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Phylogeography of a vanishing North American songbird: The painted bunting (Passerina ciris)

Connie Ann Herr
University of Nevada, Las Vegas

PHYLOGEOGRAPHY OF A VANISHING NORTH AMERICAN SONGBIRD:

THE PAINTED BUNTING (*PASSERINA CIRIS*)

by

Connie Ann Herr

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University of Nevada, Las Vegas
1988

Master of Science
University of Nevada, Las Vegas
1991

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Connie Ann Herr

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Department of Life Sciences

Brett Riddle, Committee Chair

Daniel Thompson, Committee Member

Lloyd Stark, Committee Member

Mark Buttner, Graduate College Representative

Ronald Smith, Ph. D., Vice President for Research and Graduate Studies
and Dean of the Graduate College

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ABSTRACT

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by

Connie Ann Herr

Dr. Brett Riddle, Examination Committee Chair
Professor of Biological Sciences
University of Nevada, Las Vegas

Studies of genetic variation within and between species can provide insights into their evolutionary history as well as important information for conserving biodiversity. An understanding of population processes can assist in the conservation of biodiversity by contrasting current versus historical patterns, and the processes that have generated these patterns. Genetic differentiation often coincides with significant geological or climatic changes that have shaped the sizes and locations of the species geographic range and altered the connectivity between populations over time. Phylogenetic and population genetic analyses can also provide a statistical framework for the investigation of how human processes such as habitat loss, population connectivity, overexploitation, and species introductions can affect biodiversity.

Here, I employ a suite of phylogenetic and population genetic analyses to address several questions regarding the phylogenetic relationships of the Nearctic – Neotropical migratory songbird: the Painted Bunting (*Passerina ciris*). The Painted Bunting breeds in the southeast and south central United States and winters in the Florida Keys, the Caribbean, Mexico and portions of Central America. The Atlantic Coast population of the Painted Bunting is a bird of considerable conservation concern. The biotic history of

this part of North America has been examined using a wide variety of vertebrates. Many species have had their geographic ranges shift repeatedly during Pleistocene glaciations and many geographic features have been suggested as possible barriers to gene flow.

I begin by reconstructing the phylogeny of the genus *Passerina* and three members of the closely related genus *Cyanocompsa* to address issues concerning the evolution of migration within the *Passerina* clade and the role, geographical source, and timing of range expansions within the Painted Bunting. Data presented herein support the hypotheses that the Painted Bunting split from its sister, the Varied Bunting approximately 1.5 – 2.1 million years ago during the Pleistocene and that the evolution of migration within the bunting phylogeny evolved independently two times. Additionally, the Painted Bunting is embedded within an otherwise sedentary clade of Mexican birds indicating that the Painted Buntings ancestor is of Mexican origin. Genetic analyses of populations within the breeding grounds indicate that the allopatric Painted Bunting populations diverged approximately 26,000 – 115,000 years ago and represent incipient species and as such the Atlantic Coast and interior populations should be recognized as separate management units. Hypotheses concerning the patterns of connection between the breeding and overwintering ranges also suggest a general separation between the Atlantic Coast breeding and Caribbean wintering areas from the interior breeding and Mexican/Central American wintering grounds.

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CHAPTER 1

HISTORICAL BIOGEOGRAPHY OF THE GENUS *PASSERINA*: IMPLICATIONS FOR THE EVOLUTION OF THE PAINTED BUNTING (*PASSERINA CIRIS*)

Introduction

Phylogeography is a discipline that focuses on the relationships between geography and gene genealogies, typically at the species level and below, incorporating a phylogenetic and population genetic perspective into biogeography (Avise et al. 1987). It also incorporates the effects of historical processes on population-level geographical patterns (Avise 2000; Avise 2009). Recent advances in population genetics theory, including coalescence (Tavare 1984; Hudson 1990),along with molecular techniques allow thorough investigations of the roles of gene flow, colonization, and population fragmentation in influencing the evolutionary history of a species (Hey 2005; Peters et al. 2005; Klicka et al. 2007). The focus of my dissertation project concerns the phylogeography of the Painted Bunting (*Passerina ciris*), a short to medium distance Neotropical migrant within the genus *Passerina*. By comparing distributions of genetic lineages across populations of the Painted Bunting (*Passerina ciris*), I have tested hypotheses about the biogeographic factors that may have shaped the current distribution and pattern of divergence of populations of a Neotropical migrant bird (Avise 2000; Carstens et al. 2005; Spellman and Klicka 2006). The phylogenies I construct have been used to determine whether vicariance or dispersal of Painted Bunting explains present day disjunct breeding and non-breeding distributions, and whether the ancestral range of

this migratory species encompasses its present–day breeding or non-breeding geographic area (Joseph et al. 1999; Joseph et al. 2003; Outlaw et al. 2003).

Increasingly, molecular tools are also being used to address issues such as phylogeny, historical biogeography, and population genetics of migratory species in order to address issues such as the evolution of migratory routes and the role, geographical source, and timing of recent range expansions of migrants (Joseph 2005). These tools have provided a greater understanding into the evolution of migration. In my research, I assess the general hypotheses that migratory birds evolve from ancestors in their present-day non breeding range through shifts or displacements of the breeding range, and that the seasonal subtropics have been a staging area for the evolution of migration of Painted Buntings in the Americas (Keast and Morton 1980; Cox 1985). Molecular data sets can be applied hierarchically, from the study of entire migration systems, to the evolution of migration within clades of species, and ultimately down to processes acting within a single migratory species (Joseph 2005).

Prior to engaging in a detailed discussion about the phylogenetic relationships within the Painted Bunting, I find it important to assess the relatedness of the Painted Bunting with other members of the genus. In chapter one I provide a brief overview of the members of the genus *Passerina* and its closest relative and reconstruct a phylogeny of the genus *Passerina* to establish hypotheses of an approximate time frame and location in which the Painted Bunting diverged from its sister taxon, the Varied Bunting. Additionally, I discuss migratory behavior within the genus *Passerina* with respect to the general observation that migratory behavior does not seem to be evolutionarily constrained because both residency and long distance migration have evolved repeatedly

(Helbig 2003). Evidence has also shown that a great diversity of migratory strategies, both among and within species, also exists (Sutherland 1998; Telleria et al. 2001; Perez-Tris et al. 2004).

