal, its estimation remains a challenge for many biologists. Three main modes of studying dispersal have been tracking marked or radio-tagged individuals, using genetic markers, and developing mathematical models

(Nathan 2001). All three methods have nontrivial limitations. For example, relocating marked individuals becomes less likely if individuals move long distances away from the site where they were marked, biasing the data to shorter dispersal distances (Koenig, Van Vuren & Hooge 1996). When using genetic markers for traditional measures of gene flow among populations, researchers must assume populations have attained equilibrium between mutation and genetic drift (Thompson & Goodman 1997).

Recently, two natural markers (as distinguished from artificial markers such as banding, tagging, or radio collaring) have been promoted for estimating dispersal. The first marker uses stable hydrogen isotope ratios in feathers as a signature of the area where the feathers were grown (Chamberlain *et al.* 1997, Hobson & Wassenaar 1997). The second marker uses song structure to estimate meme (a cultural unit, either an idea or behavior, passed between individuals through learning or imitation; Dawkins 1989) flow between populations (Lynch 1996). Here, these two short-term (in regards to recency of dispersal events) markers are used along with genetic markers, which estimate genetically effective dispersal over evolutionary time scales. We use this combination of methods to estimate dispersal (and gene flow) in a North American songbird, the Henslow's sparrow (*Ammodramus henslowii*, Audobon). The short-term markers can potentially reveal more about current inter-population movements, while the genetic markers estimate rates of effective dispersal over generations. Both the stable isotope and meme flow methods estimate the proportion of dispersers immigrating into the sampled population. These nongenetic markers may provide better information on contemporary rates of dispersal into focal populations.

DISPERSAL ESTIMATED BY STABLE ISOTOPE RATIOS

Stable isotope ratios of various elements have been used to identify migratory origins (e.g., Hobson 1999), trophic levels (e.g., Beaudoin *et al.* 1999), and feeding preferences of various animals (e.g., Hobson & Clark 1992; Szepanski, Ben-David & Ballenberghe 1999). This study uses deuterium/hydrogen isotope ratios (δH) in feathers to estimate dispersal, a newer application of these markers (Hobson *et al.* 2001). Because δH varies predictably along a geographic gradient in North America, δH of feathers correlates with δH of the area where the feathers were grown (Chamberlain *et al.* 1997, Hobson & Wassenaar 1997). The δH of a feather collected in the breeding grounds in spring or early summer provides a signature of the area where the feather was grown during the previous breeding season. If a bird returns to the same breeding site, then its feather δH should be similar to ratios of conspecifics in the region. However, if a bird disperses a long distance, its feathers may have a δH that differs from the ratios of conspecifics in the region. Wassenaar and Hobson (2001) have developed a contour map of δH in feathers grown in North America (Fig. 2.1). This map is used to compare the δH of feathers to determine if they likely originated from the region where the feathers were collected. Those individuals with feather isotope ratios falling outside the expected range of values are judged to have dispersed into the region.

DISPERSAL ESTIMATED BY MEME FLOW (GEOGRAPHIC VARIATION IN SONG)

Meme flow may also be used to estimate the proportion of individuals that have recently dispersed into an area. Variation in song structure is used here as an indication of meme

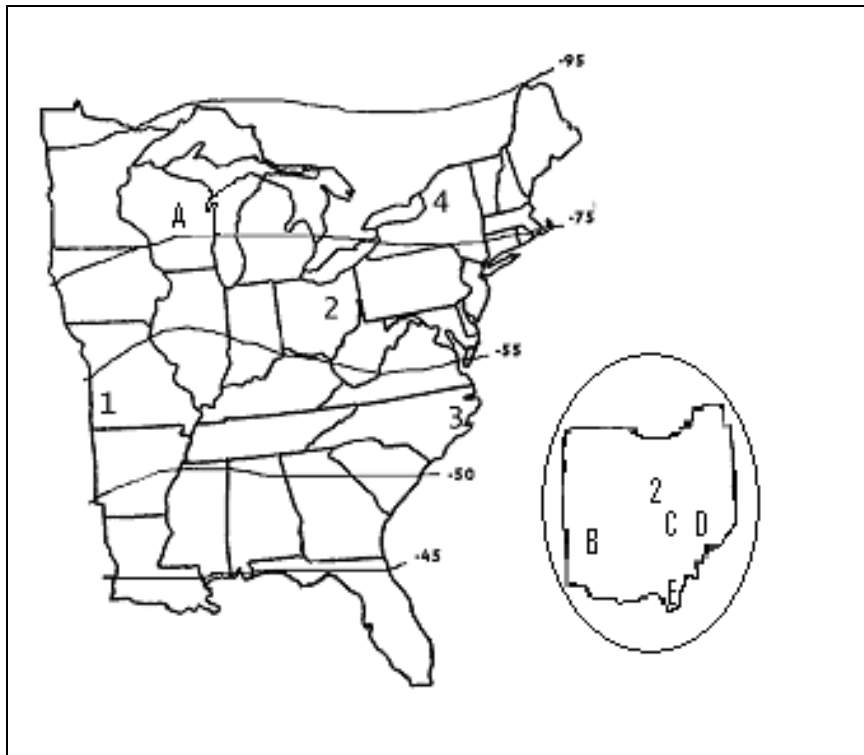


Figure 2.1. Stable deuterium/hydrogen isotope contour lines and sites where rectrices and songs (numbered sites) and additional genetic data (lettered sites) were collected from Henslow's sparrows. 1) Niawathe Prairie, Missouri 2) Woodbury Wildlife Area, Ohio 3) Voice of America Tower, North Carolina 4) Fort Drum, New York A) Buena Vista Grasslands, Wisconsin B) Wright Patterson Air Force Base, Ohio C) Tri-Valley Wildlife Area, Ohio D) Egypt Valley Wildlife Area, Ohio E) Crown City Wildlife Area, Ohio. Contour lines taken from Figure 1 in Wassenaar & Hobson 2001.

flow. This marker takes advantage of geographic variation in song characteristics. Such variation within songbird species is thought to be a byproduct of cultural evolution, with males singing songs learned early in life (Mundinger 1982). Variation in song between populations is thought to have evolved through errors and improvisations during song learning that spread through the original population (Lemon 1975). The amount and scale of geographic variation depends on the ontogeny of song in relation to dispersal

events. Here are brief descriptions of the main patterns of song ontogeny. (1) In species with an *open-ended learning phase*, males learn songs throughout their lives, adapting to any new dialects they encounter (e.g., Nottebohm & Nottebohm 1978). (2) In species with an *early-sensitive learning phase*, males learn songs heard in their natal area (e.g., Immelmann 1969). (3) In species with a *long- or late-sensitive learning phase*, males may learn songs similar to those heard in their first breeding area (e.g., Kroodsma & Pickert 1984; Nordby, Campbell & Beecher 2001). (4) In species with an *overproduction/selective mechanism*, young males learn multiple song variants and then select a variant that most resembles songs heard at the first breeding site. The selected variant then becomes crystallized in the repertoire (reviewed by Nelson 1996). All four of these learning mechanisms would result in songs being more similar within breeding areas than between breeding areas. With the open-ended learning phase mechanism, males can adjust their songs to the local dialect and so odd songs are not expected, unless they arise from errors in song learning. With the other three mechanisms, however, unique or unusual songs are expected because males' previously crystallized songs may be dissimilar to local dialect in their current breeding area. These odd songs may represent the dialects in the males' natal areas.

Unique and unusual songs may come about through either immigration of males into breeding areas or through song mutation during learning (Lynch 1996). With an odd song due to immigration, a male crystallizes his song after the end of his learning/selection phase and then disperses to a new area the following year, continuing to sing his crystallized song that may be uncharacteristic of the new site. If so, then a unique or unusual song may indicate a dispersal event. Odd songs due to learning

mutations are imperfect copies of model songs, and cultural transmission of these imperfections may often occur (Lynch 1996). If song mutation rate can be estimated, then the proportion of unusual songs not attributable to song mutation can be taken as an estimate of dispersal (Lynch 1996). This approach could be used along with other methods to refine the estimation of immigration.

The meme flow method of estimating dispersal requires geographic variation in the cultural trait (song in this study). Although geographic variation in oscine song is well known (Kroodsma 1978), most studies have focused on species with complex songs such as the white-crowned sparrow (*Zonotrichia leucophrys*, Forster; e.g., Marler & Tamura 1962, Baptista 1977, Nelson 1998). Each song can be separated into components and then each component can be measured (e.g., duration, maximal and minimal frequency, and frequency modulation rate). These measures are then used to describe song variation, which in turn has been used as an indication of song function (Kroodsma 1996), song ontogeny (Kroodsma 1996), and breeding philopatry (Lynch 1996).

The species used in this study, the Henslow's sparrow, has one of the simplest of all oscine songs. After making assumptions about this species' song ontogeny and mutation rate (see below), the estimated proportion of unique or unusual songs is used to estimate dispersal in each studied population. These estimates are then compared with corresponding estimates based on the δH method. Estimates of dispersal using these two methods may be more feasible than estimates based on other methods (e.g., tracking, tagging).

We base our assumptions regarding song ontogeny in Henslow's sparrows on evidence from other sparrow species belonging to the same family (Emberizidae). Three

such species have been studied both in the laboratory and in the field: song sparrow (*Melospiza melodia*; e.g., Nordby, Campbell & Beecher 2002), swamp sparrow (*Melospiza georgiana*; e.g., Marler & Peters 1981), and white-crowned sparrow (e.g., Nelson & Marler 1994). These studies found these species to memorize song in the first 4 months of life, with no record of a marked adult changing its song between years. It is thought that some migratory songbird species overproduce their repertoire during song development and then select a song that resembles those of the first breeding site. This crystallized song is then kept throughout the individual's life (Nelson, Marler & Morton 1996). It is therefore assumed that a male ultimately selects a song most similar to the songs he heard during his first breeding season. It is also assumed that the rate of song mutation during song development varies little among oscines. Thus, lacking estimates of song mutation rate in Henslow's sparrows, we use estimates for other species (Lynch 1996). These assumptions, of course, should be tested in future studies. Given the assumption of an overproduction/selection mechanism of song learning, any unique or unusual songs not accounted for by song mutation will be attributed to immigration.

DISPERSAL ESTIMATED BY GENE FLOW

Estimates based on the first two methods are compared to estimates based on more familiar genetic methods. Here, we use three methods for evaluating population structure (one using F_{st} , another using private alleles, and a third using a Bayesian clustering approach). The first two methods are used to estimate the number of effective migrants per generation (Nm) among populations. The third method is used to corroborate the inferences regarding population structure.

Molecular genetic tools have been used in a variety of population studies (reviews by Avise 1994, Parker *et al.* 1998). When populations become genetically isolated, allele frequencies at neutral loci may change due to genetic drift and/or natural selection (Moritz 1994). By using estimates of genetic structure (F_{st}) based on Wright's (1931) classical island model, the amount of gene flow and the time since common ancestry between populations can be estimated.

In Wright's island model, F_{st} is inversely proportional to the genetically effective migration rate, Nm ($F_{st} = 1/[4Nm+1]$; Slatkin 1985a; but see Whitlock & McCauley 1998 for a critique), where N is the effective population size and m is the migration rate between populations. Populations with low F_{st} are assumed to have high rates of dispersal, which homogenize allele frequencies across populations. Populations with high F_{st} are assumed to have few dispersers, which allow allele frequencies to differentiate through drift, mutation, and selection. High estimates of effective dispersal should correspond with high Nm (low F_{st}). Low estimates of effective dispersal should correspond with low Nm (high F_{st}), assuming the populations are at evolutionary equilibrium. If the populations are not at equilibrium, then it is possible for low levels of genetic structure to exist, despite low dispersal rates, because the genetic structure reflects recent common ancestry (Gibbs, Dawson & Hobson 2000). F_{st} estimates use differences in allele frequencies among populations as indicators of population structure.

As with F_{st} , estimates of private alleles among populations are also related to Nm among populations (Slatkin 1985b). Here, Nm is also estimated using private alleles. Through computer simulations, the average frequency of private alleles has been shown to be inversely and roughly linearly related to Nm (Slatkin 1981, 1985b; Barton & Slatkin

1986). Along with estimating Nm from the frequencies of private alleles detected among the populations, a new method is introduced to evaluate the probability of a putatively private allele's being truly private and not simply appearing so due to insufficient sampling. The same assumptions associated with F_{st} estimates are made for estimates of Nm using private alleles (Whitlock & McCauley 1998).

The third genetic analysis uses a Bayesian clustering method to evaluate genetic structure. The computer program *Structure* (Pritchard, Stephens & Donnelly 2000) estimates population allele frequencies and clusters individuals into populations based on their genotypes. Individuals are assigned prior probabilities of being from any of a number of populations, K (K can be unknown). These prior probabilities can be uniform or they can be based on information about the populations (e.g., where individuals were sampled defines the most probable origin). *Structure* also allows for admixture among populations and for allele frequencies to be correlated among populations. In addition, this program calculates the probability that each individual originated from each population. This probabilistic assignment in turn identifies individuals who likely immigrated into the population from which they were sampled. (For more details on *Structure*, see Pritchard, Stephens & Donnelly 2000.) Here this approach is used to infer the number of populations, given the observed allele frequencies. If more than one population is probable, then migrants can be identified using *Structure*. The Bayesian inferences about population structure, along with estimates of Nm based on F_{st} and private alleles, are compared with the δH and meme flow estimates of dispersal.

METHODS

DISPERSAL ESTIMATED BY STABLE ISOTOPE RATIOS

The proportion of immigrants into a local population is estimated using deuterium/hydrogen stable isotope ratios (δH) in feathers. Two or three rectrices were collected from individual Henslow's sparrows at each of four sites across the species' breeding range, in New York, North Carolina, Ohio, and Missouri, U.S.A. (Fig. 2.1). These feathers were washed, dried and weighed in silver foil capsules. The samples were equilibrated to a standard ambient δH by exposing them for 2 h to steam with a known δH . They were then combusted and δH was measured with a Micromass Optima™ dual inlet isotope-ratio mass spectrometer at the National Water Research Institute in Saskatoon, Saskatchewan, Canada.

To estimate the number of dispersers into a sampling site, an expected range of feather δH for the site was generated. For each sampling site, an expected feather δH was taken from a contour map of feather δH (Fig. 2.1) based on mean growing season precipitation δH (Wassenaar & Hobson 2001). The δH values are reported in parts per thousand deviations from Vienna Standard Mean Ocean Water Standard (VSMOW) and normalized to the VSMOW/SLAP (Standard Light Antarctic Precipitation) scale. To generate the expected range of δH for feathers originating from a region, the standard deviation of feather δH from a study on ovenbirds (*Seiurus aurocapillus*, Linnaeus; Wassenaar & Hobson 2001) was used. These birds were banded and known to have grown their feathers at the study site in Saskatchewan. By using values within ± 1.96 SD from the expected feather δH , it is assumed that 95% of the Henslow's sparrow feather δH fall within that range. Individuals with feather δH within the expected range were

deemed to be natives to the area. Individuals with δH more negative than that range were judged to be immigrants; individuals with δH far less negative than that range were considered to have feathers that had been grown on the wintering grounds. One population (New York) had feather samples that apparently originated on the wintering grounds; in this population, we also report the proportions of dispersers and non-dispersers with these samples removed.

Because many individuals were tested, some or all of the detected dispersal events might have been “false positives.” To compensate for this multiplicity problem, the Benjamini-Hochberg method was applied (Benjamini and Hochberg 1995). Specifically, any nominally significant (i.e., $P < 0.05$) test is considered to remain significant if the following condition is met: $p_i \leq (iq)/n$, where $p_1 \leq p_2 \leq \dots \leq p_n$ are the ordered P -values, q is the assigned False Discovery Rate (0.05), and n is the number of individuals tested.

DISPERSAL ESTIMATED BY MEME FLOW

The proportion of breeding males that dispersed into a site was estimated using bioacoustic analysis. Males were recorded at the same four sites where feathers were collected. Males that were recorded were not necessarily the same individuals whose feathers were sampled. Recordings in North Carolina and Ohio were made in the summer of 2000; recordings at the other sites were made in the summers of 1999 and 2000. No male was recorded if it had been banded the previous year; therefore, we assume no male was recorded in both years. A Sony TCD5 tape recorder and an AT 815 microphone were used to record songs at distances from 1 to 7 m.

Recorded songs were digitized at a sampling rate of 25 kHz and 16-bit amplitude resolution and measured using Signal Sound Analysis Software (Engineering Design 2001). Clear recordings with no interfering background noise were selected. One song was measured from each bird recorded. We are confident that one song per individual characterizes the individual's songs, as the songs of each male are uniform (pers. obs., Borror 1954).

Henslow's sparrow songs usually have five components, with some songs having an extra introductory component and others missing the third component (Fig. 2.2). For each of the five main components, the following measurements were made using a custom Signal macro: beginning, middle and ending times and frequencies; frequency and time at lowest and highest frequencies; and dominant frequency (measured on a power spectrum) (Fig. 2.2). The frequency resolution was 0.1 Hz and the temporal resolution was 0.1 ms. The third component (determined by its position relative to the other components on the spectrogram) was missing in 14 of the 73 males recorded. This missing component indicates a large structural difference. To represent this difference, these songs were assigned third-component values equal to three standard deviations from the mean. (To evaluate the effect of third components, the analysis was also performed without them.) Because the statistical analysis requires continuous data, the introductory component was not used. All tests were performed using SPSS (2001) routines.

A new meme was defined as any song that was significantly different from all the other songs in a given area. To estimate the proportion of new memes not characteristic of a given site, a discriminant analysis was performed, using a Wilk's lambda stepwise

method with a variable entry of $F = 1.25$ and a variable removal of $F = 1.00$. Each case was classified by cross-validation and any misclassified case was considered a new meme for that site. The proportion of alien memes for a site was a combination of new memes originating from learning mutation and from dispersal (Lynch 1996). In a study of four songbird species, Lynch (1996) observed that the migration and meme mutation rates were roughly equivalent (Table 2.1). The mean across these studies was used to estimate the number of dispersers. The range of dispersal estimates based on the largest and smallest dispersal estimates from the Lynch study is also reported.

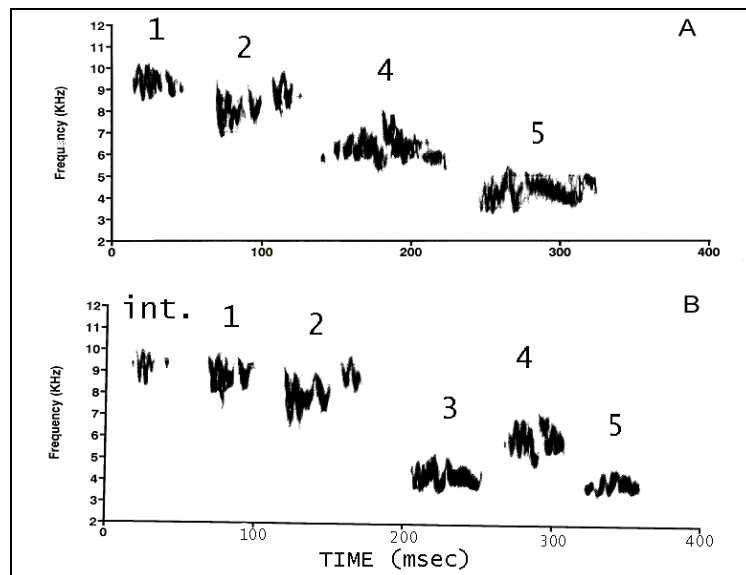


Figure 2.2. Sonograms of two representative Henslow's sparrow songs. The following 11 features were measured for each of the main song components: beginning, middle and ending times and frequencies; frequency and time at lowest and highest frequencies; and dominant frequency. Song A is missing the third component while Song B has the third component as well as an introductory note.

DISPERSAL ESTIMATED BY GENE FLOW

For the genetic analysis, blood was collected and DNA extracted from the same individuals from which feathers were collected. To find primers that would amplify polymorphic microsatellites, several primers developed for other avian species were evaluated (Table A.1). Five primers that produced polymorphic DNA fragments were chosen for the microsatellite analysis (Maμ23, Hrμ7, Mcyμ4, Pdoμ5, Dpμ16) and the polymerase chain reaction (PCR) was optimized for each primer (Table A.2). The

Species	Proportion of immigrants per generation	Proportion of song mutations per generation
Wood thrush (<i>Hylocichla mustelina</i> , Gmelin)	0.53	0.47
Lazuli bunting (<i>Passerina amoena mustelina</i> , Say)	0.58	0.42
Eurasian tree sparrow (<i>Passer montanus mustelina</i> , Linnaeus)	0.56	0.44
Chaffinch (<i>Fringilla coelebs mustelina</i>)	0.27	0.73
Mean	0.49	0.51

Table 2.1. Estimated proportions of immigrants per generation versus meme mutations per generation in four songbird species. Data taken from Table 10.1 in Lynch (1996).

lengths of base-pair fragments were first estimated by comparing them to plasmids of known length. Later samples were compared with Henslow’s sparrow DNA fragments of known size.

The data were tested for departure from Hardy-Weinberg equilibrium using estimates of the inbreeding coefficient (F_{IS} - from FSTAT [Goudet 1995]) for each locus

and population (Table A.3). (Three additional populations were used in this analysis [Fig. 2.1, sites C, D and E]; these populations were not used in the δH and bioacoustic analyses because few feather and song samples were collected at these sites.) If the proportion of heterozygotes is lower than expected under Hardy-Weinberg equilibrium, the data set may include null alleles, defined as alleles that are not amplified by the locus primers (Pemberton *et al.* 1995). The failure to amplify may be due to mutations at the allele-binding site that prevent the primer from binding to the DNA strand (Callen *et al.* 1993). Three loci (Hr μ 7, Mcy μ 4, Pdo μ 5) were judged to have null alleles. The data for these loci were corrected using a method suggested by Chakraborty *et al.* (1994), where the frequency of null alleles (r) is estimated from the observed (H_o) and expected heterozygosities (H_e): $r = (H_e - H_o)/(H_e + H_o)$

Observed and expected heterozygosities were estimated using Arlequin (Schneider, Roessli & Excoffier 2000). The data were adjusted to account for the estimated null allele frequency as done in other studies (e.g., Newman & Squire 2001). Specifically, null allele frequencies were increased to their expected frequencies, by replacing one of a pair of homozygous alleles with a null allele for the appropriate number of homozygous individuals. Table A.4 reports observed and expected heterozygosities and calculations of null allele frequencies. After this correction, differentiation within each pair of populations was estimated using an F_{st} index in GENEPOP (Raymond & Rousset 1995). Nm estimates were then calculated from these F_{st} estimates. Both corrected and uncorrected results are reported. To evaluate significance for each locus, an exact test of population differentiation was performed for each population pair using GENEPOP (Raymond & Rousset 1995).

The number of migrants per generation between populations was also estimated based on private alleles. These estimates were made using GENEPOP (Raymond & Rousset 1995). This program calculates Nm based on private alleles using the calculations described in Slatkin (1985b), and Barton and Slatkin (1986). To evaluate whether each putatively private allele was truly private and not just a result of sampling error, the probability of observing a putatively private allele by chance alone was calculated. The method is described below in detail.

Five additional populations were used in this analysis (Fig. 2.1, sites A-E). For each of the nine populations, putatively private alleles (PPAs) were defined as any allele detected in only one of the populations. Table 2.2 reports the number of PPAs found in each population. The cumulative binomial probability (P) of detecting zero PPAs, given an overall frequency of f , was calculated for each population. The assumed frequency of a PPA (f) is the number of detections of the PPA (x) across all populations (y) (some PPAs were detected multiple times in a single population). The probability of detecting a PPA with frequency f is $1-P$. To calculate the probability of detecting a private allele at least $1, 2, \dots, x$ times in each population, the probabilities of all the possible outcomes were computed for each population. Table 2.3 presents an example of the calculation, using the North Carolina data. The probability of at least z PPA detections equals the sum of all the probabilities that are less than or equal to z PPA detections. If this sum is greater than or equal to 0.95, then there is a 95% certainty that at least z private alleles exist in that population.

Nm estimates using all PPAs and using only truly private alleles (with 95% certainty) were compared. (Nm estimates were calculated across all four populations.)

For the Nm estimate based on truly private alleles, all iterations of the maximum number of private alleles for each population were used. For example, with the North Carolina population, there are at least two private alleles with 95% certainty (Table 2.3). Because it is not known which of the four PPAs are truly private, all permutations of two truly private alleles are produced, with the original number of detections for each allele. The permutations were then used in GENEPOP (Raymond & Rousset 1995) to calculate Nm across the four populations. Finally, a mean Nm estimate was calculated over all permutations.

A Mantel test (FSTAT, Goudet 1995) was used to evaluate whether genetic distance and geographic distance between Henslow's sparrow populations were associated. One population (Wisconsin) was excluded because sample size was small ($n=5$). Any significant isolation-by-distance pattern would indicate the populations are at evolutionary equilibrium (Gibbs, Dawson & Hobson 2000).

We used the program *Structure* (Pritchard, Stephens & Donnelly 2000) for the Bayesian analysis of genetic structure. Because parameters and assumptions can greatly influence the outcome, we used three sets of parameters (described in Table 2.4) to infer the number of populations (i.e., clusters). For all three sets, we assumed that allele frequencies were correlated among populations. A long burn-in period (500,000 iterations) was followed by a large number of Markov chain Monte Carlo repeats (1,000,000). For parameter Set 1, we assumed admixture among populations and uniform prior probabilities of admixture (α). For Set 2, we assumed no admixture between populations. For Set 3, we assumed that each individual's sampling site was the most probable origin. Where admixture was assumed, it was assumed to go back two

Population	Number of samples*	Number of PPAs (detected x times)	Number of private alleles with 95% certainty
Missouri (1)	224	5 (2, 1, 1, 1, 1)	≥ 2
Woodbury, Ohio (2)	227	2 (1, 1)	0
North Carolina (3)	470	4 (3, 5, 1, 1)	≥ 2
New York (4)	231	None	N/A
Wisconsin (A)	50	1 (1)	0
Wright Patterson Air Force Base, Ohio (B)	30	None	N/A
Tri-Valley, Ohio (C)	193	None	N/A
Egypt Valley, Ohio (D)	170	1 (1)	0
Crown City, Ohio (E)	197	2 (1, 1)	0
Total	1792	15	≥ 4

*Sum of all alleles found in that population.

Table 2.2. Estimated number of truly private alleles among putative private alleles (PPA) with 95% certainty in each population. Numerals and letters in parentheses beside the populations' sites indicate their geographic location (Fig. 2.1).

generations. Finally, we assumed that the prior probability of migration was 0.05. For each parameter set, the probability of the data was calculated for K populations, with K ranging from 1 to 7. These probabilities were then used to calculate the posterior probability of each K using Bayes' theorem:

$$\Pr(K = j) = \frac{\ln \Pr(X | K_j)}{\sum_{m=1}^7 \ln \Pr(X | K_m)},$$

where K = number of populations j and $j = (1, 2, \dots, 7)$,

If the largest posterior probability was observed for $K = 1$, then we inferred that the samples came from one large panmictic population. If the largest posterior probability was for some value of K exceeding unity, then the probabilities of population origin of each individual were calculated for each individual to determine the number of migrants

in each population. The genetic data from seven sites were used for the analysis (coded 1, 2, 3, 4, C, D, and E in Fig. 2.1). The data from two remaining sites (A and B, Fig. 2.1) were not used because sample sizes for these sites were judged to be too small.

Iterations of detection (Y = 1-P) or non-detection (N=P) of each PPA	P of 1st PPA	P of 2nd PPA	P of 2nd PPA	P of 2nd PPA	Product of Ps
YYYY	0.891	0.993	0.632	0.632	0.354
YYYN	0.891	0.993	0.632	0.368	0.206
YYNY	0.891	0.993	0.368	0.632	0.206
YYNN	0.891	0.993	0.368	0.368	0.120
YNY Y	0.891	0.007	0.632	0.632	0.002
YNYN	0.891	0.007	0.632	0.368	0.001
YNNY	0.891	0.007	0.368	0.632	0.001
YNNN	0.891	0.007	0.368	0.368	0.001
NYYY	0.109	0.993	0.632	0.632	0.043
NYYN	0.109	0.993	0.632	0.368	0.025
NYNY	0.109	0.993	0.368	0.632	0.025
NYNN	0.109	0.993	0.368	0.368	0.015
NNYY	0.109	0.007	0.632	0.632	0.0003
NNYN	0.109	0.007	0.632	0.368	0.0002
NNNY	0.109	0.007	0.368	0.632	0.0002
NNNN	0.109	0.007	0.368	0.368	0.0001

Table 2.3. Example of the computation procedure for estimating the number of putatively private alleles (PPA) with 95% certainty, using the North Carolina population, with 4 PPAs. The first PPA was detected two times, the second five times, the third and fourth one time each. The probability of at least 1 private allele = 0.9999 (= the sum of all the “Product of Ps” permutations with at least one Y), of at least 2 private alleles = 0.9841, at least 3 private alleles = 0.8109, and of at least 4 private alleles = 0.3537.

RESULTS

The estimated proportion of dispersers based on feather δH was similar across sites, ranging from 0.22 to 0.31 (Table 2.5). The δH s of four of the feather samples (all from the site in New York) were similar to the expected ratios (approximately -45) for the wintering grounds. Therefore, we assumed these feathers were grown over the previous winter. Identifying “false positives”

Set	Description
Same for all sets	Length of burn-in period = 500,000 Number of Markov chain Monte Carlo Repeats after burn-in = 1,000,000 Allele Frequencies assumed to be correlated among populations Assume different values of F_{st} for different subpopulations Prior Population F_{st} Means = 0.01 Prior Population F_{st} standard deviations = 0.05 Use constant $\lambda^* = 1.0$
Set 1	Assume admixture Initial $\alpha^{\#} = 1.0$ Same α for all populations Max $\alpha = 10.0$ Standard Deviation of $\alpha = 0.025$
Set 2	Assume no admixture
Set 3	Use prior population information (i.e., sampling locations) Assume admixture goes back to two generations Prior migration rate = 0.05

*The frequency of each allele. [#]Distribution of each individual’s proportion of admixture.

Table 2.4. Three sets of parameters and assumptions used in Bayesian analyses of genetic structure, using the software *Structure* (Pritchard, Stephens & Donnelly 2000).

(Benjamini & Yekutieli 2001) reduced the proportions of dispersers, with the Ohio sample containing no dispersers and the New York sample containing the most (Table 2.5).

The estimated proportion of dispersers based on bioacoustic measures varied across sites, ranging from 0 to 0.24 (Table 2.6). Discriminant analysis produced three functions that separated the four populations by song structure (Fig. 2.3). The four populations were significantly different from each other in at least one discriminant function (Mann-Whitney *U*-test, all $p < 0.001$). Song structure did not vary clinally across the breeding range, but formed a mosaic, typical of geographic variation in

Site	Missouri	Ohio	North Carolina	New York ¹
Sample size	21	9	17	17 (13)
Expected feather δH range	-66 – -39	-78 – -52	-66 – -39	-97 – -71
Proportion within the range	0.76	0.78	0.71	0.53 (0.69)
Proportion outside the range (dispersers)	0.24	0.22	0.29	0.23 (0.31)
Proportion outside the breeding range values (feathers probably grown in the wintering grounds)	0	0	0	0.24
Proportion of dispersers with 95% certainty ²	0.19	0	0.12	0.23 (0.31)

¹ Values in parentheses calculated with feathers determined to have been grown on the breeding grounds and not on the wintering grounds.

² Using values ± 1.96 SD from the expected feather δH , and using the Benjamini-Hochberg (1995) method assuming a false discovery rate of 5% (see text for details)

Table 2.5. Estimated proportions of dispersers in four sampling sites across the breeding range of the Henslow's sparrow, based on stable deuterium/hydrogen (δH) feather ratios.

Site	Missouri	Ohio	North Carolina	New York
Sample size	15	20	23	14
Proportion of songs misclassified (alien songs)	0	0.50	0.04	0.29
Proportion of alien songs due to breeding dispersal (range)	0 (0)	0.24 (0.14 – 0.29)	0.02 (0.02 – 0.01)	0.14 (0.08 – 0.16)

Table 2.6. Estimated proportions of dispersers in four sampling sites across the breeding range of the Henslow’s sparrow, based on song structure.

other songbirds. The analysis also indicated a low overall level of dispersal at the four sites, with the Missouri site having no alien songs and Ohio having the most alien songs (Table 2.6). Ohio had the highest dispersal estimates based on meme flow (Table 2.6) and the lowest dispersal estimates based on stable isotope ratios (Table 2.5). A discriminant analysis without the third song component produced qualitatively similar results (Table A.5).

The estimated number of migrants per generation, based on genetic measures, was generally high, ranging from 13.3 to 54.1 based on F_{st} analysis, and from 3.4 to 5.6 based on private allele analysis (Table 2.7). All pairwise breeding site F_{st} estimates were low and all but one of the pairwise comparisons were significant (Table 2.7), indicating little genetic structure and large numbers of migrants per generation between populations. Exact tests indicated significant differentiation between populations for only two of five loci (Maμ23 and Pdoμ5; Table A.6 reports F_{st} estimates for individual loci). The estimates of Nm based on F_{st} analysis were much larger than those based on private allele analysis. The pair of populations with the largest Nm estimate based on F_{st} analysis (New

York and North Carolina) was not the same as the pair with the largest Nm estimate based on private allele analysis (Missouri and Ohio). At least four of 15 putatively private alleles (PPAs) were truly private with 95% certainty (Table 2.2). The estimated number of private alleles ranged from 0 to 2 in the nine populations. The Nm estimate across all populations using PPAs was 7.2 and the Nm estimate using only private alleles was 7.8. The Mantel test on genetic and geographic distance produced no significant isolation-by-distance among the populations, suggesting that the populations were not at evolutionary equilibrium.

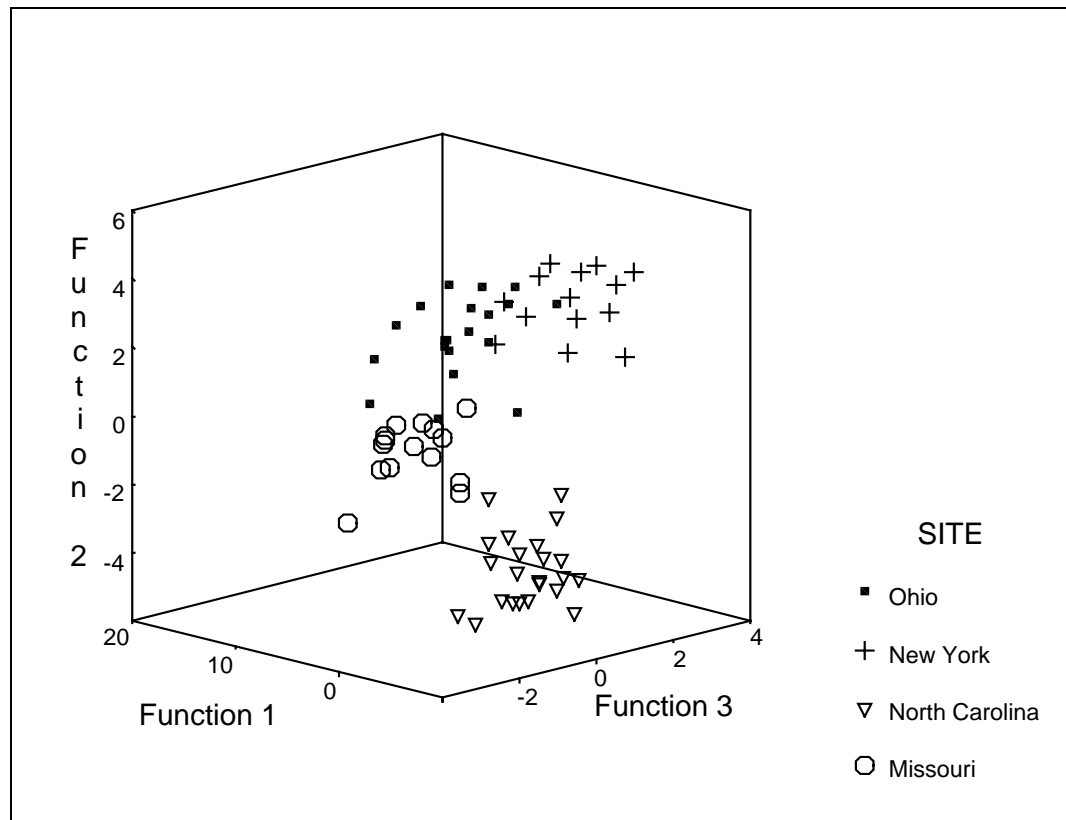


Figure 2.3. Discriminant functions of Henslow's sparrow songs from four populations. The three main discriminant functions were based on time and frequency measures.

In the Bayesian analysis of population structure, parameter Sets 2 and 3 produced the largest posterior probabilities for $K=1$ (each with probabilities of 0.999) while parameter Set 1 produced the largest posterior probability for $K=5$ (with a probability of 1.0). The initial inference for parameter Set 1 is misleading because the individual probabilities of population origin were generally extremely close to 0.2, indicating no clear genetic population structure. The analysis generated nearly uniform assignment probabilities (mean probability for $K=X$ was $1/X$; maximum variance = 0.001).

	Missouri	Ohio	North Carolina	New York
Missouri (sample size=23)		56.57 (138.64)	36.51* (23.33*)	23.11 (35.98*)
Ohio (sample size=23)	4.41		42.85 (51.83*)	13.49* (15.78*)
North Carolina (sample size=54)	4.93	5.50		20.58* (35.46*)
New York (sample size=18)	3.88	3.40	5.56	

* F_{st} value significantly different from zero after the Benjamini-Hochberg (1995) correction for multiplicity, assuming a 5% false discovery rate.

Table 2.7. Pairwise Nm calculated from F_{st} estimates (above diagonal) and Nm estimates based on private alleles (below diagonal) for four Henslow's sparrow populations. Values in parentheses were calculated without the correction for null alleles. Nm from the F_{st} estimates were calculated with the following equation: $F_{st}=1/[4Nm+1]$; Slatkin 1985a). All estimates made using GENEPOP (Raymond & Rousset 1995).

DISCUSSION

All three methods typically yielded high dispersal rates among Henslow's sparrow populations. The δH method yielded estimates ranging from 0 to 0.31 migrants/sampled individual, with three of the four populations containing large proportions of dispersers (Table 2.5). The meme flow method yielded estimates ranging from 0 to 0.24, with two of the four populations containing high proportions of dispersers (Table 2.6). The private-allele method yielded high estimates of gene flow (i.e., high genetically effective dispersal), but not as high as the gene flow estimates based on the F_{st} analysis (Table 2.6). Finally, the Bayesian clustering method produced support for the hypothesis of one large population. Although all methods produced estimates of high dispersal, some sources of biases must be kept in mind when evaluating these results.

One possible source of bias in the δH method is the directionality of the δH precipitation gradient. As shown in Fig. 2.1, environmental δH varies along a north-south gradient. Thus, this method can only detect dispersers originating from previous summer sites that are north or south of the current summer site. It may be possible to reduce this bias by refining the isotope gradient map, both by increasing the number of sites sampled for environmental δH and by searching for other elements that vary geographically, preferably along an east-west gradient. Also, studies on the geographic variation of isotopic ratios of other elements may improve isotopic signatures. Another natural marker investigators are using, rare elements found in certain regions (Amin, Bramer & Emslie 2003), may further improve the accuracy of feather chemical composition as a natural marker of origin for the specific regions where those rare elements are present.

The meme flow method could either underestimate or overestimate dispersal. If some populations share songs of very similar structure, then the discriminant analysis may assign a song to the region where it was recorded, even though the song was learned at another site, thereby producing an underestimate of dispersal. If estimates of the proportion of song differences due to meme flow (rather than learning mutation; Table 2.1) are inaccurate, then dispersal estimates may be lower or higher than the true dispersal rates.