The information gathered from this chapter will better inform hypotheses posed in the second and third chapters which specifically outline the genetic diversity within the Painted Bunting throughout its distribution and will also permit better inferences as to potential scenarios into the evolutionary history of the Painted Bunting. In chapters two and three, I use the tools of molecular population genetics to examine the finer details of population genetic structure within the Painted Bunting. Specifically, chapter two looks at the degree of genetic diversity within the allopatric Atlantic Coast and interior breeding populations and chapter three assesses the degree of connectivity between the breeding and wintering grounds of the Painted Bunting.

Overview of the genus *Passerina*

There have been quite a few studies that have addressed species relationships within the genus *Passerina*. The first fossil record of an extinct *Passerina* species was documented from Chihuahua, Mexico approximately 4 million years ago (Mya) (Steadman and McKitrick 1982). The fossil fragments identify an extinct *Passerina* species that was intermediate between the Lazuli Bunting (*P. amoena*) and the Blue Grosbeak (*P. caerulea*) in size, and from a region where they both currently occur. The genus *Passerina* (Aves: Cardinalidae) had traditionally been composed of six species of small (13-20 g), sexually dichromatic songbirds (Lazuli Bunting, Indigo Bunting, Painted Bunting, Varied Bunting, Orange Breasted Bunting and Rosita's Bunting). The collective breeding ranges of these species encompass most of Mexico, the United States

and southern Canada. Within the genus, the Indigo Bunting and Lazuli's Bunting had typically been considered sister species because they are morphologically similar and form a well-known hybrid zone where their breeding distributions overlap in the western Great Plains and eastern foothills of the Rocky Mountains in North America (Sibley and Short Jr. 1959; Emlen et al. 1975; Kroodsma 1975; Baker and Baker 1990; Baker and Boylan 1999). Additionally, some authors placed the monotypic Blue Grosbeak (*Guiraca caerulea*) within this genus (Phillips et al. 1964; Blake 1969; Mayr and Short 1970). Studies of museum skins (Ridgway, 1901) as well as a more recent study utilizing numerical phenetic analyses of both skins and skeletons (Hellack and Schnell 1977) concluded that the members of *Passerina*, as defined (Sibley and Monroe 1990, American Ornithologists' Union (AOU) 1998), form a natural group. Klicka et al. (2001) addressed the evolutionary relationships of the traditional six-member genus and closely related species, including the Blue Grosbeak and three *Cyanocompsa* species using 1143 base pairs of sequence data from the mitochondrial cytochrome *b* gene. The results of the Klicka et al. (2001) study showed strong support for the sister relationship between the Blue Grosbeak and Lazuli's Bunting, demonstrating that the Blue Grosbeak was derived from within the *Passerina* assemblage. These results led to a name change to *P. caerulea* (Banks et al. 2002). Subsequent work by Klicka et al. (2007) looked at members of the Cardinalini (cardinal-grosbeaks) tribe and found the reconfigured Cardinalini assemblage to be comprised of five well-supported major clades. The genus *Passerina* formed a monophyletic group within one of the five major clades. The relationships within the genus *Passerina* were nearly identical in both molecular phylogenies (Klicka et al. 2001; Klicka et al. 2007), differing only in the placement of the Indigo Bunting, which was not

supported in either study. In the 2001 study, approximate times of divergence were calculated using two independently derived clock calibrations (1.6 and 2% per million years). Results suggested that *Cyanocompsa* and *Passerina* buntings diverged from a common ancestor approximately 4.1 to 7.3 million years ago (Mya). This time span corresponds with a Late Miocene period of accelerated cooling and drying during which forests and woodlands gave way to a variety of grassland habitats in western North America (Riddle 1995). Faunal changes and shifts in carbon-isotope composition of herbivore-tooth enamel document this expansion of grasslands in North America during the mid to late Miocene (Webb 1984). Other well-resolved nodes within the phylogeny indicated that the Blue Grosbeak and Lazuli's Bunting diverged from each other approximately 2.4 to 3.7 Mya, and that the most recent split within the group took place between 1.5 to 2.1 Mya between the Painted Bunting and the Varied Bunting (Klicka et al. 2001). Additional comparative studies involving most or all of the members of *Passerina* have examined many life history characteristics, including studies on song (Thompson 1968), morphology (Hellack and Schnell 1977), ecological niches (Martinez-Meyer et al. 2004), and the evolution of plumage color (Stoddard and Prum 2008)

The *Passerina* clade is relatively well resolved (Klicka et al. 2001; Klicka et al. 2007), however, there have not been any studies dealing with migratory behavior (i.e. connectivity and pathways) of members of the genus *Passerina* or its sister clade. It has long been known that the migration route is a significant part of a species' range. Over one-half of the approximately 332 migratory bird species that breed in North America and winter in the tropics are affected by the obstacle to migratory flight imposed by open water across the Gulf of Mexico (Rappole and Ramos 1994). Migratory routes are

subject to continuous modification (i.e. evolution) through natural selection according to changes in a variety of pressures (Rappole et al. 1979; Richardson 1979). Members within the genus *Passerina* differ with respect to their propensity to migrate. Three species are Nearctic-Neotropical migrates, and the remaining members are considered sedentary including the closest relative, *Cyanocompsa*.

I reconstructed the bunting phylogeny of Klicka et al. (2001), adding three additional mitochondrial genes that yielded 4,169 base pairs of concatenated data. Coupling this phylogenetic hypothesis with divergence time estimates and an ancestral state construction of migratory behavior may help provide insights into the diversification of this group and a historical framework for a better understanding of various other life-history traits within the genus *Passerina*. An understanding of the historical biogeography of the regions in which the Painted Bunting occur is crucial in developing hypotheses regarding migratory pathways, ancestral areas, or phylogeographic history of this species.