The meme flow and δH methods estimate dispersal in males, but the genetic methods estimate dispersal in both sexes. Due to behavioral differences between male and female Henslow's sparrows, meme flow and δH methods sample males only (only the male sings and is easily caught using song playbacks and mist nets). The genetic methods, however, use nuclear DNA, which accounts for gene flow in both sexes. Genetic dispersal estimates may thus differ from estimates based on the other two methods due to differences in the dispersal behavior of males and females.

Pairwise comparisons of Nm estimates based on the two genetic methods revealed some differences as large as an order of magnitude (Table 2.7). These differences may derive from two sources. First, sampling error could produce the discrepancies because different numbers of alleles were analyzed by the two methods. The F_{st} estimates used 111 alleles while the private allele estimates used a subgroup of only 15 alleles. Second, although the relationship between private alleles and Nm is roughly linear (Fig. 1 in Slatkin 1985b), the relationship between F_{st} and Nm is nonlinear (Fig. 2 in Whitlock & McCauley 1998). Thus, small inaccuracies in F_{st} may produce larger inaccuracies in Nm estimates (Whitlock & McCauley 1998) than may small inaccuracies in private alleles.

Because of this nonlinear relationship, the very small F_{st} estimates among the Henslow's sparrow populations convert into extreme overestimates of Nm . One or both these sources could have contributed to the discrepancies of Nm estimates calculated with the two genetic methods.

The Nm estimates across populations (based on putatively private alleles [PPAs] and statistically confirmed private alleles) differed by less than one migrant per generation. This small difference should be interpreted cautiously. The effect of using PPAs instead of private alleles may be greater in studies where the numbers of PPAs are far greater than the numbers of truly private alleles (i.e., when allele sample sizes are smaller than the present study).

Both methods produced values greater than one migrant per generation for all pairwise combinations of populations, indicating more-than-adequate gene flow among the populations to counter any effects of genetic drift or weak natural selection (Wright 1931, Mills & Allendorf 1996, Vucetich & Waite 2000). However, caution is needed in interpreting these estimates. Estimation of Nm from F_{st} and private alleles has been criticized as being inaccurate due to weak correlations between Nm and F_{st} , and the improbable assumptions of the island model (Whitlock 1992, Whitlock & McCauley 1998). In addition, if populations were not at evolutionary equilibrium, then the high gene flow estimates may reflect recent common ancestry, not necessarily high gene flow. A Mantel test indicated no isolation-by-distance, implying that the populations were not at equilibrium (Gibbs, Dawson & Hobson 2000). This implication is supported by the Bayesian inference that the Henslow's sparrows in this study belong to a single population, with no genetic differentiation among sampling sites.

In a future analysis, we will estimate the effective population size (N_e) of each of the sampling sites. These estimates could then be used in Wright's equation as $N (F_{st} = 1/[4Nm+1]$; Slatkin 1985a). It will then be possible to have an estimate of the proportion of immigrants (m) in a population using the genetic data. These proportion estimates will be more comparable to the stable isotope ratio and meme flow estimates.

Notwithstanding the above-discussed limitations of the genetic, bioacoustic, and stable isotope methods, all estimates do reveal substantial dispersal among populations (Tables 2.5, 2.6 and 2.7). Such high dispersal is expected in species like the Henslow's sparrow that inhabit early successional habitat (Wiens 2001). Due to anthropogenic effects, many of the grassland habitats in the historical range of this species have been converted to other types of landuse (e.g., row crop agriculture). Meanwhile, grassland habitats outside this range have been created. Henslow's sparrows have colonized these new grassland habitats (Herkert, Vickery & Kroodsma 2002), indicating a strong ability to disperse into new regions. This recent expansion of the species range may be a byproduct of an evolved tendency to be highly dispersive. The high levels of dispersal estimated by all three methods also suggest that inbreeding in this species should not be a concern for managers, because these populations experience well above the traditional one-migrant-per-generation criterion recommended by conservation biologists (Mills & Allendorf 1996, Vucetich & Waite 2000).

POTENTIAL USES AND FUTURE NEEDS

Dispersal estimates based on stable isotope ratios and meme flow could contribute to a variety of fields in biology, including theoretical population biology and conservation

biology. (Table 2.8 lists some advantages, applications, and challenges of using genetic, stable isotope, and song data in estimating dispersal.) Many dispersal studies focus on the rate of successful immigration rather than emigration, an important characteristic of these two methods. Hence, these estimates may be more useful than dispersal estimates of outgoing dispersal rates, which are biased due to limited ability to detect long-distance dispersal events (Koenig, Van Vuren & Hooge 1996). Stable isotope ratios and meme flow, where appropriate, could be used to estimate contemporary dispersal rates in organisms that are not in evolutionary equilibrium (i.e., where genetic estimates of dispersal are suspect). These methods could be useful also for evaluating the relative contributions of gene flow versus natural selection as factors influencing population differentiation. Populations that are highly differentiated despite high dispersal rates are indicative of environment-specific selection regimes, with selection acting against dispersers not well adapted to their new environments (Ehrlich & Raven 1969).

In conservation biology, these dispersal estimates could be used in species that are difficult to catch or observe, reducing the need to recapture, re-sight, or track individuals as is necessary in marking studies. Dispersal estimates of endangered species, where handling animals to mark or gather tissue samples may be detrimental, could be made with song recordings and shed tissue samples (as long as precautions are taken to avoid unknowingly collecting replicate samples from the same individuals).

Conservation biologists could also use these two dispersal estimates in conjunction with genetic dispersal estimates to evaluate levels of historic and contemporary dispersal in a species. Populations with historically low gene flow (high F_{st} , low Nm) may have contemporarily high gene flow (assuming dispersal rates as

estimated with stable isotopes and meme flow correspond to gene flow). This heightened level of gene flow may be beneficial, if the avoidance of inbreeding depression is the management goal, or it may be detrimental, if maintenance of genetic differentiation is the management goal. Conversely, populations with historically high gene flow and contemporarily low gene flow could indicate a species affected by anthropogenic barriers between populations. These two natural-marker methods are relatively rapid and inexpensive tools for estimating contemporary dispersal rates and evaluate how these rates could affect inbreeding depression and genetic differentiation.

Furthermore, knowledge of contemporary dispersal rates is important in the design of species protection and recovery plans. If a species is found to have very limited dispersal, then the habitat matrix between reserves might not be an issue, since individuals would be unlikely to disperse between reserves, even if habitat connectivity were increased. For a species with high dispersal rates, connections between reserves need to be carefully planned to minimize the probability of death during dispersal.

Future research is needed to increase the accuracy of these two dispersal estimators. More refined contour maps of δH and other stable isotope ratios are needed. By producing fine-scale maps of geographic variation of several elements, it may be possible to identify the origins of individuals at many distances and from many directions. In songbirds, the geographic distribution of dialects and song ontogeny must be known to distinguish among natal, breeding, and overall dispersal estimates. More refined estimates of meme flow would be made possible by additional studies on song mutation rates and by collecting detailed measures of many songs throughout the breeding range. Another method for estimating meme flow uses private memes in a

model similar to the one developed for private alleles (Lynch 1994). Although no private memes were found in this study, this method may be useful in other songbird studies. Lastly, an evaluation of the use of these two natural markers as surrogates for genetic estimates would be useful. Comparisons could be made between gene flow and these two dispersal estimates in populations for which good estimates of gene flow already exist (e.g., estimates made using direct methods such as radio tracking or tagging). It would be interesting to see how well the dispersal estimates from these two methods agree with gene flow estimates of species known to have low gene flow (i.e., species that are generally of greater concern to conservation geneticists). These are challenging tasks, but they may allow for a clearer picture of how organisms disperse throughout the species range. Museum collections could provide additional data for these two dispersal estimates. Archived skins and song recordings provide a large source of samples for dispersal studies on a variety of species and could make estimating dispersal possible with even limited funds (relative to the funding requirements of mark-recapture or radio-tracking studies). We therefore promote the use of two markers, stable isotope ratios in tissues and song characteristics, in studies of songbird dispersal.

ACKNOWLEDGMENTS

Thanks to those who helped with the field work and analyses: John and Paula Wright, Robin Krebbs, Steven Joule, Alicia Bacci, Maiken Winter, Robert Grosholz, Elizabeth Vaughn, Jarling Ho, Allison Reid, Jose Diaz, Paul Doherty, Patricia Parker, Doug

Nelson, Sandra Gaunt, Jill Soha, Bo Bunnell, Kristin Field, and Scott Hull. Funding was provided by grants from Ohio Department of Natural Resources, Division of Wildlife; Columbus Zoo, and Mississippi Natural Heritage Fund.

Genes	Stable isotopes	Songs
Dispersal estimates are of both sexes using nuclear DNA and of females using mitochondrial DNA.	Dispersal estimates can be of both sexes.	Dispersal estimates are of the singing sex.
Can use the data to evaluate the historical relationships among populations.	Can use archived tissues to compare historical versus contemporary dispersal.	Can use archived songs to compare historical versus contemporary dispersal.
Possible to estimate the amount of genetically effective dispersal	Can estimate year-to-year dispersal.	
Less handling needed. May be able to use discarded tissues.		Less handling needed. Can record songs from a distance.
Estimates the amount of immigrants into a region rather than emigrants leaving a region.		
Populations not at evolutionary equilibrium may have little genetic distinctions.	Need to use elements that have stable isotope ratios that vary across the species' range.	Need information on the species' song ontogeny.

Table 2.8. Advantages, potential applications, and challenges of using genetic, stable isotope, and song data in estimating dispersal.

REFERENCES

- Acosta, C.A. (2002) Spatially explicit dispersal dynamics and equilibrium population sizes in marine harvest refuges. *ICES Journal of Marine Science*, **59**, 458-468.
- Alderson, G.W., Gibbs, H.L. & Sealy, S.G. (1999) Parentage and kinship studies in an obligate brood parasitic bird, the brown-headed cowbird (*Molothrus ater*), using microsatellite DNA markers. *Journal of Heredity*, **90**, 182-190.
- Alomar, O., Goula, M., Albajes, R. (2002) Colonisation of tomato fields by predatory mired bugs (Hemiptera: Heteroptera) in northern Spain. *Agriculture, Ecosystems and Environment*, **89**, 105-115.
- Amin, R., Bramer, M.A. & Emslie, R. (2003) Intelligent data analysis for conservation: experiments with rhino horn fingerprint identification. *Applications and Innovations in Intelligent Systems X* (eds A. Macintosh, R. Ellis & R. Coenen), pp. 207-222. Springer-Verlag, New York.
- Avise, J.C. (1994) *Molecular markers, natural history, and evolution*. Chapman and Hall, New York.
- Baptista, L.F. (1977) Geographic variation in song and dialects of the Puget Sound white crowned sparrow. *Canadian Journal of Zoology*, **63**, 1741-1752.
- Barton, N.H. & Slatkin, M. (1986) A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity*, **56**, 409-415.
- Beaudoin, C.P., Tonn, W.M., Prepas, E.E. & Wassenaar, L.I. (1999) Individual specialization and trophic adaptability of northern pike (*Esox lucius*): an isotope and dietary analysis. *Oecologia*, **120**, 386-396.
- Benjamini, Y. & Hochberg, Y. (1995) Controlling the false discovery rate – a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B – Statistical Methodology* **57**, 289-300.
- Benjamini, Y. & Yekutieli, D. (2001) The control of the false discovery rate in multiple testing under dependency. *The Annals of Statistics*, **29**, 1165-1188.
- Bensch, S., Price, T. & Kohn, J. (1997) Isolation and characterization of microsatellite loci in a Phylloscopus warbler. *Molecular Ecology*, **6**, 91-92.
- Borror, D.J. (1954) Analytical studies of Henslow's sparrow songs. *The Wilson Bulletin*, **66**, 243-252.
- Boulinier, T., McCoy, K.D. & Sorci, G. (2001) Dispersal and parasitism. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt & J.D. Nichols), pp. 169-179. Oxford University Press, Oxford.
- Callen, D.F., Thompson, A.D., Shen, Y., Phillips, H.A., Richards, R.I., Mulley, J.C. & Sutherland, G.R. (1993) Incidence and origin of 'null' alleles in the (AC)_n microsatellite markers. *American Journal of Human Genetics*, **52**, 922-927.
- Chakraborty, R., Zhong, Y., Jin, L. & Budowle, B. (1994) Nondetectability of restriction fragments and independence of DNA fragment sizes within and between loci in RFLP typing of DNA. *American Journal of Human Genetics*, **55**, 391-401.
- Chamberlain, C.P., Blum, J.D., Holmes, R.T., Fen, X., Sherry, T.W. & Graves, G.R. (1997) The use of isotope tracers for identifying populations of migratory birds. *Oecologia*, **109**, 132-141.
- Dawkins, R. (1976) *The Selfish Gene*. Oxford University Press, New York.

- Dawson, R.J.G., Gibbs, H.L., Hobson, K.A. & Yezerinac, S.M. (1997) Isolation of microsatellite DNA markers from a passerine bird, *Dendroica peregrina* (the yellow warbler), and their use in population studies. *Heredity*, **79**, 506-514.
- Double, M.C., Dawson, D., Burke, T. & Cockburn, A. (1997) Finding the fathers in the least faithful bird: A microsatellite-based genotyping system for the superb fairy-wren *Malurus cyaneus*. *Molecular Ecology* **6**: 691-693.
- Engineering Design (2001) Signal for DOS. Engineering Design, Belmont, MA.
- Ehrlich, P.R. & Raven, P.H. (1969) Differentiation of Populations. *Science*, **165**, 1228-1232.
- Gibbs, H.L., Tabak, L.M. & Hobson, K.A. (1999) Characterization of microsatellite DNA loci for a neotropical migrant songbird, the Swainson's thrush (*Catharus ustulatus*). *Molecular Ecology* **8**, 1551-1552.
- Gibbs, H.L., Dawson, R.J.G. & Hobson, K.A. (2000) Limited differentiation in microsatellite DNA variation among northern populations of the yellow warbler: evidence for male-biased gene flow? *Molecular Ecology*, **9**, 2137-2147.
- Goudet, J. (1995) FSTAT, version 1.2: a computer program to calculate F-statistics. *Journal of Heredity*, **86**, 485-486.
- Griffith, S.C., Steward, I.R.K., Dawson, D.A., Owens, I.P.F. & Burke, T. (1999) Contrasting levels of extra-pair paternity in mainland and island populations of the house sparrow (*Passer domesticus*); Is there an 'island effect'? *Biological Journal of the Linnean Society* **68**, 303-316.
- Herkert, J.R., Vickery, P.D. & Kroodsma, D.E. (2002) Henslow's sparrow (*Ammodramus henslowii*). *The Birds of North America* (eds A. Poole & F. Gill), 672. The Birds of North America, Inc. Philadelphia.
- Hobson, K.A., & Clark, R.G. (1992) Assessing avian diets using stable isotopes. II. Factors influencing diet-tissue fractionation. *Condor*, **94**, 181-188.
- Hobson, K.A. & Wassenaar, L.I. (1997) Linking breeding and wintering grounds of neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. *Oecologia*, **109**, 142-148.
- Hobson, K.A. (1999) Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia*, **120**, 314-326.
- Hobson, K.A., McFarland, K.P., Wassenaar, L.I., Rimmer, C.C. & Goetz, J.E. (2001) Linking breeding and wintering grounds of Bicknell's Thrushes using stable isotope analyses of feathers. *Auk*, **118**, 16-23.
- Immelmann, K. (1969) Song development in the zebra finch and other estrildid finches. *Bird Vocalizations: Their Relation to Current Problems in Biology and Psychology* (ed R.A. Hinde), pp. 61-74. Cambridge University Press, London.
- Koenig, W. D., Van Vuren, D. & Hooge, P. N. (1996) Detectability, philopatry, and the distribution of dispersal distances in vertebrates. *Trends in Ecology and Evolution*, **11**, 514-517.
- Kroodsma, D.E. (1978) Aspects of learning in the ontogeny of birds song: where, from whom, when, how many, which, and how accurately? *Comparative and Evolutionary Aspects of Behavioral Development* (eds G. Burghardt & M. Bekoff), pp. 215-230. Garland Press, New York.

- Kroodsma, D.E. (1996) Ecology of passerine song development. *Ecology and Evolution of Acoustic Communication in Birds* (eds D.E. Kroodsma & E.H. Miller), pp. 3-19. Cornell University Press, Ithaca, NY.
- Kroodsma, D.E. Pickert, R. (1984) Sensitive phases for song learning: effects of social interaction and individual variation. *Animal Behaviour*, **32**, 389-394.
- Lemon, R.E. (1975) How birds develop song dialects. *Condor*, **77**, 385-406.
- Lynch, A. (1996) The population memetics of birdsong. *Ecology and Evolution of Acoustic Communication in Birds* (eds D.E. Kroodsma & E.H. Miller), pp. 181-197. Cornell University Press, Ithaca, NY.
- Lynch, A. (1994) A population memetics approach to cultural evolution in Chaffinch song: differentiation among populations. *Evolution*, **48**, 351-359.
- Marler, P. & Tamura, M. (1962) Song 'dialects' in three populations of white-crowned sparrows. *Condor*, **64**, 368-377.
- Marler, P. & Peters, S. (1981) Sparrows learn adult song and more from memory. *Science*, **213**, 780-782.
- Martinez, J.G., Soler, J.J., Soler, M., Moller, A.P. & Burke, T. (1999) Comparative population structure and gene flow of a brood parasite, the great spotted cuckoo (*Clamator glandarius*), and its primary host, the magpie (*Pica pica*). *Evolution* **53**, 269-278.
- McDonald, D.B. & Potts, W.K. (1994) Cooperative display and relatedness among males in a lek-mating bird. *Science*, **266**, 1030-1032.
- Mills, L.S. & Allendorf, F.W. (1996) The one-migrant-per-generation rule in conservation and management. *Conservation Biology*, **10**, 1509-158.
- Moritz, C. (1994) Defining 'evolutionarily significant units' for conservation. *Trends in Ecology and Evolution*, **9**, 373-375.
- Mundinger, P.C. (1982) Microgeographic and macrogeographic variation in acquired vocalizations of birds. *Acoustic Communication in Birds Vol. 2* (eds D.E. Kroodsma & E.H. Miller), pp. 147-208. Academic Press, New York.
- Nathan, R. (2001) The challenges of studying dispersal. *Trends in Ecology and Evolution*, **16**, 481-483.
- Nelson, D.A. (1996) Social interaction and sensitive phases for song learning: a critical review. *Social Influences on Vocal Development* (eds C.T. Snowdon & M. Hausberger), pp. 7-21. Cambridge University Press, Cambridge, MA.
- Nelson, D.A. (1998) Geographic variation in song of Gambel's white-crowned sparrow. *Behaviour*, **135**, 321-342.
- Nelson, D.A. & Marler, P. (1994) Selection-based learning in bird song development. *Proceedings of the National Academy of Sciences, U.S.A*, **91**, 10498-10501.
- Nelson, D.A., Marler, P. & Morton, M.L. (1996) Overproduction in song development: an evolutionary correlate with migration. *Animal Behaviour*, **51**, 1127-1140.
- Newman, R.A. & Squire, T. (2001) Microsatellite variation and fine-scale population structure in the wood frog (*Rana sylvatica*). *Molecular Ecology*, **10**, 1087-1100.
- Nordby, J.C., Campbell, S.E. & Beecher, M.D. (2001) Late song learning in song sparrows. *Animal Behaviour*, **61**, 835-846.
- Nordby, J.C., Campbell, S.E. & Beecher, M.D. (2002) Adult song sparrows do not alter their song repertoires. *Ethology*, **108**, 39-50.

- Nottebohm, F. & Nottebohm, M.E. (1978) Relationship between song repertoire and age in the canary, *Serinus canarius*. *Zeitschrift fur Tierpsychologie*, **46**, 298-305.
- Parker, P.G., Snow, A.A., Schug, M.D., Booton, G.C. & Fuerst, P.A. (1998) What molecules can tell us about populations: choosing and using a molecular marker. *Ecology*, **79**, 361-382.
- Pemberton, J.M., Slate, J., Bancroft, D.R. & Barrett, J.A. (1995) Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology*, **4**, 249-252.
- Petren, K. (1998) Microsatellite primers from *Geospiza fortis* and cross-species amplification in Darwin's finches. *Molecular Ecology*, **7**, 1782-1784.
- Primmer, C.R., Moller, A.P. & Ellegren, H. (1996) A wide-range survey of cross-species microsatellite markers: a parentage testing system for the swallow *Hirundo rustica*. *Molecular Ecology*, **4**, 493-498.
- Pritchard, J.K., Stephens, M. & Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945-959.
- Piertney, S.B., Marquiss, M. & Summers, R. (1998) Characterization of tetranucleotide microsatellite markers in the Scottish crossbill (*Loxia scotica*). *Molecular Ecology*, **7**, 1248-1251.
- Raymond, M. & Rousset, F. (1995) GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248-249.
- Schneider, S., Roessli, D. & Excoffier, L. (2000) Arlequin ver. 2000: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
- Slatkin, M. (1981) Estimating levels of gene flow in natural populations. *Genetics*, **99**, 323-335.
- Slatkin, M. (1985a) Gene flow in natural populations. *Annual Review of Ecology and Systematics*, **16**, 393-430.
- Slatkin, M. (1985b) Rare alleles as indicators of gene flow. *Evolution*, **39**, 53-65.
- SPSS for Windows (2001) Rel. 11.0.1. Chicago: SPSS Inc.
- Szepanski, M.M., Ben-David, M. & Van Ballenberghe, V. (1999) Assessment of anadromous salmon resources in the diet of the Alexander Archipelago wolf using stable isotope analysis. *Oecologia*, **120**, 327-335.
- Thompson, P.M., & Goodman, S. (1997) Direct and indirect estimates of dispersal distances. *Trends in Ecology and Evolution*, **12**, 195-196.
- Vucetich, J.A. & Waite, T.A. (2000) Is one migrant per generation sufficient for the genetic management of fluctuating populations? *Animal Conservation*, **3**, 261-266.
- Warwick, S.I., Beckie, H.J. & Small, E. (1999) Transgenic crops: new weed problems for Canada? *Phytoprotection*, **80**, 71-84.
- Wassenaar, L.I. & Hobson, K.A. (2001) A stable-isotope approach to delineate geographical catchment areas of avian migration monitoring stations in North America. *Environmental Science and Technology*, **35**, 1845-1850.
- Weisser, W.W. (2001) The effects of predation on dispersal. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt & J.D. Nichols), pp. 180-188. Oxford University Press, Oxford.
- Whitlock, M.C. (1992) Temporal fluctuations in demographic parameters and the genetic variance among populations. *Evolution*, **46**, 608-615.

- Whitlock, M.C & McCauley, D.E. (1998) Indirect measures of gene flow and migration: $F_{st} \approx 1/(4Nm+1)$. *Heredity*, **82**, 117-125.
- Wiens, J.A. (2001) The landscape context of dispersal. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt & J.D. Nichols), pp. 96-109. Oxford University Press, Oxford.
- Wright, S. (1931) Evolution in Mendelian populations. *Genetics*, **16**, 97-159.

CHAPTER 3

HOW DO DISPERSAL ESTIMATES COMPARE? A REVIEW AND PROSPECTUS

SUMMARY

1. We compare three methods for estimating dispersal across five migratory bird species: red-winged blackbird (*Agelaius phoeniceus*), Bicknell's thrush (*Catharus bicknelli*), Henslow's sparrow (*Ammodramus henslowii*), black-throated blue warbler (*Dendroica caerulescens*), and Wilson's warbler (*Wilsonia pusilla*). These methods use genetic population structure, deuterium/hydrogen stable isotope ratios (δH) in feathers, and spatial autocorrelation of population densities.
2. Genetic population structure has been used to estimate dispersal by taking advantage of Wright's (1931) classical equation relating F_{st} and the number of migrants per generation, Nm . This method estimates the average genetically effective rate of dispersal over generations, thus it potentially estimates dispersal over the long term.

3. Stable isotope ratios could be useful indicators of dispersal. δH in flight feathers correlates with environmental δH . In these species, feathers are molted and regenerated in late summer. Because precipitation δH varies along a geographic gradient, feather δH provides a signature of an individual's previous summer site. The strength of association (r^2) between feather δH and local environmental δH is assumed to correlate negatively with dispersal rate. This method estimates inter-annual dispersal, thus it is an estimate of dispersal over the short term.
4. Spatial autocorrelation (SAC), the synchrony of population abundances that decreases over time and space, may be caused by density-dependent dispersal or density-independent factors (including the Moran effect). Detection of SAC may indicate high dispersal rates in a region, provided dispersal can be identified as a synchronizing agent. This method does not provide a direct estimate of dispersal, but has the potential to determine whether dispersal contributes to synchrony of population dynamics. Inferences pertain to the years of data collection, thus it is a short-term method.
5. Published data on genetic structure and δH along with new SAC analyses are compared. Two or three dispersal "estimates" are generated for each of the five species.
6. The SAC analysis provided no definitive evidence that dispersal was a major synchronizing agent. However, this method may be inadequate for the typical life histories of migratory birds.

7. Red-winged blackbirds were found to have limited genetic structure (possibly high historical dispersal rates) and high r^2 for δH values (low current dispersal rates). In general, this pattern could indicate newly formed dispersal barriers.
8. Estimates of genetic structure in the black-throated blue warbler are currently unavailable. However, this species was found to have high r^2 for δH values. If the genetic structure is limited, then there may be newly formed dispersal barriers. If the genetic structure is significant, then this species has historically and currently limited dispersal.
9. Bicknell's thrushes, Henslow's sparrows, and Wilson's warblers all had limited genetic structure and low r^2 for the δH values. The limited structure may be due to the currently or historically high dispersal rates. If the former, this species might have had historically low dispersal and is now experiencing the loss of dispersal barriers or a range expansion. If the latter, then dispersal rates have not changed dramatically over time.
10. Comparing multiple estimates using these and additional methods may help increase our understanding of dispersal. Various dispersal estimates could be integrated using Bayesian analysis to make stronger inferences about population connectivity.

INTRODUCTION

Dispersal affects a suite of phenomena and thus estimates of dispersal are in high demand in a variety of biological fields. In ecology and evolutionary biology, dispersal influences how a species or population is affected by natural selection, genetic drift, and inbreeding. In populations with high dispersal rates, weak natural selection may be overwhelmed, as immigrants that were not byproducts of the same selective forces come into an area and emigrants that were byproducts of these selective forces leave the area (Whitlock 2001). Variable dispersal among demes can reduce the overall effective population size, thereby increasing the effects of genetic drift (Whitlock 2001). Depleted populations of species that are infrequent or short-distance dispersers may have increased chance of suffering from inbreeding depression (O’Riain & Braude 2001) or competition (Lambin, Aars & Piertney 2001). Organisms with sluggish dispersal may be more strongly affected by high densities of predators or parasites in their habitat (Boulinier, McCoy & Sorci 2001; Weisser 2001). Because dispersal rates have broad implications, their accurate estimation is important to conservation biologists making decisions on species conservation and recovery plans.

For conservation planning, biologists must evaluate how individuals of the species of concern dispersed before human disturbance and how those dispersal behaviors have changed due to habitat fragmentation, introduction of exotics, exploitation, or exposure to pesticides and chemical wastes (Macdonald & Johnson 2001). Reserve plans that take dispersal behavior into account may better ensure that areas with depleted or extinct populations will be infused or recolonized by immigrants. Species with high dispersal rates may also be more likely to recolonize restored habitat or

expand into areas outside the reserve. Inbreeding depression may also be less likely in reserves where enough individuals disperse among appropriate habitats. Dispersal could allow individuals to find refuge from predation, disease, or inclement environmental conditions. While dispersal may have many beneficial effects, the potential negative effects of high dispersal rates must also be taken into account; high dispersal rates may allow for diseases or predators to invade a reserve, potentially wiping out the species of concern (Macdonald & Johnson 2001).

Dispersal has been studied in many taxa using a variety of techniques. Estimates of dispersal patterns have been made using mark-recapture data (e.g., Mennechez, Schtickzelle & Baguette 2003), radio tracking (e.g., Blouin-Demers & Weatherhead 2002), and genetic population structure (e.g., Gibbs, Dawson & Hobson 2000). Recently, spatial autocorrelation of population dynamics has also been used to estimate overall dispersal in a region (e.g., Hanski & Woiwod 1993, Swanson & Johnson 1999). Another new technique uses tissue stable isotope ratios to identify regional origins of individuals and hence has the potential to yield dispersal estimates (e.g., Hobson et al. 2001).

Adequate estimation of dispersal usually requires enormous effort, expense, and technical expertise. In mark-recapture techniques, an enormous number of individuals first need to be marked, in order to resight/recapture/recover enough individuals to produce robust dispersal estimates. Radio-tracking requires expensive equipment. Gene flow estimates work best with polymorphic genetic markers, which often have to be developed for the particular species under study. Spatial autocorrelation analysis requires census data that cover large ranges and long periods (Koenig 1997). Stable isotope ratios

are estimated with a mass spectrophotometer and the cost per sample may limit the number of individuals analyzed, although the cost may decrease as procedures become more cost-effective (Wassenaar & Hobson 2003).

These various methods for estimating dispersal may produce different, even contradictory, estimates. This is troublesome, because a species' estimated rate of dispersal may greatly affect the design of conservation or recovery plans. To evaluate how different dispersal estimates correspond with each other, we compare estimates made using genetic markers, stable isotope ratios, and spatial autocorrelation in five avian species: red-winged blackbird (*Agelaius phoeniceus*), Bicknell's thrush (*Catharus bicknelli*), black-throated blue warbler (*Dendroica caerulescens*), Wilson's warbler (*Wilsonia pusilla*) and Henslow's sparrow (*Ammodramus henslowii*). These particular species were chosen because stable isotope ratio data were available for each of them.

METHODS OF ESTIMATING DISPERSAL

Genetic population structure

Genetic structure is commonly described with the estimator, F_{st} , defined as “the variance in allele frequencies among populations...standardized by the mean allele frequency...” (Whitlock & McCauley 1998). The relationship between F_{st} and the number of migrants per generation, Nm , under Wright's (1931) island model is given by:

$$F_{st} = 1/(Nm + 1),$$

where N = the effective population size and m = the migration rate between populations.

This method estimates average gene flow among populations, and has been widely used as an alternative to direct methods (e.g., mark-recapture, radio-tracking). Populations

with low F_{st} estimates are assumed to have high dispersal rates, while populations with high F_{st} estimates are assumed to have low dispersal rates. It is assumed that the populations are at evolutionary equilibrium. If populations are not at equilibrium, then it is possible for low levels of genetic structure to exist, despite low dispersal rates, because the genetic structure reflects recent common ancestry (Gibbs, Dawson & Hobson 2000).

As with all methods, this one has advantages and disadvantages. One advantage is that it provides an estimate of successful dispersal; it is the result of the flow of new genes into a population through the reproduction of immigrants (Slatkin 1987). In addition, the data for evaluating the genetic population structure may potentially be used to detect other evolutionary patterns (e.g., evaluating patterns of a species' range expansion or its phylogenetic status). These advantages are balanced with criticisms of this method's inaccuracy, due to its unrealistic assumptions, and to the nonlinear relationship between F_{st} and Nm , where any inaccuracy in an F_{st} estimate is amplified in the conversion to an estimate of Nm (Whitlock & McCauley 1998). The few studies that have compared these estimates with independent dispersal estimates have found either rough agreement or substantial discrepancy (Rousset 2001). With these shortcomings in mind, we will compare qualitative, general outcomes, rather than quantitative results.

Data on genetic population structure were available for four of the five species (red-winged blackbird, Bicknell's thrush, Wilson's warbler and Henslow's sparrow). A parallel study is currently being performed on the black-throated blue warbler and should be completed in 2004 (M. Webster, pers. comm.).

Stable isotope ratios

This method involves hydrogen/deuterium stable isotope ratios (δH) in feathers. Stable isotope ratios of various elements have been promoted as natural indicators of breeding/wintering origins of migratory birds (using hydrogen and carbon; e.g., Marra, Hobson & Holmes 1998; Chapter 1), trophic levels (using carbon and nitrogen; e.g., Beaudoin et al. 1999), and feeding preferences (using carbon and nitrogen; e.g., Szepanski, Ben-David & Van Ballenberghe 1999). Another use of δH in feathers is as an indicator of dispersal (Hobson et al. 2001). This application takes advantage of two facts: 1) the environmental ratio of deuterium to hydrogen changes across a gradient in North America, and 2) the ratios in tissues correspond to the ratios of the environment in which the tissues were grown (Chamberlain et al. 1997, Hobson & Wassenaar 1997). Feathers collected on the breeding grounds reflect the ratios of the region where those feathers were grown during the preceding late summer. Birds returning to the same natal/breeding region will have feather δH similar to the ratios of other birds originating from that region, while birds immigrating into the region may have δH that differ from the ratios of birds native to the area. The relationship between feather δH and environmental precipitation δH may be an indication of the amount of dispersal occurring in the region. Species with weaker correlations of feather δH and precipitation δH may comprise populations with higher immigration rates (Hobson et al. 2001). That is, immigration rate should be inversely related to the coefficient of determination (r^2) for the correlation between feather δH and precipitation δH .

This method has several noteworthy limitations. It does not detect dispersers that have come from regions with δH values similar to those at the sampling site. Also, the

δH environmental gradient is based on the kriging of precipitation δH from relatively few sites and does not take into account other possible sources of water δH , such as lakes with paleowaters and oceanic waters, which may have very different δH than the ratios of precipitation water (Hobson & Wassenaar 1997). In addition, differences in precipitation δH may be due to altitude (Poage & Chamberlain 2001). The published δH gradient, therefore, may not accurately represent the local environmental δH . Finally, the gradient can only represent the general region of δH origins and thus the r^2 values may only represent long-distance dispersers and not dispersers from neighboring areas. Estimates of r^2 are available for all five species.

Spatial autocorrelation

Spatial autocorrelation (SAC), the synchrony of population abundances that decreases over time and space, is generally thought to be the result of any or all of three processes: 1) nomadic predators that move from areas of low to high prey densities, 2) density-dependent dispersal rates that decline with distance, and 3) density-independent factors (e.g., environmental synchrony) that affect population densities across a region, also known as the Moran effect (Koenig 1999, Swanson & Johnson 1999). The focus here will be on the latter two processes. Distinguishing between dispersal and the Moran effect, as synchronizing agents, has been challenging and produced much discussion in the biological literature (e.g., Ripa 2000).

Briefly, dispersal is thought to result in SAC that decays quickly with distance, whereas the Moran effect is thought to result in large-scale SAC (Ranta et al. 1995). Comparison between SAC of population abundances and SAC of environmental factors

(e.g., rainfall, temperature) may indicate the relative importance of the Moran effect versus dispersal (Nott et al. 2002). However, environmental synchrony may affect population SAC in general, and dispersal may simply be an additional level of SAC (Ripa 2000). To further complicate matters, the levels of SAC need not be constant over time. Thus, depending on the time series being compared, SAC may or may not be detected (Ranta, Kaitala & Lundberg 1997).

In a metapopulation, the process causing SAC can also produce different persistence times. If the SAC is at least partly due to dispersal, then subpopulations may be more rapidly recolonized after local extinctions (Hanski 1989). However, if the SAC is mostly or entirely due to the Moran effect, then the entire metapopulation is affected by environmental stochasticity, which may compromise persistence prospects (Ebenhard 1991).

SAC levels should also affect how species or regions are monitored and managed. Areas with low SAC would require more monitoring sites, for one site's decline/incline may not reflect a neighboring site's status. Areas with high SAC would require fewer monitoring sites, as the population status at one site will likely reflect the status at a neighboring site (Koenig 2001).

A set of criteria has been developed to help distinguish which synchronizing agent, dispersal or the Moran effect, has more influence (Swanson & Johnson 1999). The method uses two criteria. The first criterion evaluates the density-dependent pattern among synchronous populations. If the Moran effect is operating, then synchronous populations are expected to have similar (i.e., homogeneous) density-dependent structures. If dispersal is contributing to synchrony, the synchronous populations are

expected to have heterogeneous density-dependent structure (Swanson & Johnson 1999). The second criterion evaluates the synchrony-distance relationship among populations. If dispersal is important, then synchronous populations are expected to have a significant relationship between synchrony and interpopulation distance. The four possible outcomes of these two criteria, along with the four possible conclusions, are presented in Table 3.1 (Swanson & Johnson 1999). Determining the most influential agent for SAC may help managers understand population dynamics and devise appropriate management plans.

Although SAC analysis can help with conservation management and may be a useful estimate of dispersal, the amount of data needed to detect SAC in a region is enormous and therefore difficult to acquire. Fortunately, the Breeding Bird Survey (BBS) is an excellent source of data for SAC analysis of North American birds. This survey, started in 1966, covers the United States and southern Canada and is available to the public through the BBS web site (<http://www.pwrc.usgs.gov/bbs>). The census is performed each breeding season, with participants collecting data every 0.5 miles along 24.5 mile-long roadside routes. During each stop, all birds seen within a quarter-mile radius or heard over three minutes are recorded. With over 4100 routes across a large part of North America, this long-term data set has been used for other SAC analyses (e.g., Koenig 1998, 2001). For this paper, SAC analysis was performed on three of the five species (red-winged blackbird, black-throated blue warbler, and Wilson's warbler). The BBS data for the other two species were insufficient for SAC analysis.

Density-dependent structure	Synchrony-distance correlation	
	Significant	Non-significant
Heterogeneous	Dispersal	No main Moran effect
Homogeneous	Confounded	Possible main Moran effect

Table 3.1 Decision criteria for the most influential agent of spatial autocorrelation. Adapted from Table 1 in Swanson & Johnson 1999.

HISTORICAL VERSUS CURRENT LEVELS OF GENE FLOW

The three methods for estimating dispersal do so at different time scales. Genetic methods estimate long-term (historical) levels of gene flow, estimating the average gene flow over tens to thousands of generations, depending on the genetic markers used and on the current and historical characteristics of the population (Parker et al. 1998). Stable isotope methods estimate short-term (current) levels of dispersal, from one breeding season to the next. Spatial autocorrelation methods estimate levels of (potential) dispersal that go as far back as the census data used in the analysis (36 years in the case of the Breeding Bird Survey).

Dispersal estimates at these different time scales may reveal how anthropogenic changes have affected a species' dispersal rate. Table 3.2 presents the possible patterns of dispersal using the three methods. Below is a discussion of the potential causes and consequences of each pattern.

Pattern A: The dispersal rate of a species with both historically and currently high levels of dispersal has probably not been greatly affected by anthropogenic changes. Other than ensuring that high levels of dispersal are not disrupted by further anthropogenic change, management aimed at altering dispersal rate should be unnecessary for a species with this pattern. However, the limited genetic structure may

not reflect historically high dispersal but rather recent common ancestry of the populations (Gibbs, Dawson & Hobson 2000). If recent common ancestry exists, then low long-term dispersal rates would not be revealed by genetic structure. Research is needed to confirm that limited genetic structure reflects high dispersal rather than recent common ancestry.

Pattern B: A species with historically high gene flow and currently low dispersal may be experiencing new barriers between populations that reduce dispersal. If dispersal rates are too low, then there is a chance of inbreeding depression within populations. The conventional recommendation for avoiding inbreeding is to satisfy the one-migrant-per-generation rule (Wright 1931). This rule refers to one *genetically effective* migrant per generation. To satisfy this rule, it may often be necessary to ensure at least 10 actual migrants per generation (Mills & Allendorf 1996, Vucetich & Waite 2000). In species with this dispersal pattern, conservation biologists should strive to reduce the likelihood of inbreeding depression by removing migration barriers and developing migration corridors between populations. Again, researchers need to be confident that the limited genetic structure is due to historically high dispersal rates and not recent common ancestry.

Pattern C: Species that historically had low levels of gene flow and now have high levels of dispersal may experience outbreeding depression in the previously isolated populations (Allendorf & Leary 1986, Templeton 1986). If dispersal barriers have been removed, then populations that are well adapted to an environment may suffer from an influx of immigrants not as well suited to the area. If selection is overwhelmed by the high migration rate, then locally deleterious genes may inundate the population,

depressing population performance. In such cases, barriers would need to be re-established to reduce dispersal rates. This pattern may be more common in freshwater systems, where dams, manipulation of waterways and hatcheries routinely remove historical barriers to migration (e.g., Brown et al. 2000).