Analyses of the genus *Passerina*

I examined the phylogenetic patterns in the evolution of migratory behavior and estimated divergence times of members within this clade. The Klicka et al. (2001) study examined the group with a single mitochondrial marker cytochrome-*b* (cyt-*b*, 1143 base pairs). I obtained sequences (Klicka, unpublished data) of the mitochondrial genes NADH subunits 2 (ND2, 1038 base pairs) and 6 (ND6, 519 base pairs), control region (cr, 1469 base pairs) and combined them with the original dataset. Bayesian inference (BI) analyses were performed with the combined 4169 base pair dataset. Bayesian inference of phylogeny constructs evolutionary relationships by using a maximum

likelihood framework, and posterior probability values are approximated using a Markov Chain Monte Carlo (MCMC) algorithm (Huelsenbeck and Ronquist 2001; Huelsenbeck and Imennow 2002). The Akaike information criterion (AIC; Akaike 1973) as implemented in MrModeltest v.2.3 (Nylander 2004) with default parameters and maximum likelihood optimizations, was used to choose the appropriate models of sequence evolution. I determined the sequence evolution model for the concatenated dataset as well as data partitioned by gene. I used the selected models in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) for BI, incorporating Bayesian posterior probabilities as evidence of nodal support. Nodes having posterior probability values of 95% or greater on these trees were deemed significantly supported (after Huelsenbeck and Ronquist 2001). MCMC analyses were run for 4×10^6 generations using the default parameters of four Markov chains per generation, with random starting trees and subsequent trees sampled every 1000 generations. Diagnostic tests were performed to evaluate mixing and convergence of MCMC chains. The burn-in was determined from visual inspection of the likelihood plots in the program Tracer v 1.4 (Drummond and Rambaut 2007). After excluding those trees generated during the "burn-in" period prior to stable equilibrium (10,000 trees), a 50% majority-rule consensus tree was generated.

I evaluated evolutionary patterns of migratory behavior via parsimony optimization of migratory character states onto the topology inferred from the concatenated dataset. Optimization was performed with Mesquite v. 2.73 (Maddison and Maddison 2010). Migratory behavior was coded as a binary character. Species were coded as sedentary or migratory according to the AOU, Checklist of the Birds of North America (American

Ornithologists' Union 1998) and Distribution and Taxonomy of Birds of the World (Sibley and Monroe Jr. 1990).

To obtain divergence estimates for the *Passerina* clade, I used a Bayesian approach as implemented in the phylogenetic program BEAST v.1.4.8 (Drummond and Rambaut 2007). I used the ND2 dataset in this analysis so that a more accurate comparison could be made between these estimates and the previously analyzed dataset (using ND2) of the allopatric breeding populations of the Painted Bunting (Herr et al., 2011).

Phylogenetic reconstructions: genus level

Ancestral states reconstruction of migratory behavior indicates that migration within the group evolved independently two times. The Painted Bunting (*P. ciris*) is embedded within an otherwise sedentary clade of Mexican birds (Fig. 1). Migratory behavior occurs in one other clade within the Bunting phylogeny. Members within this clade include the Indigo Bunting (*P. cyanea*), Lazuli Bunting (*P. amoena*), and the Blue Grosbeak (*P. caerulea*). Divergence time estimates for this clade occurred much earlier than the clade that includes the Painted and Varied Bunting (see Fig. 2).

Bayesian analysis of the concatenated dataset of 4169 base pairs yielded species-level relationships of *Passerina* congruent with those presented in the Klicka et al. (2001) study. The tree was fully resolved with nodal support of 100% at all nodes with the exception of the clade including the Indigo Bunting and its sister, the Lazuli Bunting, and the Blue Grosbeak (Fig. 2). This node was poorly resolved in previous studies as well (Klicka et al. 2001; Klicka et al. 2007).

Divergence time estimates suggest that the *Passerina* buntings diverged from their common ancestor (*Cyanocompsa*) between 5.5 to 8 Mya during the late Miocene (Fig. 2).

Once diverged from their common ancestor, mean divergence time estimates show that genetic diversification of the group occurred rapidly between 6 and 2.2 Mya respectively. The most recent split occurring between the Painted Bunting and the Varied Bunting during the late Pliocene early Pleistocene; between 1.6 to 2.9 Mya. The divergence estimates are consistent with those reported by Klicka et al. (2001).

Phylogenetic relationships within the genus *Passerina*

The phylogenetic relationships among members of the genus *Passerina* and its sister group, *Cyanocompsa*, concur with previous studies (Klicka et al. 2001; Klicka et al. 2007). My data indicate that the *Passerina* clade is most likely derived from a Mesoamerican ancestor. Distribution within the clade and the sister group, are all confined to Central America, Mexico, and the United States. Members of the sister clade are sedentary and are distributed throughout Central and South America (Fig. 1). The general pattern suggests that the earliest speciation within the clade was in the southern part of its distribution, followed by later expansion toward the north. Specifically, the clade that includes the Painted Bunting (Varied Bunting, Orange Breasted Bunting and Rosita's Bunting), are for the most part, sedentary, and are distributed throughout Mexico (Fig. 3). Migratory behavior in the Painted Bunting is independent of other members within the genus *Passerina.*

Information gathered from the reconstruction of the *Passerina* clade will allow for better hypothesis testing as to the evolutionary history of the Painted Bunting and possible origin of the Painted Bunting ancestor, as addressed in chapter three.

Fig. 1 Ancestral states characterization of migratory behavior mapped on Bayesian topology. Characters mapped as discrete. Black lines indicate migratory and white indicate sedentary species. A map of the year round distribution of each species is shown next to each tip of the tree.

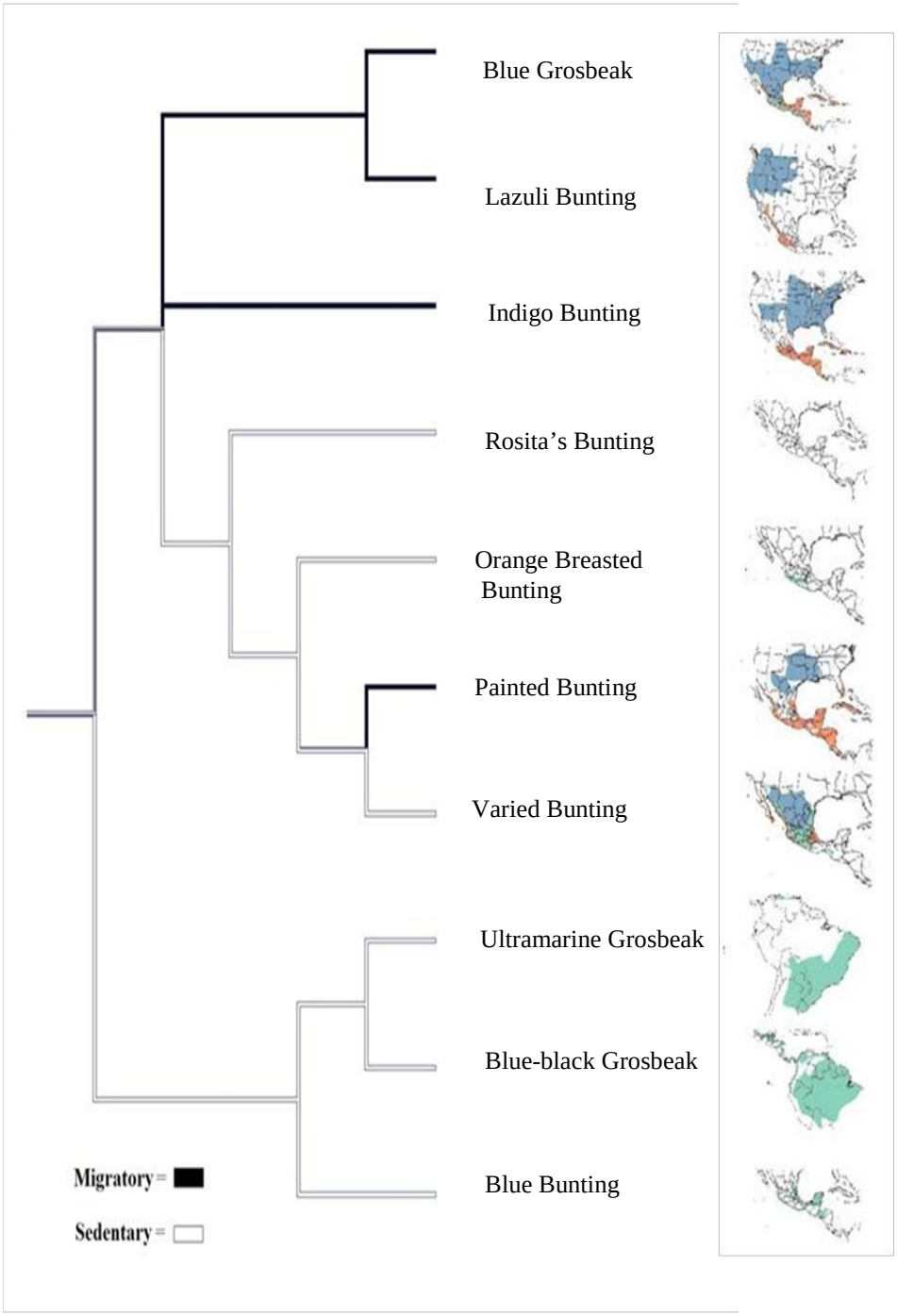


Fig. 2 Bayesian inference tree from ND2 sequences depicting the relationships within genus *Passerina* and members of the sister group. Numbers below each node represent divergence time estimates (My). Green bars represent the 95% confidence intervals surrounding each estimate. Numbers above nodes indicate Bayesian posterior probabilities using standard models of evolution.

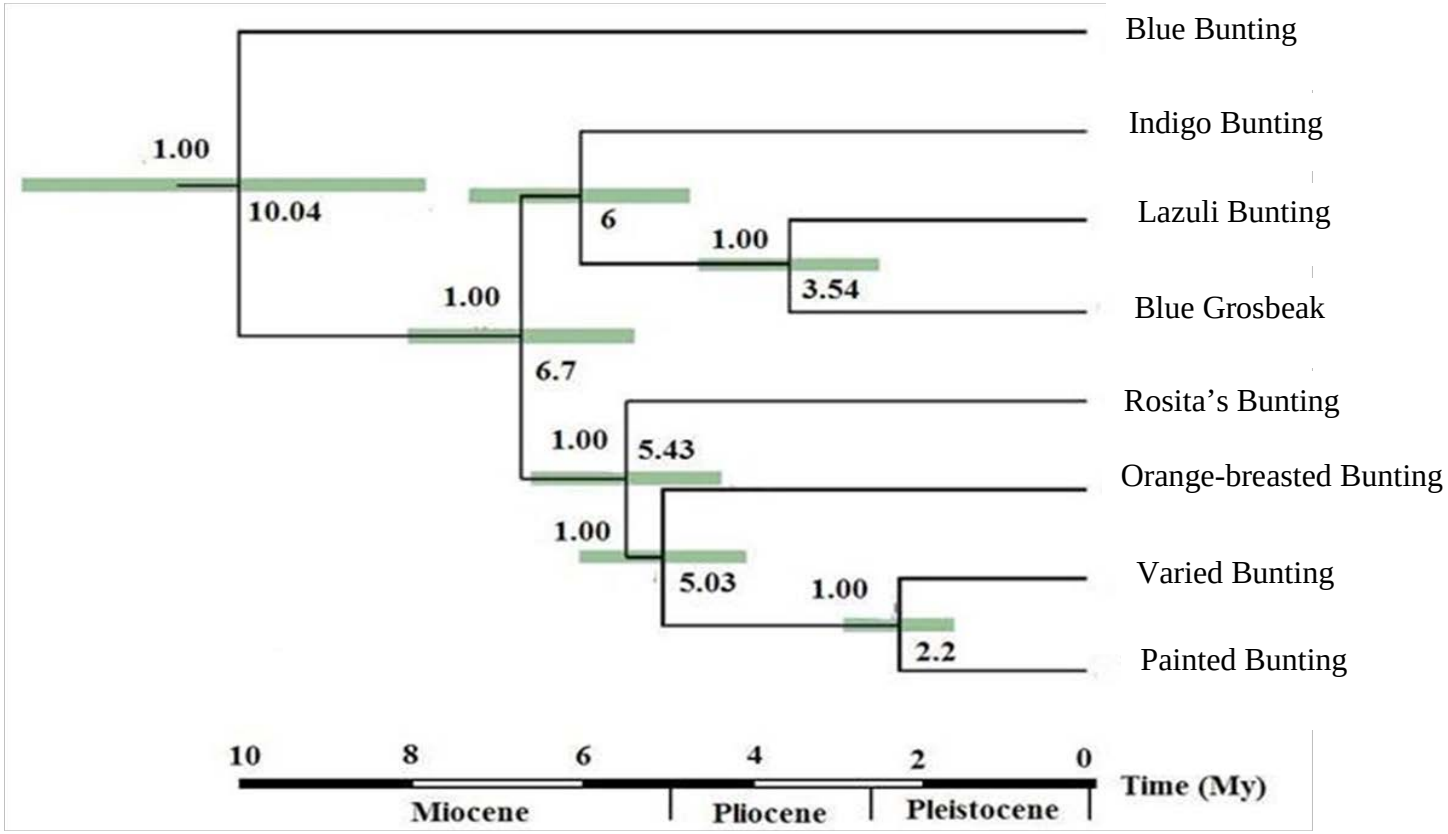
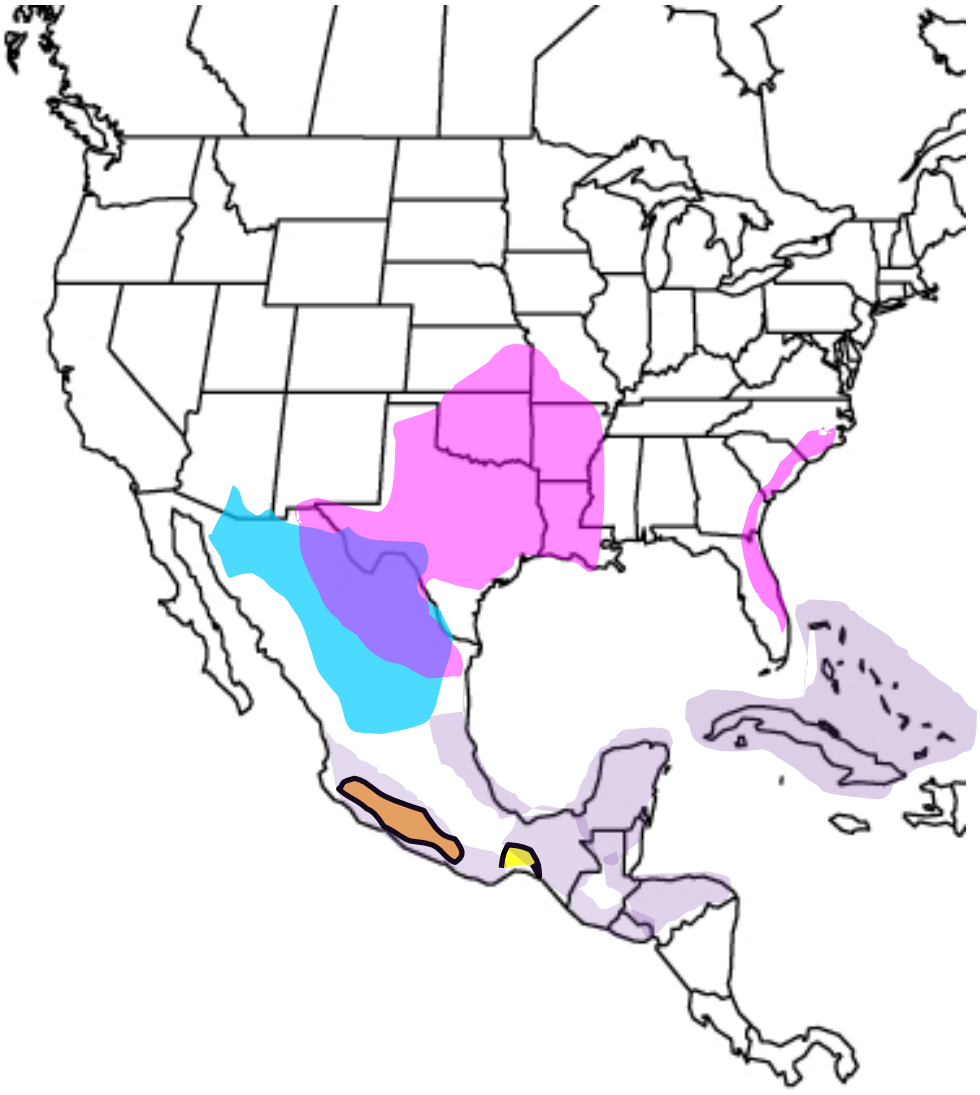