Pattern D: Species with historically and currently low dispersal may not suffer from inbreeding depression, provided deleterious genes have already been purged (Hedrick 1994). As with Pattern A, management should be needed only to maintain current levels of dispersal. Species that experience a change in dispersal rate over time need to be evaluated to determine the cause (natural versus anthropogenic) and consequences.

Dispersal rate pattern			Genetic structure	δH feather:location r^2	Spatial autocorrelation
<i>ID</i>	<i>Long-term</i>	<i>Short-term</i>			
A	High	High	Limited	Small	High
B	High	Low	Limited	Large	Low
C	Low	High	Limited	Small	High
D	Low	Low	Extensive	Large	Low

Table 3.2 Four possible dispersal rate patterns using long-term (i.e., historical) and short-term (i.e., current) dispersal estimates.

METHODS

Dispersal estimates were generated using all three methods for Henslow’s sparrow. Because these estimates represent original research, the methods for this species are described in detail. The SAC analyses also represent original research and so are also described in detail. Dispersal estimates based on genetic structure and stable isotope

ratios for the other four species were gathered from the literature and so the methods are not described in detail. Brief descriptions of these five species are incorporated into the RESULTS.

HENSLOW'S SPARROW DISPERSAL ESTIMATES BASED ON GENETIC DATA

DNA samples were extracted from 176 individuals sampled at seven sites across the breeding range (Table 3.3). Five primers (developed for other species) were selected based on their ability to amplify polymorphic microsatellites in Henslow's sparrow (Table A.1). The polymerase chain reaction was optimized for each primer. Base-pair fragment lengths were first estimated by comparing them to plasmids of known length and then fragments were compared to Henslow's sparrow DNA fragments of known size.

The microsatellite data were tested for null alleles, defined as alleles that are not amplified by locus primers (Pemberton et al. 1995), by using estimates of the inbreeding coefficient (F_i from FSTAT [Goudet 1995]) to test for departure from Hardy-Weinberg equilibrium. When null alleles are present, a data set tends to have a lower proportion of heterozygotes than expected under Hardy-Weinberg equilibrium (Pemberton et al. 1995). Three loci (Hr μ 7, Mcy μ 4, Pdo μ 5) were determined to have null alleles and thus the data were corrected using the following method. First, the frequency of null alleles (r) was estimated from the calculated (H_o) and expected heterozygosities (H_e) for each locus:

$$r = (H_e - H_o) / (H_e + H_o).$$

Then the frequencies of null alleles were increased to their expected frequencies by replacing one of a pair of homozygotes with a null allele until the expected frequency was attained (Chakraborty et al. 1992, Newman & Squire 2001). Observed and expected

heterozygosities were estimated using Arlequin (Schneider, Roessli & Excoffier 2000). After this correction, the genetic structure was estimated using an F_{st} index in FSTAT (Goudet 1995). Both corrected and uncorrected results are reported. These F_{st} estimates represent the average amount of gene flow among populations and thus are assumed to indicate the amount of dispersal among populations.

HENSLOW'S SPARROW DISPERSAL ESTIMATES BASED ON STABLE ISOTOPES

Feather deuterium ratios were estimated from 129 individuals sampled at 13 sites across the breeding range (Table 3.3). A rectrix from each individual was washed, dried, weighed, and placed in a silver foil cup. Because a proportion of keratin's hydrogen is exchangeable with ambient hydrogen, the samples were exposed to steam for 2h to equilibrate that proportion to a known ratio of ambient hydrogen (Wassenaar & Hobson 2000). The samples were then combusted and the resulting H₂O was reduced to H₂ gas. The δH of this gas was measured with a Micromass Optima™ dual inlet isotope-ratio mass spectrometer at the National Water Research Institute in Saskatoon, Saskatchewan, Canada. The δH are reported in parts per thousand deviations from Vienna Standard Mean Ocean Water Standard (VSMOW) and normalized to the VSMOW/SLAP (Standard Light Antarctic Precipitation) scale.

Each site's expected precipitation δH was taken from a contour map of feather δH (Fig. 3.1). The data were evaluated for potential outliers, defined here as samples whose standardized residuals fell outside three standard deviations from zero (Montgomery 1991). R^2 values based on a Pearson correlation between the feather and site δH (using SPSS, 2001) were estimated, using the data set with and without outliers.

DISPERSAL ESTIMATES BASED ON SPATIAL AUTOCORRELATION ANALYSIS

This analysis was performed on three species: red-winged blackbird, black-throated blue warbler and Wilson’s warbler using data from the North American Breeding Bird Survey (BBS). In the BBS data set, the numbers of detections for Henslow’s sparrows and Bicknell’s thrushes were insufficient for analysis.

For a meaningful comparison of the stable isotope dispersal estimates, we used BBS routes from the same states and provinces sampled for stable isotope analysis.

Study site	Location	Number of blood samples	Number of feather samples
Niawathe Prairie, Missouri	37°41’N, 93°45’W	23	21
Woodbury Wildlife Area, Ohio	40°20’N, 82°00’W	23	9
Voice of America Tower, North Carolina	35°37’N, 77°29’W	53	17
Fort Drum, New York	44°07’N, 75°18’W	18	17
Tri-Valley Wildlife Area, Ohio	40°04’N, 81°58’W	20	10
Egypt Valley Wildlife Area, Ohio	40°05’N, 81°10’W	18	10
Crown City Wildlife Area, Ohio	38°35’N, 82°16’W	21	10
Buena Vista Grasslands, Wisconsin	44°20’N, 89°50’W	0	10
Shawnee National Forest, Illinois	37°30’N, 88°40’W	0	5
Big Oaks National Wildlife Refuge, Indiana	38°44’N, 85°22’W	0	9
Wright Patterson Air Force Base, Ohio	39°45’N, 84°11’W	0	3
Clarion County, Pennsylvania	41°05’N, 79°26’W	0	4
Whooping Crane Foundation, Nebraska	40°49’N, 98°35’W	0	4
Total		176	129

Table 3.3 Sample sizes and locations where Henslow’s sparrow blood and feathers were collected.

Routes were grouped into hexagons in a uniform sampling grid. Routes were included if they met the following criteria. Over the 36 years of the survey, the focal species must have been detected at least twice and during at least 10% of the years. After the ineligible routes were removed, hexagons with fewer than five routes sampled for at least five years were removed.

For each year, the average number of detections per hexagon (μ) was log transformed ($\log(\mu+1)$). For each hexagon, these data were detrended by using the residuals from the regression of year on μ . This procedure removes any long-term population trends (Koenig 1997). Pearson correlation coefficients were calculated for all pairwise combinations of hexagons with overlapping surveys during at least five years (Koenig 1997).

The correlation coefficients were used to determine clusters of hexagons exhibiting spatial autocorrelation. To equalize the sample size among the three species, clusters with equal numbers of hexagons were used. For each species, one cluster of nine synchronous hexagons was determined. For black-throated blue warblers and Wilson's warblers, the hexagons were adjacent (Fig. 3.2a and 3.2b respectively), while for the red-winged blackbird, the nine clustered hexagons were adjoining or separated by just one hexagon (Fig. 3.2c). These clusters were then evaluated to determine the synchronizing agent.

To evaluate whether or not the Moran effect was a main synchronizing agent, we analyzed the density-dependent structure of each cluster. First, the fit of two density-dependence models was evaluated for each hexagon. The first model is a direct density-dependence model, where the current population size is predicted from the preceding

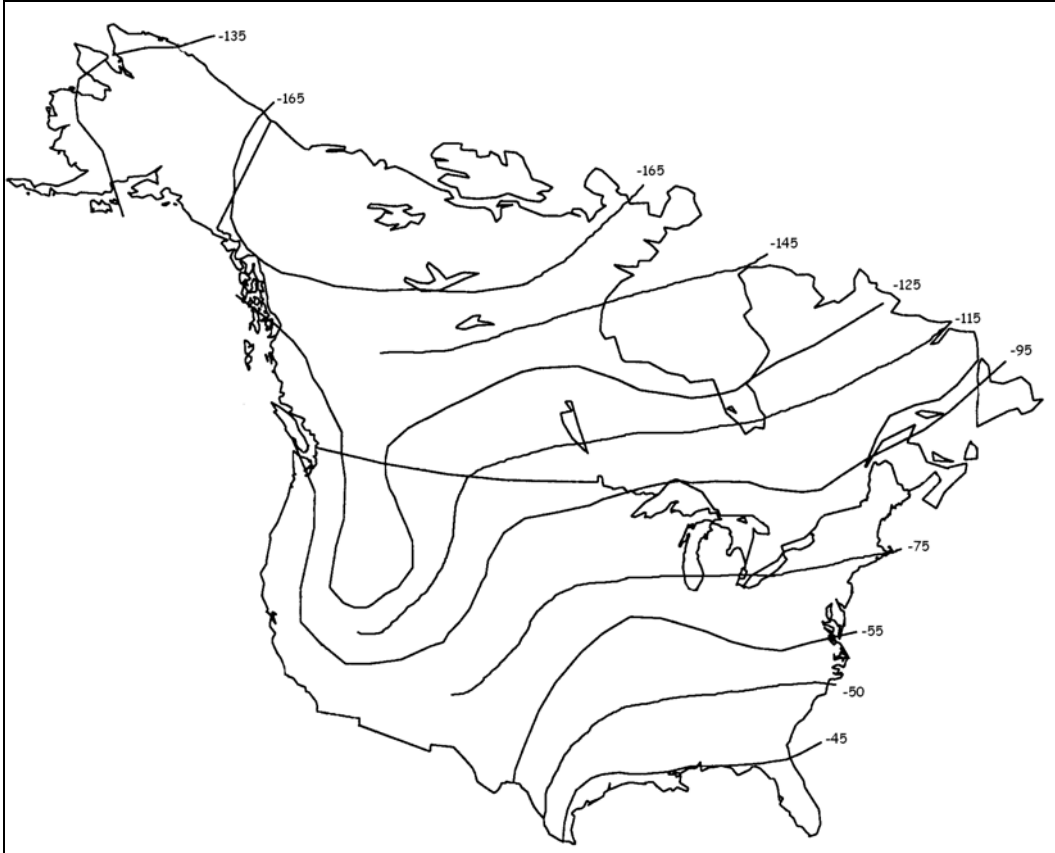


Figure 3.1 Contour map of feather isotope ratios. Adapted from Figure 1 in Hobson and Wassenaar 2000.

year's population size. The second model is an indirect density-dependence model, where the current population size is predicted by the population size during each of the two preceding years. The fit of the two models was evaluated using Akaike Information Criteria (AIC) values. This evaluation was performed as follows. For the first model, the sum of squares of a regression analysis of the population size at time t versus time $t-1$ was calculated. For the second model, the sum of squares of a regression analysis of the population size at time t versus times $t-1$ and $t-2$ was calculated. These sums of squares were then used to calculate the AIC value using the equation:

$$AIC = N_x * Ln\left(\frac{RSS_x}{N_x}\right) + 2K_x + \left(\frac{2K_x(K_x + 1)}{N_x - K_x - 1}\right),$$

where N_x = number of years for model x , RSS_x = sum of squares for model x , K_x = the number of free parameters for model x .

The model with the lowest AIC value was judged to be the best. If the same model was judged to be best for all hexagons in a cluster, then the cluster was categorized as homogeneous in its density-dependent structure. If not, then the cluster was categorized as heterogeneous.

To evaluate whether dispersal was a main synchronizing agent, we analyzed the relationship between synchrony and interpopulation distance within each cluster of hexagons using a Mantel test. Because a Mantel test compares two dissimilarity matrices, the synchrony matrix consisted of pairwise Pearson correlation coefficients subtracted from one. The interpopulation distance matrix used great circle distances between centers of hexagons. For each Mantel test, 999 iterations were performed. A significant positive correlation between the two dissimilarity matrices indicated dispersal as a synchronizing agent.

For each species, density-dependence structure and synchrony-distance relationship were used to evaluate which factor produced synchrony among the populations. Table 3.1 (Swanson & Johnson 1999) presents the decision rules for the four possible outcomes of these criteria. Mean Pearson r -values for the pairwise-comparisons of the hexagons in a cluster are presented as a crude indicator of the potential influence of dispersal as a synchronizing agent. In future work, estimates of instability of local population dynamics could be used to evaluate the influence of density-dependent dispersal on SAC (Ripa 2000, Lundberg et al. 2000).

RESULTS

SPECIES ACCOUNTS

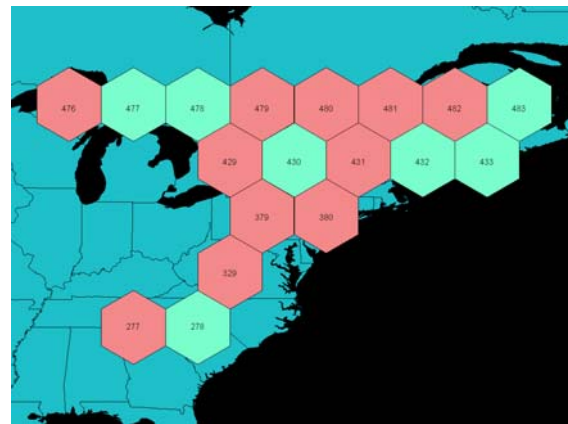
Below are brief descriptions of each of the five species. Table 3.4 presents dispersal estimates derived from the three methods.

Red-winged blackbirds

This species is one of the most successful of North America's landbirds (Ehrlich, Dobkin & Wheye 1988) with a range across most of the continent, limited only by the cold northern reaches of Canada and Alaska (Sauer, Hines & Fallon 2003). They overwinter in large flocks in most of the United States and extending south into Costa Rica (Ehrlich, Dobkin & Wheye 1988). This socially polygynous species (Searcy & Yasukawa 1995) tends to inhabit riparian habitat (Ehrlich, Dobkin & Wheye 1988), but over the last few decades has become one of the most abundant species in upland habitat in some areas of its breeding range (S. Hull, pers. comm.). Genetic analysis was based on mitochondrial DNA restriction-site variation from 127 samples collected in the United States (Arizona, California, Colorado, Florida, Georgia, Illinois, Louisiana, Minnesota, New York, Ohio, Pennsylvania, South Carolina, Texas, and Washington), Mexico (Puebla and Moreles), and Canada (Alberta). Results indicate small genotypic differences among populations, and genotypes and phylogenetic assemblages are found across the sampling range. There may be slight phylogeographic structure, with one genotype assemblage found in the Mexican sites only and one assemblage absent from the western sites (Ball et al. 1988). The δH analysis was based on sampling of feathers gathered from 64 territorial males



A



B



C

Figure 3.2 Maps for A) Wilson's warbler, B) black-throated blue warbler, C) red-winged blackbird. Like-colored hexagons indicate synchronous fluctuations of population density over time. The nine clustered pink hexagons (all adjacent for black-throated blue warblers and Wilson's warblers, one hexagon away for red-winged blackbirds) were used in the SAC analysis. Population density data were taken from North American Breeding Bird Survey.

Species	Genetic Population Structure		δH feather:location r^2		Spatial Autocorrelation Agent	Apparent Dispersal Pattern
	Estimate	Source	Estimate	Source		
Red-winged blackbird	Limited	Ball et al. 1988	0.83	Hobson & Wassenaar 2000	No main Moran effect (0.502)*	B
Bicknell's thrush	Limited	Ellison 2001	0.12	Hobson et al. 2001	Insufficient BBS data	A or C
Henslow's sparrow	Limited	This paper	0.14	This paper	Insufficient BBS data	A or C
Black-throated blue warbler	Currently unavailable	M. Webster, pers. com.	0.86	Chamberlain et al. 1997	No main Moran effect (0.643)*	B or D
Wilson's warbler	Low across range Limited in western range	Clegg et al. 2003	0.14	Clegg et al. 2003	No main Moran effect (0.035)*	A or C

*Mean r^2 value of SAC pairwise comparisons

Table 3.4 Dispersal estimates and patterns of five North American passerines.

from 10 locations in the United States (in Louisiana, Mississippi, Missouri, Illinois, Kansas, Iowa, and North Dakota) and one location in Saskatchewan, Canada. The samples' exchangeable hydrogen was equilibrated to steam with a known δH , and the total hydrogen ratio values are used. The feather δH correlated with precipitation δH values with an r^2 of 0.83 (Hobson & Wassenaar 2000). For this species, there was a heterogeneous density-dependent structure and a weak, nonsignificant synchrony-distance correlation (Table 3.5).

Bicknell's thrush

While red-winged blackbirds are found across the North American continent, breeding Bicknell's thrushes are constricted to the mountaintops of northeastern United States and

southeastern Canada. In winter, this long distance migrant is found in the Greater Antilles (Ouellet 1993). This habitat specialist inhabits fir-dominated forest at high elevations (900m and above) and is thought to select areas of regenerating forest. A conservative estimate of its abundance is 100,000 individuals and it is recognized by conservation organizations as one of the most vulnerable species of the region. Males and females mate with multiple partners with more than 75% of studied broods having more than one father (Goetz 2001). Genetic analysis was based on mitochondrial DNA sequences of control region III from 43 samples taken from New York, Vermont and New Hampshire. There was a modest but nonsignificant ($P>0.05$) population structure, with dispersal estimates (number of migrants per generation) being greater than unity among sites (Ellison 2001). The δH analysis was done for 64 feather samples from sites in Nova Scotia, New Brunswick, Quebec, Vermont and New York. The reported feather δH are for nonexchangeable hydrogen. The combination of rectrices from birds in their second year of life (SY) and after their second year (ASY) produced a weak correlation between feather δH and precipitation δH (r^2 of 0.12). If only ASY rectrices are included, the r^2 is 0.23 (Hobson et al. 2001).

Black-throated blue warbler

This species is a long-distance migrant, breeding in southeastern Canada and northeastern United States, west to the Great Lakes region and down through the Appalachian Mountains (Sauer, Hines & Fallon 2003) and wintering in Central America, Caribbean and Bahamas (Ehrlich, Dobkin & Wheye 1988). Although not as restricted as Bicknell's thrush, this species does specialize in continuous deciduous-coniferous forests with dense

undergrowth (Holmes 1994; Ehrlich, Dobkin & Wheye 1988). It is a socially monogamous species, with high rates of extra-pair fertilization (Chuang, Webster & Holmes 1999). Genetic analysis is currently underway for this species (Michael Webster, pers. comm.). The δH analysis was done on 154 feather samples gathered from nine sites across the breeding range (in the United States: Georgia, Michigan, New Hampshire, New York, Virginia, and West Virginia; in Canada: New Brunswick and Ontario). The feathers equilibrated to the environmental δH for several months, thus the exchangeable δH should be the same for all samples. The reported δH s are for both exchangeable and nonexchangeable δH . The correlation between feather δH and precipitation and surface water δH produced an r^2 value of 0.74 (Chamberlain et al. 1997). For this species, there was a heterogeneous density-dependent structure and a weak, nonsignificant synchrony-distance correlation (Table 3.5).

Wilson's warbler

The Wilson's warbler has a larger breeding range than the black-throated blue warbler. It extends across southern Canada and down through the United States' Sierra Nevada and Rocky Mountains (Sauer, Hines & Fallon 2003). This socially monogamous species inhabits wet thickets and woodland over a wide range of elevations (Ehrlich, Dobkin & Wheye 1988). Genetic analysis was done on eight microsatellite markers from 159 samples gathered from eight breeding ground sites in the United States (Alaska, California, Colorado, Oregon, and Washington) and Canada (Alberta, British Columbia, and Quebec). The analysis produced significant but low populations structure ($F_{st} = 0.035$, 95% C.I.: 0.007-0.091). Among the seven western sites, there was little

population structure ($F_{st} = 0.005$, 95% C.I.: 0.001-0.009; Clegg et al. 2003). The feather δH analysis was done on 117 breeding adults from six sites in the United States (Alaska, California, Colorado, and Oregon) and Canada (Alberta and Quebec). The samples were exposed to known ambient water δH for at least 10 days prior to isotopic analysis. The isotopic ratios of both exchangeable and nonexchangeable δH were reported. Unlike the other studies, the reported correlation is between feather δH and latitude instead of precipitation δH . The precipitation ratios for the sites are not available and could not be estimated with confidence using the contour map developed by Wassenaar and Hobson (2001), because the contours on the western part of the North American continent are not as well-defined as those on the eastern part of the continent (Fig. 3.1). The reported correlation has an r^2 of 0.14 (Clegg et al. 2003). For this species, there was a heterogeneous density-dependent structure and a weak, nonsignificant synchrony-distance correlation (Table 3.5).