Fig. 3 Distribution of the clade within the genus *Passerina* that includes the Painted Bunting. Color highlights on the map correspond to the breeding distribution of the Painted Bunting (pink), wintering distribution (violet), and year round distribution of the sedentary members of the clade, the Orange Breasted Bunting (orange), Rosita's Bunting (yellow) and Varied Bunting (light blue).



CHAPTER TWO

GENETIC DIVERSITY WITHIN THE ALLOPATRIC BREEDING DISTRIBUTION OF THE PAINTED BUNTING (*PASSERINA CIRIS*)

Abstract

The breeding distribution of the Painted Bunting (*Passerina ciris*) is comprised of two allopatric populations separated by a 550-km distributional gap in the southeastern United States. Curiously, the boundary between the two recognized *P. ciris* subspecies does not separate the two allopatric breeding populations but instead runs roughly through the center of the interior population. Genetic relationships among these subspecies, and the allopatric breeding populations of Painted Bunting, have not been assessed. Given the recent decline in overall abundance of this species, such an assessment is warranted. I sampled birds from 15 localities (138 individuals) and identified 35 distinct haplotypes, six belonging to the Atlantic Coast population and 26 to the interior population, with three shared by both populations. AMOVA results showed that a significantly greater portion of the total genetic variance is explained when grouping birds by the interior and Atlantic Coast populations rather than by subspecies. Furthermore, my data indicate that the Atlantic Coast and interior populations represent independently evolving taxa, with no measureable gene flow between them. Although recently diverged (26,000 – 115,000 years ago), these isolated bunting populations represent incipient species. For development of conservation strategies, I suggest that the Atlantic Coast and interior populations be recognized as separate management units.

Introduction

The Painted Bunting (*Passerina ciris*) is a small, brightly colored, songbird that breeds in the southeast and south central United States and winters in the Florida Keys, the Caribbean, Mexico and portions of Central America (Fig. 4). Its breeding distribution is comprised of two disjunct populations, separated by a 550-km gap. The interior breeding population is mainly distributed throughout Kansas, Oklahoma, Texas, Arkansas, and Louisiana while the Atlantic Coast population is limited to coastal portions of North Carolina, South Carolina, Georgia, and northeastern Florida (Sykes and Holzman 2005). Two subspecies of Painted Bunting are currently recognized based on geographic variation in wing length and plumage color (*ciris, pallidior,* American Ornithologists' Union 1957). On average, the western subspecies *pallidior* is paler ventrally (both sexes) and has longer wing length (males) than does *ciris* (Mearns 1911; Storer 1951). The boundary between these two subspecies does not separate the two allopatric breeding populations but instead runs roughly through the center of the interior population (Fig. 4), from east Texas northward between 96° and 97° west longitude (American Ornithologists' Union 1957).