Henslow's sparrow

These birds are short-distance migrants, breeding in western New York to eastern Oklahoma, and overwintering in the southeastern United States (Sauer, Hines & Fallon 2003). They inhabit moist grasslands, often with some standing vegetation used as singing perches (Herkert, Vickery & Kroodsma 2002). Although persistent breeding populations have been observed, researchers have also noted local extinctions and recolonizations (Pruitt 1996). This species is socially monogamous (Robins 1971) and often nests in loose “colonies” (Pruitt 1996). Genetic analysis revealed little population

Species	Density-dependent structure	Synchrony-distance correlation Pearson's r (P-value)	Conclusion	Mean r-value of pairwise comparisons
Red-winged blackbird	Heterogeneous	0.007 (0.51)	No main Moran effect	0.502
Black-throated blue warbler	Heterogeneous	-0.109 (0.29)	No main Moran effect	0.643
Wilson's warbler	Heterogeneous	0.051 (0.61)	No main Moran effect	0.035

Table 3.5 Outcomes of spatial autocorrelation analysis of three North American passerines. Data for the analysis taken from the North American Breeding Bird Survey.

structure ($F_{st} = 0.01$, 95% C.I.: 0.003 – 0.025, corrected for null alleles; $F_{st} = 0.008$, 95% C.I.: -0.001 – 0.024, not corrected for null alleles). The feather δH analysis produced an $r^2 = 0.15$ when outliers were included, and $r^2 = 0.18$ when outliers were excluded.

DISCUSSION

Various possible dispersal patterns emerged from the three methods (Table 3.4). For the four species with already-completed genetic analysis, the results indicate limited population structure. The stable isotope analyses indicate species with either high (low r^2) or low (high r^2) dispersal rates. In the SAC analysis, the synchrony-dispersal correlations were not significant, thus we found no evidence that dispersal is the main agent causing the SAC. However, according to the criterion in Table 3.5, the Moran effect should be excluded as a main synchronizing agent (Table 3.5). This inference should be revisited following reanalysis based on new statistical methods (Ripa 2000,

Lundberg et al. 2000). The SAC analysis also indicates high r^2 in the pairwise-comparisons between hexagons in two species and a low r^2 in the third species (Table 3.5).

Another indication that dispersal is not a main agent for the SAC patterns is the conflict between the mean r^2 values of the pairwise-comparisons of the hexagons and the r^2 values from the δH analysis (Table 3.4). If dispersal were an important SAC agent, then the red-winged blackbird's relatively high mean r^2 value of the pairwise comparisons of the SAC hexagons would indicate a high rate of short-term dispersal. However, the high r^2 of the δH analysis indicates low short-term dispersal. In black-throated blue warblers, the high SAC mean r^2 value and high r^2 of the δH analysis also conflict in their dispersal estimates. Finally, for Wilson's warbler, the low SAC mean r^2 value and low r^2 of the δH analysis conflict. Although we found no support for dispersal as a main agent for SAC, it may be influential at smaller scales and a main agent in other species. Future analyses will address this issue.

Although we found no evidence that either dispersal or the Moran effect was a major synchronizing agent, our analysis should be interpreted with caution. The criteria used to evaluate these two phenomena as synchronizing agents may not be well suited to migrating birds. If birds disperse to new breeding grounds during spring migration, then a synchrony-distance correlation may not exist. (If a wintering bird moves around a lot when in the wintering range, then the location of its new breeding site may be more related to the distance from its last wintering site.) In regards to the Moran effect, if individuals from the same breeding region migrate to different wintering sites, then the various environmental conditions at those wintering sites might also affect the breeding

region's population density during the following breeding season. Thus, the hexagon clusters would not be expected to have homogeneous density-dependence structures. A theoretical investigation incorporating these biological realities is needed to develop new criteria to evaluate the influence of dispersal and the Moran effect on the SAC of migratory birds. In the near future, though, reanalysis using techniques described by Lundberg et al. (2000) should help clarify the relative influence of dispersal and the Moran effect.

When comparing the long-term and short-term dispersal estimates, the three species with high short-term dispersal rates and apparently high long-term dispersal rates fall into either dispersal Patterns A or C (Table 3.4). (Pattern C is possible because short-term high dispersal may remove any population structure that could have accumulated in a species with historically low dispersal.) The management needs of a species experiencing the dispersal history represented by Pattern A are minimal. However, if the species is experiencing Pattern C, then managers should investigate the possibility that historical barriers have been removed and evaluate the populations for any signs of outbreeding depression.

The dispersal history for red-winged blackbirds is likely to be Pattern B, with limited long-term and short-term dispersal (Table 3.4). If so, this species may be experiencing recent dispersal barriers. Although this species appears to be doing quite well, it is possible that populations will start to experience inbreeding depression in the future.

Because data on genetic structure are currently unavailable for the black-throated blue warbler, there are two possible dispersal patterns for this species, Pattern B or

Pattern D (Table 3.4). If black-throated blue warblers have limited population structure (i.e., high long-term dispersal), then this species may be at risk for inbreeding depression. (It has a low short-term dispersal rate, according to the stable isotope data.) If this species has strong population structure (i.e., low long-term dispersal), then Pattern D is more likely and little management of this species' dispersal is needed.

For these patterns to reflect the dispersal history of these species, several assumptions must be true. Our first assumption is that the genetic population structure reflects long-term dispersal history. Various models have been devised to estimate dispersal from genetic data. However, the estimates made using these methods have rarely been compared with independent dispersal estimates (Rousset 2001).

Our second assumption is that the r^2 values from the stable isotope analysis reflect interannual dispersal in a local population. The low r^2 values found in Bicknell's thrush, Henslow's sparrow, and Wilson's warblers are assumed to indicate high recent dispersal for these species. These r^2 values contrast greatly with the high r^2 values found in red-winged blackbirds and black-throated blue warblers (Table 3.4). The high r^2 values found in these two species are more similar to r^2 values found in studies where the sampled individuals were known to have grown their feathers in the sampling area (e.g., Cooper's hawks $r^2=0.83$, Meehan et al. 2001; raptors inland generalists $r^2=0.59$ Lott, Meehan & Heath 2003). Hence, the high r^2 values are assumed to indicate low interannual dispersal in red-winged blackbirds and black-throated blue warblers.

Our third assumption is that the dispersal estimates based on stable isotope data reflect dispersal by reproductively successful individuals and thus are comparable to the genetically effective dispersal rates estimated using genetic population structure. Four of

the isotope ratio studies sampled adults exhibiting territorial behavior (red-winged blackbirds, Bicknell's thrushes, and Henslow's sparrows) or reproductive traits (Wilson's warbler), while the fifth study did not specify the reproductive status of the sampled birds (black-throated blue warblers). Although exhibiting territorial behaviors and reproductive traits does not ensure that the individuals were reproductively successful, the relative dispersal rates estimated with δH data are assumed to correspond to the relative dispersal rates estimated with genetic data.

The four genetic analyses revealed limited population structure. This limited structure may be due to the relatively recent expansion of birds into the northern reaches of North America, after the end of the last ice age (Gibbs, Dawson & Hobson 2000, Pielou 1991). The populations of these species may not be at evolutionary equilibrium. If so, then we cannot conclude that the limited population structure is due solely to high long-term dispersal rates; it may be due to recent common ancestry (Gibbs, Dawson & Hobson 2000; Pielou 1991)

How different estimates of dispersal correspond is an important issue in the study of dispersal. One estimate may reflect long-term dispersal while another estimate reflects short-term dispersal. A given method may not indicate dispersal at all, if its assumptions are violated. By comparing multiple methods of estimating dispersal, we may come closer to deciphering true underlying patterns of dispersal. Along with the three methods discussed here, studies using other methods can also be performed and added to the comparisons. Elements other than hydrogen may have stable isotope ratios that vary geographically and could be used as another natural individual marker of the area where an individual's tissues were grown. Other natural markers could also be utilized. Trace

elements in an individual's tissues may also be characteristic of the area (e.g., Amin, Bramer & Emslie 2003). Additional genetic markers and any morphometric characteristics unique to certain regions would also be useful natural markers. Meme flow can also be used in identifying dispersing songbirds (Chapter 2). Finally, advances in technology have allowed for the production of lighter, longer lasting, less expensive tracking devices, such as small global positioning satellite units (Reynolds & Riley 2002, von Hünenbein et al. 2000) and satellite Doppler tracking transmitters (Argos 2001). As these technologies advance they promise to revolutionize our knowledge of avian dispersal.

Multiple estimates of dispersal may be combined to produce an overall estimate of the probability that an individual has dispersed into a population. Bayesian methods allow for the integration of data to make probability estimates for multiple hypotheses (i.e., the probabilities of origin from various sites including the sampling location). Any pre-existing information could be used to develop prior probability distributions. Dispersal estimates can also be updated as additional dispersal estimates are acquired, thus refining our understanding of a species' dispersal rates.

By estimating dispersal using various methods on the same populations, the different estimates can be compared and combined to improve our understanding of the rates and distances of dispersal. Additional studies on these and other species, combining new techniques and Bayesian analysis, should yield much-improved estimates of dispersal and substantial advances in the methodology.

ACKNOWLEDGMENTS

Thanks to those who helped with the field work and analyses: John and Paula Wright, Robin Krebs, Steven Joule, Alicia Bacci, Maiken Winter, Robert Grosholz, Elizabeth Vaughn, Jarling Ho, Paul Doherty, Patricia Parker, Bo Bunnell, Kristin Field, and Scott Hull. Funding was provided by grants from Ohio Department of Natural Resources, Division of Wildlife; Columbus Zoo, and Mississippi Natural Heritage Fund.

REFERENCES

- Allendorf, F.W. & Leary, R.F. (1986) Heterozygosity and fitness in natural populations of animals. *Conservation biology: the science of scarcity and diversity* (ed M.E. Soulé), pp 57-76. Sinauer Associates, Sunderland, Massachusetts.
- Amin, R., Bramer, M.A. & Emslie, R. (2003) Intelligent data analysis for conservation: experiments with rhino horn fingerprint identification. *Applications and Innovations in Intelligent Systems X* (eds A. Macintosh, R. Ellis & R. Coenen), pp. 207-222. Springer-Verlag, New York.
- Argos website (2001) http://www.argosinc.com/system_overview.htm.
- Ball, R.M., Freeman, S., James, F.C., Bermingham, E., & Avise, J.C. (1988) Phylogeographic population structure of Red-winged Blackbirds assessed by mitochondrial DNA. *Proceedings of the National Academy of Sciences USA* **85**, 1558-1562.
- Barton, N.H. (2001) The evolutionary consequences of gene flow and local adaptation: future approaches. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt, & J.D. Nichols), pp. 329-340. Oxford University Press, Oxford.
- Barton, N.H. & Slatkin, M. (1986) A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity* **56**, 409-415.
- Beaudoin, C.P, Tonn, W.M., Prepas, E.E. & Wassenaar, L.I. (1999) Individual specialization and trophic adaptability of northern pike (*Esox lucius*): an isotope and dietary analysis. *Oecologia* **120**, 386-396.
- Blouin-Demers, G. and Weatherhead, P.J. (2002) Implications of movement patterns for gene flow in black rat snakes (*Elaphe obsoleta*). *Canadian Journal of Zoology* **80**, 1162-1172.
- Boulinier, T., McCoy, K.D. & Sorci, G. (2001) Dispersal and parasitism. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt, & J.D. Nichols), pp. 169-179. Oxford University Press, Oxford.
- Brown, B.L., Gunter, T.P., Waters, J.M., & Epifanio, J.M. (2000) Evaluating genetic diversity associated with propagation-assisted restoration of American shad. *Conservation Biology* **14**, 294-303.
- Chakraborty, R., Zhong, Y., Jin, L. & Budowle, B. (1994) Nondetectability of restriction fragments and independence of DNA fragment sizes within and between loci in RFLP typing of DNA. *American Journal of Human Genetics* **55**, 391-401.
- Chamberlain, C.P., Blum, J.D., Holmes, R.T., Feng, X., Sherry, T.W., & Graves, G.R. (1997) The use of isotope tracers for identifying populations of migratory birds. *Oecologia* **109**, 132-141.
- Chuang, H.C., Webster, M.S., and Holmes, R.T. (1999) Extrapair paternity and local synchrony in the black-throated blue warbler. *Auk* **116**, 726-736.
- Clegg, S.M., Kelly, J.F., Kimura, M., & Smith, T.B. (2003) Combining genetic markers and stable isotopes to reveal population connectivity and migration patterns in a Neotropical migrant, Wilson's warbler (*Wilsonia pusilla*). *Molecular Ecology* **12**, 819-830.

- Ebenhard, T. (1991) Colonization in metapopulations: a review of theory and observations. *Metapopulation Dynamics* (eds M. Gilpin & I. Hanski), pp. 105-121. Academic Press, London.
- Ellison, W.G. (2001) *Population structure and gene flow in two long distance migrant birds, the Bicknell's Thrush (Catharus bicknelli) and Veery (C. fuscescens)*. PhD thesis, University at Albany, State University of New York, Albany, U.S.A.
- Ehrlich, P.R., Dobkin, D.S., & Wheye, D. (1988). *The Birders Handbook*. Simon & Schuster, New York.
- Gibbs, H.L., Dawson, R.J.G., and Hobson, K.A. (2000) Limited differentiation in microsatellite DNA variation among northern populations of the yellow warbler: evidence for male-biased gene flow? *Molecular Ecology* **9**, 2137-2147.
- Goetz, J.E. (2001) Parentage and parental care in Bicknell's Thrush, *Catharus bicknelli*. M.S. thesis, State University of New York, Syracuse.
- Goudet, J. (1995). FSTAT, version 1.2: a computer program to calculate F-statistics. *Journal of Heredity* **86**, 485-486.
- Hanski, I. (1989) Metapopulation dynamics – does it help to have more of the same? *Trends in Ecology & Evolution* **4**, 113-114.
- Hanski, I. & Woiwod, P. (1993) Spatial synchrony in the dynamics of moth and aphid populations. *Journal of Animal Ecology* **62**, 656-668.
- Hedrick, P.W. (1994) Purging inbreeding depression and the probability of extinction: full-sib mating. *Heredity* **73**, 363-372.
- Herkert, J.R., Vickery, P.D. & Kroodsmas, D.E. (2002) Henslow's sparrow (*Ammodramus henslowii*). *Birds of North America* (eds A. Poole & F. Gill), **672**. The Birds of North America, Inc. Philadelphia, PA.
- Hobson, K.A., & Wassenaar, L.I. (1997) Linking breeding and wintering grounds of Neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. *Oecologia* **109**, 142-148.
- Hobson, K.A., & Wassenaar, L.I. (2000) Stable-carbon and hydrogen isotope ratios reveal breeding origins of Red-winged Blackbirds. *Ecological Applications* **10**, 911-916.
- Hobson, K.A., McFarland, K.P., Wassenaar, L.I., Rimmer, C.C. & Goetz, J.E. (2001) Linking breeding and wintering grounds of Bicknell's Thrushes using stable isotope analyses of feathers. *The Auk* **118**, 16-23.
- Holmes, R.T. (1994) Black-throated Blue Warbler (*Dendroica caerulescens*). *Birds of North America* (eds A. Poole & F. Gill), **87**. The Birds of North America, Inc. Philadelphia, PA.
- Koenig, W.D. (1998) Spatial Autocorrelation in California Land Birds. *Conservation Biology* **12**, 612-620.
- Koenig, W.D. (1999) Spatial Autocorrelation of ecological phenomena. *Trends in Ecology and Evolution* **14**, 22-26.
- Koenig, W.D. (2001) Spatial autocorrelation and local disappearances in wintering North American birds. *Ecology* **82**, 3519-3530.
- Lambin, X., Aars, J., and Pieltney, S.B. (2001) Dispersal, intraspecific competition, kin competition and kin facilitation: a review of the empirical evidence. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt, & J.D. Nichols), pp. 110-122. Oxford University Press, Oxford.

- Lott, C.A., Meehan, T.D. & Heath J.A. (2003) Estimating the latitudinal origins of migratory birds using hydrogen and sulfur stable isotopes in feathers: influence of marine prey base. *Oecologia*, **134**, 505-510.
- Lundberg, P., Ranta E., Ripa, J. & Kaitala, V. (2000) Population variability in space and time. *Trends in Ecology and Evolution* **15**, 460-464.
- Macdonald, D.W. and Johnson, D.D.P. (2001) Dispersal in theory and practice: consequences for conservation biology. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt, & J.D. Nichols), pp. 358-372. Oxford University Press, Oxford.
- Marra, P.P., Hobson, K.A., & Holmes, R.T. (1998) Linking winter and summer events in a migratory bird by using stable-isotopes. *Science* **292**, 1884-1886.
- Meehan, T.D., Lott, C.A., Sharp, Z.D., Smith, R.B., Rosenfield, R.N, Stewart, A.C. & Murphy, R.K. (2001) Using hydrogen isotope geochemistry to estimate the natal latitudes of immature Cooper's hawks migrating through the Florida Keys. *Condor*, **103**, 11-20.
- Mennechez, G., Schtickzelle, N., and Baguette, M. (2003) Metapopulation dynamics of the bog fritillary butterfly: Comparison of demographic parameters and dispersal between a continuous and a highly fragmented landscape. *Landscape Ecology* **18**, 279, 291.
- Mills, L.S. & Allendorf, F.W. (1996) The one-migrant-per-generation rule in conservation and management. *Conservation Biology*, **10**, 1509-158.
- Montgomery, D.C. (1991) *Design and Analysis of Experiments*, p. 98 John Wiley & Sons, New York.
- Newman, R.A. & Squire, T. (2001) Microsatellite variation and fine-scale population structure in the wood frog (*Rana sylvatica*). *Molecular Ecology* **10**, 1087-1100.
- Nott, M.P., Desante, D.F., Siegel, R.B., & Pyle, P. (2002) Influences of the El Niño/Southern Oscillation and the North Atlantic Oscillation on avian productivity in forests of the Pacific Northwest of North America. *Global Ecology & Biogeography* **11**, 333-342.
- Ouellet, H.R. (1993) Bicknell's Thrush: Taxonomic status and distribution. *Wilson Bulletin* **105**, 545-572.
- O'Riain, M.J. and Braude, S. (2001) Inbreeding versus outbreeding in captive and wild populations of naked mole-rats. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt, & J.D. Nichols), pp. 143-154. Oxford University Press, Oxford.
- Parker, P.G., Snow, A.A., Schug, M.D., Booton, G.C. & Fuerst, P.A. (1998) What molecules can tell us about populations: choosing and using a molecular marker. *Ecology* **79**, 361-382.
- Pemberton, J.M., Slate, J., Bancroft, D.R. & Barrett, J.A. (1995) Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology* **4**, 249-252.
- Perrin, N. and Goudet, J. (2001) Inbreeding, kinship, and the evolution of natal dispersal. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt, & J.D. Nichols), pp. 123-142. Oxford University Press, Oxford.
- Pielou E.C. (1991) *After the Ice Age: the Return of Life to Glaciated North America*. University of Chicago Press, Chicago, Illinois.

- Poage, M.A. and Chamberlain, C.P. (2001) Empirical relationships between elevation and stable isotope composition of precipitation and surface waters: Considerations for studies of paleoelevation change. *American Journal of Science* **301**, 1-15.
- Pruitt, L. (1996) Henslow's Sparrow Status Assessment. U.S. Fish and Wildlife Service, Bloomington, IN.
- Ranta, E., Kaitala, V., Lindström, J., & Lindén, H. (1995) Synchrony in population dynamics. *Proceedings of the Royal Society of London B* **262**, 113-118.
- Ranta, E., Kaitala, V., & Lundberg, P. (1997) The spatial dimension in population fluctuations. *Science* **278**, 1621-1623.
- Reynolds, D.R. & Riley J.R. (2002) Remote-sensing, telemetric and computer-based technologies for investigating insect movement: a survey of existing and potential techniques. *Computers and Electronics in Agriculture* **35**, 271-307.
- Ripa, J. (2000) Analysing the Moran effect and dispersal: their significance and interaction in synchronous population dynamics. *Oikos* **89**, 175-187.
- Robins, J.D. (1971) A study of Henslow's Sparrow in Michigan. *Wilson Bulletin* **83**:39-48.
- Rousset, F. (2001) Genetic approaches to the estimation of dispersal rates. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt, & J.D. Nichols), pp. 18-28. Oxford University Press, Oxford.
- Sauer, J.R., Hines, J.E., & Fallon, J. (2003) The North American Breeding Bird Survey, Results and Analysis 1966-2002. Version 2003.1, USGS Patuxent Wildlife Research Center, Laurel, MD
- Schneider, S., Roessli, D. & Excoffier, L. (2000) Arlequin ver. 2000: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
- Searcy, W.A. and Yasukawa, K. (1995) Monographs in Behavior and Ecology: Polygyny and sexual selection in red-winged blackbirds. (eds J. Krebs & T.H. Clutton-Brock) Princeton University Press, Princeton, New Jersey.
- Slatkin, M. (1987) Gene flow and the geographic structure of natural populations. *Science* **236**, 787-792.
- SPSS for Windows (2001) Rel. 11.0.1. Chicago: SPSS Inc.
- Swanson, B.J. & Johnson, D.R. (1999). Distinguishing causes of intraspecific synchrony in population dynamics. *Oikos* **86**, 265-274.
- Szepanski, M.M., Ben-David, M. & Van Ballenberghe, V. (1999) Assessment of anadromous salmon resources in the diet of the Alexander Archipelago wolf using stable isotope analysis. *Oecologia* **120**, 327-335.
- Templeton, A.R. (1986) Coadaptation and outbreeding depression. *Conservation biology: the science of scarcity and diversity* (ed M.E. Soulé), pp 105-116. Sinauer Associates, Sunderland, Massachusetts.
- Von Hünerbein, K., Hamann, H.J., Rüter, E. & Wiltshko, W. (2000) A GPS-based system for recording the flight paths of birds. *Naturwissenschaften* **87**, 278-279.
- Vucetich, J.A. & Waite, T.A. (2000) Is one migrant per generation sufficient for the genetic management of fluctuating populations? *Animal Conservation*, **3**, 261-266.
- Wassenaar, L.I. & Hobson, K.A. (2000) Improved method for determining the stable-hydrogen isotopic composition (delta D) of complex organic materials of environmental interest. *Environmental Science and Technology* **34**, 2354-2360.

- Wassenaar, L.I. & Hobson, K.A. (2001) A stable-isotope approach to delineate geographical catchment areas of avian migration monitoring stations in North America. *Environmental Science and Technology* **35**, 1845-1850.
- Wassenaar, L.I. & Hobson, K.A. (2003) Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for the use in animal migration studies. *Isotopes in Environmental and Health Studies* **39**, 211-217.
- Weisser, W.W. (2001) The effects of predation on dispersal. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt, & J.D. Nichols), pp. 180-188. Oxford University Press, Oxford.
- Whitlock, M.C. (2001) Dispersal and the genetic properties of metapopulations. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt, & J.D. Nichols), pp. 273-282. Oxford University Press, Oxford.
- Whitlock, M.C & McCauley, D.E. (1998) Indirect measures of gene flow and migration: $F_{st} \approx 1/(4Nm+1)$. *Heredity* **82**, 117-125.
- Wright, S. (1931) Evolution in Mendelian populations. *Genetics* **16**, 97-159

APPENDIX A

TABLES OF ADDITIONAL GENETIC AND BIOACOUSTIC INFORMATION FOR HENSLOW'S SPARROW

Primer Name	Reference	Primer Name	Reference
<i>Maμ23</i>	Alderson <i>et al.</i> 1999	POCC2	Bensch <i>et al.</i> 1997
<i>Hrμ7</i>	Primmer <i>et al.</i> 1996	Asμ09	Lisle Gibbs, unpublished
<i>Mcyμ4</i>	Double <i>et al.</i> 1997	Asμ15	Lisle Gibbs, unpublished
<i>Pdoμ5</i>	Griffith <i>et al.</i> 1999	Asμ18	Lisle Gibbs, unpublished
<i>Dpμ16</i>	Dawson <i>et al.</i> 1997	Fhμ2	Primmer <i>et al.</i> 1996
GF01	Petren <i>et al.</i> 1998	Caμ2	Gibbs <i>et al.</i> 1999
GF12	Petren <i>et al.</i> 1998	Caμ4	Gibbs <i>et al.</i> 1999
GF14	Petren <i>et al.</i> 1998	Caμ5	Gibbs <i>et al.</i> 1999
Hrμ5	Primmer <i>et al.</i> 1996	Caμ6	Lisle Gibbs, unpublished
Lox1	Piertney <i>et al.</i> 1998	Caμ9	Lisle Gibbs, unpublished
LTMR16	McDonald & Potts 1994	Caμ10	Gibbs <i>et al.</i> 1999
LTMR6	McDonald & Potts 1994	Caμ28	Gibbs <i>et al.</i> 1999
LTMR8	McDonald & Potts 1994	Caμ32	Gibbs <i>et al.</i> 1999
Ppi2	Martinez <i>et al.</i> 1999	Dpμ01	Dawson <i>et al.</i> 1997

Table A.1. Primers screened for microsatellite analysis. Of 24 primers screened, five were deemed polymorphic enough to use in the genetic analysis (first five primers in italics).

Primer Names					
PCR Components	Maμ23	Hrμ7	Mcyμ4	Pdoμ5	Dpμ16
DNA	3-ng	30 ng	30 ng	30 ng	30 ng
Buffer* (10X)	1.0 μ L	1.0 μ L	1.0 μ L	1.0 μ L	1.0 μ L
MgCl₂*	2.5mM	2.5mM	2.5mM	2.5mM	2.5mM
DNTPs*	0.2mM	0.2mM	0.2mM	0.2mM	0.2mM
Primers*	2 μ M	2 μ M	2 μ M	2 μ M	2 μ M
Bovine Serum	0 μ L	1. μ L	0 μ L	1. μ L	0 μ L
Albumin (10 mg/μL)[#]					
Taq DNA	0.2	0.2	0.2	0.2	0.2
Polymerase*	units	units	units	units	units
PCR annealing	48°	48°	50°	60°	60°
temperature (°C) and	35	35	35	3 cycles	30
number of cycles	cycles	cycles	cycles	58°	cycles
				32	
				cycles	

* From Invitrogen

From Fisher Scientific

Table A.2. PCR protocols for the five primer sets used in the analysis.

Site Code [#]	Locus				
	Pdop5	Hrμ7	Mcyμ4	Dppμ16	Maμ23
1	0.255 (0.0014)*	0.315 (0.0014)*	0.307 (0.0014)*	-0.045 (0.7843)	-0.363 (1.00)
2	0.262 (0.0029)	0.107 (0.0457)	0.251 (0.0014)*	-0.042 (0.7286)	-0.591 (1.00)
3	0.223 (0.0014)*	0.223 (0.0014)*	0.129 (0.0043)	-0.099 (0.9443)	-0.316 (1.00)
4	-0.036 (0.8314)	0.080 (0.1400)	0.102 (0.1314)	0.033 (0.4729)	-0.329 (1.00)
C	0.277 (0.0014)*	0.107 (0.0057)	0.255 (0.0029)	-0.033 (0.6871)	-0.500 (1.00)
D	0.032 (0.4300)	0.366 (0.0014)*	0.122 (0.1000)	-0.024 (0.7171)	-0.221 (1.00)
E	0.157 (0.0443)	0.400 (0.0014)*	0.061 (0.2600)	0.002 (0.5771)	-0.299 (1.00)

[#]Codes correspond to the sites labeled in Fig. 1.1.

*Significant after Benjamini-Hochberg (1995) correction assuming a false discovery rate of 5%

Table A.3. The F_{IS} (P -values) of each locus across seven Henslow's sparrow breeding sites (sites 1, 2, 3, 4, C, D, and E in Fig. 1.1). F_{IS} values were calculated using FSTAT (Goudet 1995).

Site Code	Locus	H _o	H _e	r*	Total number of alleles	Number of null alleles [#]
1	Pd μ 5	0.69565	0.9343	0.146416	46	7
	Hr μ 7	0.64706	0.95187	0.190634	34	6
	Mcy μ 4	0.65217	0.93816	0.179831	46	8
2	Pd μ 5	0.68182	0.92178	0.149638	44	7
	Mcy μ 4	0.69565	0.92947	0.143879	46	7
3	Pd μ 5	0.71698	0.92147	0.124807	106	13
	Hr μ 7	0.7551	0.97433	0.126764	98	12
	Mcy μ 4	0.80392	0.92137	0.068076	102	7
4	Hr μ 7	0.88889	0.96667	0.041917	36	2
	Mcy μ 4	0.83333	0.94286	0.061666	36	2
C	Pd μ 5	0.68421	0.94737	0.161292	38	6
	Mcy μ 4	0.7	0.94872	0.150856	40	6
D	Hr μ 7	0.625	0.89744	0.089496	40	4
E	Pd μ 5	0.75	0.89744	0.089496	40	4

$$*r = (H_e - H_o) / (H_e + H_o)$$

Table A.4. The observed and expected heterozygosities (H_o and H_e respectively) and calculations of null allele frequencies (of loci determined to have null alleles) for seven Henslow's sparrow breeding sites (sites 1, 2, 3, 4, C, D, and E in Fig. 1.1). The heterozygosities were calculated with Arlequin (Schneider, Roessli & Excoffier 2000). Only loci where H_o significantly differed from H_e ($P < 0.05$) were considered to have null alleles.

#Number of null alleles = r(total number of alleles)Site	Missouri	Ohio	North Carolina	New York
Sample size	15	20	23	14
Proportion of songs misclassified (alien songs)	0.07	0.48	0.09	0.43
Proportion of alien songs due to breeding dispersal (range)	0.03 (0.02 – 0.04)	0.23 (0.13 – 0.28)	0.04 (0.02 – 0.05)	0.21 (0.12 – 0.25)

Table A.5. Estimated proportion of breeding dispersers in four sampling sites across the breeding range of the Henslow's sparrow, based on song structure, but without the third song component.

		Ohio	North Carolina	New York
Missouri	Dpμ16	-0.0031	0.0110	-0.0166
	Pdoμ5	-0.0066	0.0138*	0.0217
	Maμ23	0.0369	0.0108	0.0263
	Hrμ7	0.0112	-0.0047	0.0114
	Mcyμ4	-0.0055	0.0036	0.0110
Ohio	Dpμ16		0.0034	0.0010
	Pdoμ5		0.0021	0.0049
	Maμ23		0.0301	0.1188*
	Hrμ7		0.0051	-0.0071
	Mcyμ4		-0.0018	0.0032
North Carolina	Dpμ16			0.0082
	Pdoμ5			0.0129
	Maμ23			0.0633*
	Hrμ7			0.0039
	Mcyμ4			-0.0060

*Significant after Benjamini-Hochberg (1995) correction assuming a false discovery rate of 5%

Table A.6. Pairwise F_{st} estimates calculated with the correction for null alleles. All estimates made using GENEPOP (Raymond & Rousset 1995).

BIBLIOGRAPHY

- Acosta, C.A. (2002) Spatially explicit dispersal dynamics and equilibrium population sizes in marine harvest refuges. *ICES Journal of Marine Science*, **59**, 458-468.
- Alderson, G.W., Gibbs, H.L. & Sealy, S.G. (1999) Parentage and kinship studies in an obligate brood parasitic bird, the brown-headed cowbird (*Molothrus ater*), using microsatellite DNA markers. *Journal of Heredity*, **90**, 182-190.
- Alisauskas, R.T. & Hobson, K.A. (1993) Determination of lesser snow goose diets and winter distribution using stable isotope analysis. *Journal of Wildlife Management*, **57**, 49-54.
- Alisauskas, R.T., Klass, E.E., Hobson, K.A. & Ankney, C.D. (1998) Stable carbon isotopes support use of adventitious color to discern winter origins of lesser snow geese. *Journal of Field Ornithology*, **69**, 262-268.
- Allendorf, F.W. & Leary, R.F. (1986) Heterozygosity and fitness in natural populations of animals. *Conservation biology: the science of scarcity and diversity* (ed M.E. Soulé), pp 57-76. Sinauer Associates, Sunderland, Massachusetts.
- Alomar, O., Goula, M., Albajes, R. (2002) Colonisation of tomato fields by predatory mired bugs (Hemiptera: Heteroptera) in northern Spain. *Agriculture, Ecosystems and Environment*, **89**, 105-115.
- Amin, R., Bramer, M.A. & Emslie, R. (2003) Intelligent data analysis for conservation: experiments with rhino horn fingerprint identification. *Applications and Innovations in Intelligent Systems X* (eds A. Macintosh, R. Ellis & R. Coenen), pp. 207-222. Springer-Verlag, New York.
- Anderson, J.L. (1998) Embracing uncertainty: The interface of Bayesian statistics and cognitive psychology. *Conservation Ecology* [online], **2**, 2.
<http://www.consecol.org/vol2/iss1/art2>.
- Argos website (2001) http://www.argosinc.com/system_overview.htm.
- Avise, J.C. (1994) *Molecular markers, natural history, and evolution*. Chapman and Hall, New York.
- Ball, R.M., Freeman, S., James, F.C., Bermingham, E., & Avise, J.C. (1988) Phylogeographic population structure of Red-winged Blackbirds assessed by mitochondrial DNA. *Proceedings of the National Academy of Sciences USA* **85**, 1558-1562.

- Baptista, L.F. (1977) Geographic variation in song and dialects of the Puget Sound white crowned sparrow. *Canadian Journal of Zoology*, **63**, 1741-1752.
- Barton, N.H. & Slatkin, M. (1986) A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity*, **56**, 409-415.
- Barton, N.H. (2001) The evolutionary consequences of gene flow and local adaptation: future approaches. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt, & J.D. Nichols), pp. 329-340. Oxford University Press, Oxford.
- Beaudoin, C.P., Tonn, W.M., Prepas, E.E. & Wassenaar, L.I. (1999) Individual specialization and trophic adaptability of northern pike (*Esox lucius*): an isotope and dietary analysis. *Oecologia*, **120**, 386-396.
- Benjamini, Y. & Hochberg, Y. (1995) Controlling the false discovery rate – a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B – Statistical Methodology* **57**, 289-300.
- Benjamini, Y. & Yekutieli, D. (2001) The control of the false discovery rate in multiple testing under dependency. *The Annals of Statistics*, **29**, 1165-1188.
- Bensch, S., Price, T. & Kohn, J. (1997) Isolation and characterization of microsatellite loci in a *Phylloscopus* warbler. *Molecular Ecology*, **6**, 91-92.
- Blouin-Demers, G. and Weatherhead, P.J. (2002) Implications of movement patterns for gene flow in black rat snakes (*Elaphe obsoleta*). *Canadian Journal of Zoology* **80**, 1162-1172.
- Borrer, D.J. (1954) Analytical studies of Henslow's sparrow songs. *The Wilson Bulletin*, **66**, 243-252.
- Boulinier, T., McCoy, K.D. & Sorci, G. (2001) Dispersal and parasitism. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt & J.D. Nichols), pp. 169-179. Oxford University Press, Oxford.
- Brown, B.L., Gunter, T.P., Waters, J.M., & Epifanio, J.M. (2000) Evaluating genetic diversity associated with propagation-assisted restoration of American shad. *Conservation Biology* **14**, 294-303.
- Callen, D.F., Thompson, A.D., Shen, Y., Phillips, H.A., Richards, R.I., Mulley, J.C. & Sutherland, G.R. (1993) Incidence and origin of 'null' alleles in the (AC)_n microsatellite markers. *American Journal of Human Genetics*, **52**, 922-927.
- Chakraborty, R., Zhong, Y., Jin, L. & Budowle, B. (1994) Nondetectability of restriction fragments and independence of DNA fragment sizes within and between loci in RFLP typing of DNA. *American Journal of Human Genetics*, **55**, 391-401.
- Chamberlain, C.P., Blum, J.D., Holmes, R.T., Fen, X., Sherry, T.W. & Graves, G.R. (1997) The use of isotope tracers for identifying populations of migratory birds. *Oecologia*, **109**, 132-141.
- Chuang, H.C., Webster, M.S., and Holmes, R.T. (1999) Extrapaternality and local synchrony in the black-throated blue warbler. *Auk* **116**, 726-736.
- Clegg, S.M., Kelly, J.F., Kimura, M., & Smith, T.B. (2003) Combining genetic markers and stable isotopes to reveal population connectivity and migration patterns in a Neotropical migrant, Wilson's warbler (*Wilsonia pusilla*). *Molecular Ecology* **12**, 819-830.
- Dawkins, R. (1976) *The Selfish Gene*. Oxford University Press, New York.