Across their breeding distribution, abundance estimates indicate that Painted Buntings are in decline. Overall numbers recorded on both Breeding Bird Surveys (BBS) and Christmas Bird Counts have dropped steeply since the early 1970's (Cox 1996). Recent BBS results indicated a long-term decline at an average rate of 1.6% per year. The species was listed as a highly ranked "Species at Risk" by Partners in Flight (PIF; Hunter et al. 1993) and later placed on watch list as a "Species of Continental Importance" (Rich et al. 2004). The decline in numbers appears to be most severe within the Atlantic Coast

population. BBS data indicate a 3% decline per year in this population while Christmas counts show a significant decrease in 12 of 25 counts (Sauer et al. 1997). As a consequence, the Atlantic Coast population of Painted Bunting is recognized as one of the most locally occurring, steeply declining, high priority species, within the southeastern United States (Hunter et al. 1993). Presently, no population genetic study has been carried out on this species. Whether genetic differences exist between the subspecies *pallidior* and *ciris* or between the allopatric coastal and interior birds, are unknown. A genetic assessment of these birds is therefore warranted, to measure the degree of differentiation and the level of genetic connectivity that exists between the subspecies and the allopatric breeding populations.

Studies of genetic variation within and among populations can provide insights into a lineage's evolutionary history (Avise 2004) and information important for conserving biodiversity (DeSalle and Amato 2004; Hedrick 2004). From a conservation perspective, accurate assessments of demographic history are important for making informed management decisions (DeSalle and Amato 2004). There have been recent major advances in assessing population genetic structure using a number of different molecular techniques (Smith and Wayne 1996; Emerson et al. 2001; Pearse and Crandall 2004; Manel et al. 2005). These methods allow researchers to use current geographic patterns of genetic variation to infer evolutionary history (phylogeography). This information is valuable for studies of conservation because it allows for informed speculation regarding the processes that led to current distribution of genetic variation (Johnson et al. 2007). Population genetic studies also allow for assessment of contemporary population genetic structure, population size, and degree of gene flow among breeding populations. This

study is the first to use molecular genetic techniques to assess the genetic structure within the Painted Bunting across its breeding range in order to understand the factors that have shaped present day genetic patterns and current distributions.

Herein, I use phylogeographic methods and mitochondrial DNA (mtDNA) sequence data to characterize and quantify the amount of genetic variation within the Painted Bunting. From a conservation perspective, it is important to discern whether the threatened Atlantic Coast bunting population is both geographically *and* genetically separate from other Painted Buntings, and therefore worthy of recognition as a distinct evolutionary unit. Thus, the specific goal of this study is to test the following alternative phylogeographic hypotheses: 1) no genetic structure exists and gene flow within the Painted Bunting species is regular and ongoing; 2) genetic structure exists between the subspecies *ciris* and *pallidior* and the Atlantic Coast population is not genetically distinctive; and, 3) genetic structure exists across the 550-km distributional gap in the breeding distribution indicating that the Atlantic Coast population is genetically distinct.

Methods

Sampling and Laboratory Methods

I sampled 138 individuals from 15 localities with a focus on maximizing geographical coverage of the breeding range. My sampling scheme allowed for the evaluation of genetic diversity within and among both subspecies and disjunct breeding populations (Fig. 4). Samples were obtained through scientific collecting and augmented with feather and blood material obtained from the Patuxent Wildlife Research Center.

I sequenced the protein-coding mitochondrial gene NADH dehydrogenase subunit 2 (ND2). I acknowledge recent concerns over studies that are based on a single locus

(Edwards et al. 2005, Bazin et al. 2006). However, my objective is to potentially determine the geographic and genetic limits of recently evolved groups. The high variability and rapid coalescence time of mtDNA make it the marker of choice for addressing such questions (Zink and Barrowclough 2008). All 1041 base pairs were amplified via polymerase chain reaction (PCR) and sequenced using the primers L5215 (5'-TATCGGGCCCATACCCCGAATAT-3') (Hackett 1996) and HTrpC (5'-CGGACTTTACGACAAACTAAGAG-3') (Perez-Eman 2005; DaCosta et al. 2008). All fragments were amplified in 12.5 uL reactions under the following conditions: denaturation at 94ºC, followed by 40 cycles of 94ºC for 30s, 54ºC for 45s, and 72ºC for one minute. This was followed by a 10-minute extension at 72º C and 4ºC soak. Products were purified with Exosap-IT (USB Corporation, Cambridge, MA) purification following the manufacturer's protocols. I performed 20 uL BigDye (Applied Biosystems, Foster City, CA) sequencing reactions using 20 to 40 ng of purified and concentrated PCR product following standard protocols. Sequencing reactions were purified using a magnetic bead clean-up procedure (Clean-Seq, Agencourt Biosciences, Beverly, MA) and analyzed on an ABI 3100-*Avant* (Applied Biosystems) automated sequencer. Complementary strands of each gene were unambiguously aligned using the program Sequencher (Gene Codes Corporation, Ann Arbor, MI).

Because I used blood as a mtDNA source for some of my samples, I carefully examined our data for the presence of pseudogenes (or "numts", see Bensasson et al. 2001). Both light and heavy strands were sequenced for all PCR fragments and no gaps, insertions, or deletions were apparent in the aligned sequences. The ND2 protein coding sequences were translated into amino acids using MacClade 4 (Maddison and Maddison

2000) and compared to an existing ND2 sequence of *Passerina ciris* (GenBank accession number ABU45623) to insure the correct reading frame and to check for the premature presence of stop codons.

Phylogenetic analyses and population structure

I examined the genetic structure within the Painted Bunting using a series of analyses that focus on genetic patterns at different temporal scales, thereby employing both phylogenetic and population genetic approaches. This combination of techniques provides a more thorough exploration of the data, generating statistically rigorous phylogeographic conclusions (Knowles and Maddison 2002). I used a median joining network to visualize relationships among haplotypes (program Network 4.1.1.2; Bandelt et al. 1999). The Network software reconstructs all shortest maximum parsimony trees from a given data set. Median networks provide a useful representation of intraspecific data that are characterized by having few base substitutions between sequences. In contrast to standard tree representation, where only the tips of the tree are labeled, nodes in a median network represent either sampled haplotypes or inferred intermediates.

I used the programs Arlequin (Excoffier et al. 2005) and DnaSP (Rozas et al. 2003) to analyze patterns of genetic variation within and among sampling sites. Population genetic parameters were calculated for all sampling sites (14) from which I had ≥ 9 individuals. Genetic diversity within each site was characterized by calculating the number of unique haplotypes and the number of private haplotypes. I also performed a number of statistical tests used to estimate past demographic processes such as population expansion. Historical events (i.e. population expansion) can leave a genetic "footprint" that may be detected in sequence data (Ramos-Onsins and Rozas 2002). Mismatch

distributions (i.e. pairwise differences between haplotypes) were generated to test for historical population expansion events within populations by comparing the observed frequency distribution of pairwise nucleotide differences among individuals with distributions expected from a population expansion (Rogers and Harpending 1992). Populations at demographic equilibrium or in decline should exhibit a multimodal distribution of pairwise differences, whereas populations that have experienced a sudden demographic expansion should display a star-shaped phylogeny and a unimodal distribution (Rogers and Harpending 1992; Slatkin and Hudson 1991). However, mismatch analyses employ a number of assumptions (e.g. random mating, an infinite alleles model) that may not be met (Wakeley and Hey 1997; Schneider and Excoffier 1999) in many populations. Because of these limitations, mismatch analyses were coupled with Tajimas's *D* to test for localized population expansion (Tajima 1989) and a test of selective neutrality using Fu's *Fs* test (Fu 1997). Significantly negative *D* or *Fs* values indicate a relative excess of rare haplotype variants, suggesting expansion in population size; positive values suggest a relative excess of intermediate-frequency alleles, which is expected under a model of population subdivision or balancing selection, coincident with stable population size over time.

Population variability was estimated as haplotype diversity (h) and nucleotide diversity (π). These measures of haplotype and nucleotide diversity are useful in examining the demographic history of a lineage (Grant and Bowen 1998). Centers of origin should be more diverse in haplotype and nucleotide diversity than more recently founded populations (Althoff and Pellmyr 2002). I used analysis of molecular variance (AMOVA) to determine genetic structure at hierarchical geographic levels. I performed

two nested AMOVA with sequences grouped by region and then by individual population within each region (i.e. sampling locality) to explore whether significant genetic variation exists at multiple geographic levels. My first analysis included samples from sampling sites separated by currently recognized subspecies boundaries (i.e. morphological assignment). I based the second analysis on the allopatric separation of populations (i.e. Atlantic Coast populations vs. interior populations). Inter-population genetic variation and significance was assessed with pairwise population Φ_{ST} values. I used the Mantel correlation coefficient (Mantel 1967; Smouse et al. 1986) to test the significance of isolation by distance for sampling sites on the Atlantic Coast, and in the interior. The significance of the Mantel test was assessed using 1000 random permutations.

Coalescent analyses/isolation-with-migration

A lack of reciprocal monophyly between two genetically structured lineages may be due to ongoing gene flow or to incomplete lineage sorting. Simulations have shown that even a single non-recombining genetic locus can provide substantial power to test the hypothesis of no ongoing gene flow between two populations (Nielsen and Wakeley 2001; Hey and Nielsen 2004). I used the coalescent program Isolation with Migration (IM; Hey and Nielsen 2004) to determine if the observed pattern of genetic variation was a result of historical divergence or limited contemporary migration between interior and Atlantic Coast Painted Buntings. I also used it to estimate effective populations' sizes, migration rates, divergence time and time to most recent common ancestor (TMRCA). IM is a Bayesian Markov chain Monte Carlo (MCMC) method that tests for length of genetic isolation and levels of migration. The assumptions include selective neutrality, a sister taxon relationship among study populations, and random sampling from a

panmictic population (Hey and Nielsen 2004). IM estimates 6 parameters, including the effective number of females in each daughter population and the ancestral population (θ_1, θ_2, and θ_a), immigration rates into each daughter population (m_1 and m_2), and time since divergence (t) (Hey and Nielsen 2004). IM estimate's parameters are scaled to the neutral mutation rate (μ).

The program was run for 20,000,000 steps using 20 chains following a 500,000 step burn-in. To verify convergence, multiple IM runs were completed using a different random number seed. The program produced similar parameter estimates from each run. I report the mode and the 95% highest posterior densities (HPD) for each parameter estimate from the run that produced the highest effective sample sizes (ESS; Hey and Nielsen 2004) for all parameter estimates.

I estimated the mutation rate for mtDNA ND2 by assuming a standard passerine clock for cytochrome-*b* (cyt-*b*) of 1.9% divergence per million years (R. Fleischer, unpublished data) and determining the relative rate of ND2. Specifically, I compared mutation rate between cyt-*b* and ND2 within six *Passerina* species (J. Klicka, unpublished data). I converted t to real time (t) using $t = t/\mu$, and I calculated effective population size (N_e) using $\theta = 4 N_e\mu$. θ is scaled to the substitution rate per generation rather than per year; therefore, I multiplied my μ by a generation time of 2.2 for Painted Buntings. This generation time was calculated using the equation $T = \alpha + [s/ (1-s)]$ from Lande et al. (2003), where α is the age at maturity, and s is the annual adult survival rate. The effective number of female migrants between populations was calculated using $M_1 = \theta_1 * m/2$ and $M_2 = \theta_2 * m/2$ (Hey and Nielsen 2004).

Results

Phylogenetic analyses

I sequenced the complete ND2 gene (1041 base pairs) for 138 individuals, 78 from the Atlantic Coast and 60 from the interior. No insertions or deletions were present. The sequences yielded 40 variable sites of which 18 were phylogenetically informative. I found that ND2 is evolving ~ 1.5 times as fast as cyt-*b*, suggesting a divergence rate for ND2 of approximately 3.0% per million years. A number of studies have shown that the methodology of phylogenetics often lacks resolving power and may obscure the evolutionary relationships of lineages that are relatively recent (Crandall 1994; Crandall and Templeton 1996; Smouse 1998). The use of a bifurcating tree, may be misleading, especially when the ancestral haplotypes are extant (Althoff and Pellmyr 2002), and the use of a haplotype network may more accurately portray the true evolutionary history of a lineage (Smouse 1998; Posada and Crandall 2001). I therefore displayed the data with a haplotype network instead of a phenogram. The median joining network contained 35 haplotypes, six belonging to the Atlantic Coast population, 26 to the interior population, and three shared by both populations (Fig. 5). Two of the three shared haplotypes were common and widespread. The most common haplotype was shared by 38 individuals, 36 of which occurred in the Atlantic Coast population. The second most common haplotype was found in 28 individuals, 24 of which were from the interior population. The third shared haplotype occurred in only two individuals, one from Louisiana and the other from Georgia. An additional common haplotype (20 individuals) occurred exclusively in the Atlantic Coast population. Of the remaining haplotypes, 23 were unique to single individuals found in the interior. The remaining eight haplotypes were shared among

individuals, three among only interior birds and five among birds of the Atlantic Coast. The most divergent haplotypes were found in the interior population.