- Dawson, R.J.G., Gibbs, H.L., Hobson, K.A. & Yezerinac, S.M. (1997) Isolation of microsatellite DNA markers from a passerine bird, *Dendroica perechia* (the yellow warbler), and their use in populations studies. *Heredity*, **79**, 506-514.
- Double, M.C., Dawson, D., Burke, T. & Cockburn, A. (1997) Finding the fathers in the least faithful bird: A microsatellite-based genotyping system for the superb fairy-wren *Malurus cyaneus*. *Molecular Ecology* **6**: 691-693.
- Ebenhard, T. (1991) Colonization in metapopulations: a review of theory and observations. *Metapopulation Dynamics* (eds M. Gilpin & I. Hanski), pp. 105-121. Academic Press, London.
- Ehrlich, P.R. & Raven, P.H. (1969) Differentiation of Populations. *Science*, **165**, 1228-1232.
- Ehrlich, P.R., Dobkin, D.S., & Wheye, D. (1988). *The Birders Handbook*. Simon & Schuster, New York.
- Ellison, W.G. (2001) *Population structure and gene flow in two long distance migrant birds, the Bicknell's Thrush (Catharus bicknelli) and Veery (C. fuscescens)*. PhD thesis, University at Albany, State University of New York, Albany, U.S.A.
- Ellison, A.M. (1996) An introduction to Bayesian inference for ecological research and environmental decision-making. *Ecological Applications*, **6**, 1036-1046.
- Engineering Design (2001) Signal for DOS. Engineering Design, Belmont, MA.
- Forero, M.G. & Hobson, K.A. (2003) Using stable isotopes of nitrogen and carbon to study seabird ecology: Applications in the Mediterranean seabird community. *Scientia Marina*, **67**, 23-32.
- Gibbs, H.L., Tabak, L.M. & Hobson, K.A. (1999) Characterization of microsatellite DNA loci for a neotropical migrant songbird, the Swainson's thrush (*Catharus ustulatus*). *Molecular Ecology* **8**, 1551-1552.
- Gibbs, H.L., Dawson, R.J.G. & Hobson, K.A. (2000) Limited differentiation in microsatellite DNA variation among northern populations of the yellow warbler: evidence for male-biased gene flow? *Molecular Ecology*, **9**, 2137-2147.
- Gill, J.A., Norris, K., Potts, P.M., Gunnarsson, T.G., Atkinson P.W. & Sutherland W.J. (2001) The buffer effect and large-scale population regulation in migratory birds. *Nature* **412**, 436-438.
- Goetz, J.E. (2001) Parentage and parental care in Bicknell's Thrush, *Catharus bicknelli*. M.S. thesis, State University of New York, Syracuse.
- Goudet, J. (1995) FSTAT, version 1.2: a computer program to calculate F-statistics. *Journal of Heredity*, **86**, 485-486.
- Graves, G.R., Romanek, C.S. & Navarro, A.R. (2002) Stable isotope signature of philopatry and dispersal in a migratory songbird. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 8097-8100.
- Griffith, S.C., Steward, I.R.K., Dawson, D.A., Owens, I.P.F. & Burke, T. (1999) Contrasting levels of extra-pair paternity in mainland and island populations of the house sparrow (*Passer domesticus*); Is there an 'island effect'? *Biological Journal of the Linnean Society* **68**, 303-316.
- Gustafson, M.E. & Hildebrand, J. (1999) Bird Banding Laboratory Homepage, ver 12/02/99. [Online]. Available: <http://www.pwrc.usgs.gov/bbl/> [Access date: 10/22/03]

- Hanski, I. (1989) Metapopulation dynamics – does it help to have more of the same? *Trends in Ecology & Evolution* **4**, 113-114.
- Hanski, I. & Woiwod, P. (1993) Spatial synchrony in the dynamics of moth and aphid populations. *Journal of Animal Ecology* **62**, 656-668.
- Hedrick, P.W. (1994) Purging inbreeding depression and the probability of extinction: full-sib mating. *Heredity* **73**, 363-372.
- Herkert, J.R. (1991) Prairie birds in Illinois: populations response to two centuries of habitat change. *Illinois Natural History Survey Bulletin*, **34**, 393-399.
- Herkert, J.R. (1994) Status and habitat selection of the Henslow's Sparrow in Illinois. *Wilson Bulletin*, **106**, 35-45.
- Herkert, J.R., Vickery, P.D. & Kroodsma, D.E. (2002) Henslow's sparrow (*Ammodramus henslowii*). *The Birds of North America* (eds A. Poole & F. Gill), 672. The Birds of North America, Inc. Philadelphia.
- Hobson, K.A., & Clark, R.G. (1992) Assessing avian diets using stable isotopes. II. Factors influencing diet-tissue fractionation. *Condor*, **94**, 181-188.
- Hobson, K.A. & Wassenaar, L.I. (1997) Linking breeding and wintering grounds of neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. *Oecologia*, **109**, 142-148.
- Hobson, K.A., McFarland, K.P., Wassenaar, L.I., Rimmer, C.C. & Goetz, J.E. (2001) Linking breeding and wintering grounds of Bicknell's Thrushes using stable isotope analyses of feathers. *Auk*, **118**, 16-23.
- Hobson, K.A. (1999) Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia*, **120**, 314-326.
- Hoefs, J. (1980) *Stable isotope geochemistry*. Springer-Verlag, Berlin.
- Holmes, R.T. (1994) Black-throated Blue Warbler (*Dendroica caerulescens*). *Birds of North America* (eds A. Poole & F. Gill), **87**. The Birds of North America, Inc. Philadelphia, PA.
- Immelmann, K. (1969) Song development in the zebra finch and other estrildid finches. *Bird Vocalizations: Their Relation to Current Problems in Biology and Psychology* (ed R.A. Hinde), pp. 61-74. Cambridge University Press, London.
- Kelly, J.F. & Finch, D.M. (1998) Tracking migrant songbirds with stable isotopes. *Trends in Ecology and Evolution*, **13**, 48-49.
- Kelly, J.F., Atudorei, V., Sharp, Z.D. & Finch, D.M. (2001) Insights into Wilson's warbler migration from analyses of hydrogen stable-isotope ratios. *Oecologia*, **130**, 216-221.
- Koenig, W.D. (1998) Spatial Autocorrelation in California Land Birds. *Conservation Biology* **12**, 612-620.
- Koenig, W.D. (1999) Spatial Autocorrelation of ecological phenomena. *Trends in Ecology and Evolution* **14**, 22-26.
- Koenig, W.D. (2001) Spatial autocorrelation and local disappearances in wintering North American birds. *Ecology* **82**, 3519-3530.
- Koenig, W. D., Van Vuren, D. & Hooge, P. N. (1996) Detectability, philopatry, and the distribution of dispersal distances in vertebrates. *Trends in Ecology and Evolution*, **11**, 514-517
- Körner, C.H., Farquhar, G.D. & Wong, S.C. (1991) Carbon isotope discrimination by plants follows latitudinal and altitudinal trends. *Oecologia*, **88**, 30-40.

- Kroodsma, D.E. (1978) Aspects of learning in the ontogeny of birds song: where, from whom, when, how many, which, and how accurately? *Comparative and Evolutionary Aspects of Behavioral Development* (eds G. Burghardt & M. Bekoff), pp. 215-230. Garland Press, New York.
- Kroodsma, D.E. (1996) Ecology of passerine song development. *Ecology and Evolution of Acoustic Communication in Birds* (eds D.E. Kroodsma & E.H. Miller), pp. 3-19. Cornell University Press, Ithaca, NY.
- Kroodsma, D.E. Pickert, R. (1984) Sensitive phases for song learning: effects of social interaction and individual variation. *Animal Behaviour*, **32**, 389-394.
- Lambin, X., Aars, J., and Pieltney, S.B. (2001) Dispersal, intraspecific competition, kin competition and kin facilitation: a review of the empirical evidence. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt, & J.D. Nichols), pp. 110-122. Oxford University Press, Oxford.
- Lemon, R.E. (1975) How birds develop song dialects. *Condor*, **77**, 385-406.
- Lott, C.A., Meehan, T.D. & Heath J.A. (2003) Estimating the latitudinal origins of migratory birds using hydrogen and sulfur stable isotopes in feathers: influence of marine prey base. *Oecologia*, **134**, 505-510.
- Lundberg, P., Ranta E., Ripa, J. & Kaitala, V. (2000) Population variability in space and time. *Trends in Ecology and Evolution* **15**, 460-464.
- Lynch, A. (1996) The population memetics of birdsong. *Ecology and Evolution of Acoustic Communication in Birds* (eds D.E. Kroodsma & E.H. Miller), pp. 181-197. Cornell University Press, Ithaca, NY.
- Lynch, A. (1994) A population memetics approach to cultural evolution in Chaffinch song: differentiation among populations. *Evolution*, **48**, 351-359.
- Macdonald, D.W. and Johnson, D.D.P. (2001) Dispersal in theory and practice: consequences for conservation biology. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt, & J.D. Nichols), pp. 358-372. Oxford University Press, Oxford.
- Marler, P. & Tamura, M. (1962) Song 'dialects' in three populations of white-crowned sparrows. *Condor*, **64**, 368-377.
- Marler, P. & Peters, S. (1981) Sparrows learn adult song and more from memory. *Science*, **213**, 780-782.
- Marra, P.P., Hobson, K.A. & Holmes, R.T. (1998) Linking winter and summer events in a migratory bird by using stable-isotopes. *Science*, **292**, 1884-1886.
- Martinez, J.G., Soler, J.J., Soler, M., Moller, A.P. & Burke, T. (1999) Comparative population structure and gene flow of a brood parasite, the great spotted cuckoo (*Clamator glandarius*), and its primary host, the magpie (*Pica pica*). *Evolution* **53**, 269-278.
- McCutchan, J.H., Jr., Lewis, W.M., Jr., Kendall, C. & McGrath, C.C. (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, **102**, 378-390.
- McDonald, D.B. & Potts, W.K. (1994) Cooperative display and relatedness among males in a lek-mating bird. *Science*, **266**, 1030-1032.
- Meehan, T.D., Lott, C.A., Sharp, Z.D., Smith, R.B., Rosenfield, R.N, Stewart, A.C. & Murphy, R.K. (2001) Using hydrogen isotope geochemistry to estimate the natal latitudes of immature Cooper's hawks migrating through the Florida Keys. *Condor*, **103**, 11-20.

- Mennechez, G., Schtickzelle, N., and Baguette, M. (2003) Metapopulation dynamics of the bog fritillary butterfly: Comparison of demographic parameters and dispersal between a continuous and a highly fragmented landscape. *Landscape Ecology* **18**, 279, 291.
- Mills, L.S. & Allendorf, F.W. (1996) The one-migrant-per-generation rule in conservation and management. *Conservation Biology*, **10**, 1509-158.
- Montgomery, D.C. (1991) *Design and Analysis of Experiments*, p. 98 John Wiley & Sons, New York.
- Moritz, C. (1994) Defining 'evolutionarily significant units' for conservation. *Trends in Ecology and Evolution*, **9**, 373-375.
- Mundinger, P.C. (1982) Microgeographic and macrogeographic variation in acquired vocalizations of birds. *Acoustic Communication in Birds Vol. 2* (eds D.E. Kroodsmma & E.H. Miller), pp. 147-208. Academic Press, New York.
- Nathan, R. (2001) The challenges of studying dispersal. *Trends in Ecology and Evolution*, **16**, 481-483.
- Nelson, D.A. (1996) Social interaction and sensitive phases for song learning: a critical review. *Social Influences on Vocal Development* (eds C.T. Snowdon & M. Hausberger), pp. 7-21. Cambridge University Press, Cambridge, MA.
- Nelson, D.A. (1998) Geographic variation in song of Gambel's white-crowned sparrow. *Behaviour*, **135**, 321-342.
- Nelson, D.A. & Marler, P. (1994) Selection-based learning in bird song development. *Proceedings of the National Academy of Sciences, U.S.A.*, **91**, 10498-10501.
- Nelson, D.A., Marler, P. & Morton, M.L. (1996) Overproduction in song development: an evolutionary correlate with migration. *Animal Behaviour*, **51**, 1127-1140.
- Newman, R.A. & Squire, T. (2001) Microsatellite variation and fine-scale population structure in the wood frog (*Rana sylvatica*). *Molecular Ecology*, **10**, 1087-1100.
- Nordby, J.C., Campbell, S.E. & Beecher, M.D. (2001) Late song learning in song sparrows. *Animal Behaviour*, **61**, 835-846.
- Nordby, J.C., Campbell, S.E. & Beecher, M.D. (2002) Adult song sparrows do not alter their song repertoires. *Ethology*, **108**, 39-50.
- North American Breeding Bird Survey website (2003)USGS-<http://www.mp2-pwrc.usgs.gov/bbs/about/index.html>.
- Nott, M.P., Desante, D.F., Siegel, R.B., & Pyle, P. (2002) Influences of the El Niño/Southern Oscillation and the North Atlantic Oscillation on avian productivity in forests of the Pacific Northwest of North America. *Global Ecology & Biogeography* **11**, 333-342.
- Nottebohm, F. & Nottebohm, M.E. (1978) Relationship between song repertoire and age in the canary, *Serinus canarius*. *Zeitschrift fur Tierpsychologie*, **46**, 298-305.
- Ouellet, H.R. (1993) Bicknell's Thrush: Taxonomic status and distribution. *Wilson Bulletin* **105**, 545-572.
- O'Riain, M.J. and Braude, S. (2001) Inbreeding versus outbreeding in captive and wild populations of naked mole-rats. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt, & J.D. Nichols), pp. 143-154. Oxford University Press, Oxford.
- Parker, P.G., Snow, A.A., Schug, M.D., Booton, G.C. & Fuerst, P.A. (1998) What molecules can tell us about populations: choosing and using a molecular marker. *Ecology*, **79**, 361-382.

- Pemberton, J.M., Slate, J., Bancroft, D.R. & Barrett, J.A. (1995) Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology*, **4**, 249-252.
- Perrin, N. and Goudet, J. (2001) Inbreeding, kinship, and the evolution of natal dispersal. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt, & J.D. Nichols), pp. 123-142. Oxford University Press, Oxford.
- Petren, K. (1998) Microsatellite primers from *Geospiza fortis* and cross-species amplification in Darwin's finches. *Molecular Ecology*, **7**, 1782-1784.
- Pielou E.C. (1991) *After the Ice Age: the Return of Life to Glaciated North America*. University of Chicago Press, Chicago, Illinois.
- Plentovich, S., Holler, N.R. & Hill, G.E. (1999) Habitat requirements for Henslow's sparrows wintering in silvicultural lands of the Gulf Coastal Plain. *Auk*, **116**, 109-115.
- Poage, M.A. and Chamberlain, C.P. (2001) Empirical relationships between elevation and stable isotope composition of precipitation and surface waters: Considerations for studies of paleoelevation change. *American Journal of Science* **301**, 1-15.
- Primmer, C.R., Moller, A.P. & Ellegren, H. (1996) A wide-range survey of cross-species microsatellite markers: a parentage testing system for the swallow *Hirundo rustica*. *Molecular Ecology*, **4**, 493-498.
- Pritchard, J.K., Stephens, M. & Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945-959.
- Piertney, S.B., Marquiss, M. & Summers, R. (1998) Characterization of tetranucleotide microsatellite markers in the Scottish crossbill (*Loxia scotica*). *Molecular Ecology*, **7**, 1248-1251.
- Pruitt, L. (1996) Henslow's Sparrow Status Assessment. U.S. Fish and Wildlife Service, Bloomington, IN.
- Raymond, M. & Rousset, F. (1995) GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248-249.
- Ranta, E., Kaitala, V., Lindström, J., & Lindén, H. (1995) Synchrony in population dynamics. *Proceedings of the Royal Society of London B* **262**, 113-118.
- Ranta, E., Kaitala, V., & Lundberg, P. (1997) The spatial dimension in population fluctuations. *Science* **278**, 1621-1623.
- Ripa, J. (2000) Analysing the Moran effect and dispersal: their significance and interaction in synchronous population dynamics. *Oikos* **89**, 175-187.
- Robins, J.D. (1971) A study of Henslow's Sparrow in Michigan. *Wilson Bulletin* **83**:39-48.
- Rousset, F. (2001) Genetic approaches to the estimation of dispersal rates. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt, & J.D. Nichols), pp. 18-28. Oxford University Press, Oxford.
- Rubenstein, D.R., Chamberlain, C.P., Holmes, R.T., Ayres, M.P., Waldbauer, J.R., Graves, G.R. & Tuross, N.C. (2002) Linking breeding and wintering ranges of a migratory songbirds using stable isotopes. *Science*, **295**, 1061-1065.
- Sample, D.W. (1989) *Grassland birds in southern Wisconsin: habitat preference, population trends, and response to land use changes*. MS thesis, University of Wisconsin, Madison, U.S.A.

- Sauer, J.R., Hines, J.E., & Fallon, J. (2003) The North American Breeding Bird Survey, Results and Analysis 1966-2002. Version 2003.1, USGS Patuxent Wildlife Research Center, Laurel, MD
- Sauer, J.R., Hines, J.E. & Fallon, J. (2001) The North American Breeding Bird Survey, results and analysis 1966-2000. Version 2001.2, USGS Patuxent Wildlife Research Center, Laurel, MD. <http://www.mbr-pwrc.usgs.gov/bbs/bbs.html>
- Schaffner, F.C. & Swart, P.K. (1991) Influence of diet and environmental water on the carbon and oxygen isotopic signatures of seabird eggshell carbonate. *Bulletin of Marine Science*, 48, 23-38.
- Schneider, S., Roessli, D. & Excoffier, L. (2000) Arlequin ver. 2000: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
- Searcy, W.A. and Yasukawa, K. (1995) Monographs in Behavior and Ecology: Polygyny and sexual selection in red-winged blackbirds. (eds J. Krebs & T.H. Clutton-Brock) Princeton University Press, Princeton, New Jersey.
- Slatkin, M. (1981) Estimating levels of gene flow in natural populations. *Genetics*, **99**, 323-335.
- Slatkin, M. (1985a) Gene flow in natural populations. *Annual Review of Ecology and Systematics*, **16**, 393-430.
- Slatkin, M. (1985b) Rare alleles as indicators of gene flow. *Evolution*, **39**, 53-65.
- Slatkin, M. (1987) Gene flow and the geographic structure of natural populations. *Science* **236**, 787-792.
- SPSS for Windows (2001) Rel. 11.0.1. Chicago: SPSS Inc.
- Swanson, B.J. & Johnson, D.R. (1999). Distinguishing causes of intraspecific synchrony in population dynamics. *Oikos* **86**, 265-274.
- Swengel, S.C. (1996) Management responses of three species of declining sparrows in tallgrass prairie. *Bird Conservation International*, **6**, 241-253.
- Szepanski, M.M., Ben-David, M. & Van Ballenberghe, V. (1999) Assessment of anadromous salmon resources in the diet of the Alexander Archipelago wolf using stable isotope analysis. *Oecologia*, **120**, 327-335.
- Templeton, A.R. (1986) Coadaptation and outbreeding depression. *Conservation biology: the science of scarcity and diversity* (ed M.E. Soulé), pp 105-116. Sinauer Associates, Sunderland, Massachusetts.
- Thompson, P.M., & Goodman, S. (1997) Direct and indirect estimates of dispersal distances. *Trends in Ecology and Evolution*, **12**, 195-196.
- Tucker, J.W. & Robinson, W.D. (2003) Influence of season and frequency of fire on Henslow's Sparrows (*Ammodramus henslowii*) wintering on Gulf Coast pitcher plant bogs. *Auk*, **120**, 96-106.
- USGS Patuxent Wildlife Research Center (2001) North American Breeding Bird Survey ftp data set, version 2001.0. <ftp://pwrcftp.er.usgs.gov/mp/bbs/datafiles/>.
- USGS Patuxent Wildlife Research Center (2003a) North American Breeding Bird Survey About BBS, 1, December 2003. (<http://www.mp2-pwrc.usgs.gov/bbs/about/index.html>).
- USGS Patuxent Wildlife Research Center (2003b) North American Breeding Bird Survey Internet data set, 26, November 2003. (<http://www.mp2-pwrc.usgs.gov/bbs/retrieval/>).

- Van der Merwe, N.J., Lee-Thorpe, J.A., Thackeray, J.F., Hall-Margin, A., Kruger, F.J., Coetzee, H., Bell, R.H.V. & Lindeque, M. (1990) Source-area determination of elephant ivory by isotopic analysis. *Nature*, **346**, 744-746.
- Von Hünerbein, K., Hamann, H.J., Rüter, E. & Wiltshko, W. (2000) A GPS-based system for recording the flight paths of birds. *Naturwissenschaften* **87**, 278-279.
- Vucetich, J.A. & Waite, T.A. (2000) Is one migrant per generation sufficient for the genetic management of fluctuating populations? *Animal Conservation*, **3**, 261-266.
- Warwick, S.I., Beckie, H.J. & Small, E. (1999) Transgenic crops: new weed problems for Canada? *Phytoprotection*, **80**, 71-84.
- Wassenaar, L.I. & Hobson, K.A. (2000a) Evaluating the use of stable-carbon ($\delta^{13}\text{C}$) and H (δD) isotope ratios in feathers to infer breeding origins of Red-winged Blackbirds. *Ecological Applications*, **10**, 911-916.
- Wassenaar, L.I. & Hobson, K.A. (2000b) Improved method for determining the stable-hydrogen isotopic composition (δD) of complex organic materials of environmental interest. *Environmental Science and Technology* **34**, 2354-2360.
- Wassenaar, L.I. & Hobson, K.A. (2001) A stable-isotope approach to delineate geographical catchment areas of avian migration monitoring stations in North America. *Environmental Science and Technology* **35**, 1845-1850.
- Wassenaar, L.I. & Hobson, K.A. (2003) Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for the use in animal migration studies. *Isotopes in Environmental and Health Studies* **39**, 211-217.
- Webster, M.S., Marra, P.P., Haig, S.M, Bensch, S. & Holmes, R.T. (2002) Links between worlds: unraveling migratory connectivity. *Trends in Ecology and Evolution* **17**, 76-83.
- Weisser, W.W. (2001) The effects of predation on dispersal. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt & J.D. Nichols), pp. 180-188. Oxford University Press, Oxford.
- Whitlock, M.C. (1992) Temporal fluctuations in demographic parameters and the genetic variance among populations. *Evolution*, **46**, 608-615.
- Whitlock, M.C. (2001) Dispersal and the genetic properties of metapopulations. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt, & J.D. Nichols), pp. 273-282. Oxford University Press, Oxford.
- Whitlock, M.C & McCauley, D.E. (1998) Indirect measures of gene flow and migration: $F_{st} \neq 1/(4Nm+1)$. *Heredity* **82**, 117-125.
- Wright, S. (1931) Evolution in Mendelian populations. *Genetics* **16**, 97-159.