The data on the genetic diversity within sampling sites from the Atlantic Coast and the interior are presented in Table 1. Nucleotide diversity was low in all samples relative to levels seen in other songbird studies (see Spellman et al. 2007; Zink et al. 2008), ranging from a low of 0.001 to a high of 0.003 (Table 1). All unique haplotypes (private alleles) were restricted to the interior population, with each sampling locality in the interior having at least one private haplotype (range one-seven). Haplotype diversity was high in all populations ranging from 0.667 to 0.978 (Table 1). Mismatch distributions (not shown) for all sampled sites were unimodal and with the exception of Louisiana ($p<0.01$), and did not differ from that expected of an expanding population. In contrast, the less conservative Tajima's *D* and Fu's *Fs* tests suggest different histories for the interior and Atlantic Coast populations. Tajima's *D* values were significant in five of the six interior sampling localities and in only one from the Atlantic Coast. Significant Fu's *Fs* values were obtained for the same four interior localities while only one was obtained for any sampling locality in the Atlantic Coast. Fu's *Fs* has been shown to be the most powerful of these three tests for detecting population growth (Table 1; Ramos-Onsins and Rozas 2002).

Although most of the variation was found within populations in both analyses (subspecies groupings, Atlantic vs. interior groupings; 70% and 71% respectively), hierarchical AMOVA indicated a significant portion of the total genetic variance is due to differences among populations (Table 2). Approximately 20% of the variation is explained when the recognized subspecies groups are compared whereas 28% of the

variation is explained when the data were partitioned into Atlantic Coast and interior populations. A Mantel test failed to find a correlation between geographic distance and genetic distance among all Atlantic Coast sampling sites ((r = -0.043, p = 0.49) Fig. 6). However, genetic and geographic distance was positively correlated when comparing all interior sites (r = 0.686, p = 0.001). Because of its distance from all other interior sites, TX3 could be driving the positive correlation between genetic and geographic distances. To explore this possibility, I performed an additional Mantel test omitting this site and a positive correlation was still obtained (r = 0.855, p = 0.006).

I partitioned the molecular variation in pairwise comparisons of populations into within-population and total-variance components to obtain pairwise Φ_{ST} values (Table 3). The majority of the Φ_{ST} values found to be significant were when comparing Atlantic Coast sampling sites to interior sites. The highest Φ_{ST} values of 0.576, 0.571, and 0.532 were observed in comparisons of GA2 with TX1, OK, and TX2 respectively. There were no significant differences between interior sampling sites (where the subspecies boundary is located) and only two significant Φ_{ST} values in pairwise comparisons of Atlantic Coast sites. The two significant pairwise comparisons of Atlantic Coast sites involved GA2 with another Atlantic Coast population. The first significant comparison was between GA2 and GA1 with a Φ_{ST} value of 0.322. A second significant pairwise comparison involved GA2 with SC1; with a Φ_{ST} value of 0.193.

Coalescent analysis

IM analyses estimated $\theta_{Atlantic}$ (scaled effective size of the Atlantic Coast population) to be 5.6, $\theta_{Interior}$ (scaled effective size of the interior population) to be 200, $\theta_{Ancestral}$ (scaled effective size of the hypothesized ancestral population) to be 5.8, m_1 to be 0.323,

m_2 to be 0, *t* to be 0.6 and TMRCA to be 2.6. Estimated posterior distributions for these model parameters are shown in Figure 7. My coalescent analyses (IM; Hey and Nielsen 2004) indicate that my parameter estimates had strongly unimodal posterior distributions (Fig. 7), however, the tail of the posterior distribution of $\theta_{interior}$ did not reach zero on the x-axis, which is common when sample sizes are small or the data are not able to identify the model (Hey, 2005; Hey and Nielsen 2007), and therefore the 95% HPDs were not calculated. The model parameters, parameter values, demographic parameter estimates and 95% confidence intervals calculated using these estimates are listed in Table 4. Effective population sizes (Ne) calculated indicate that the interior population seems to have grown substantially following divergence, with a current estimate of 1,500,000, while the Atlantic Coast population has remained relatively small (41,000, range 18,000 to 106,000) relative to the estimated ancestral population size (43,000, range 4,000 to 230,000; Table 4). Confidence intervals surrounding all these estimates are large (see Table 4) because the estimates are based on a single marker. I examined the posterior distributions of migration rates to determine whether ongoing gene flow might explain the observed phylogenetic patterns. The posterior distribution of m_1 (scaled rate of migration rate into the Atlantic Coast population from the interior population) peaked at 0.323 (95% HPD = 0 to 2.5), and the posterior distribution of m_2 (scaled rate of migration rate into the interior population from the Atlantic Coast population) peaked at 0 (95% HPD = 0 to 5), indicating that there is no detectable gene flow between these allopatric populations. The posterior distributions of *t* (scaled divergence time) peaked at 0.6 (95% HPD 0.4 to 1.8). Estimates of *t* were converted to actual values of time using the equation $t = t/\mu$ (Hey and Nielsen 2004), where μ is the locus specific neutral mutation

rate and *t* is the estimate provided by IM. When converted to time in years, this analysis suggests that Atlantic Coast and interior Painted Buntings began diverging about 38,000 years before present ($_{BP}$); 95% HPD 26,000 to 115,000 $_{BP}$. Posterior probabilities of TMRCA peaked at 2.6 (95% HPD = 1 to 5), indicating that all sampled haplotypes coalesce at approximately 166,000 $_{BP}$ (range = 64,000 – 320,000 $_{BP}$).

Discussion

Overall phylogeography and population structure patterns

Nearly all Atlantic Coast sampling sites differ significantly from interior sampling sites (see Φ_{ST} values, Table 3). In contrast; I found no significant differences between interior buntings presumed to belong to different subspecies (western *pallidior* and interior forms of *ciris*), an indication that the morphological differences, as presently defined, are not concordant with the genetic differences. My analyses indicate that significant genetic structuring is apparent between the allopatric Atlantic Coast and interior breeding populations of the Painted Bunting (Fig. 3) and these lineages appear to have diverged recently (~ 38,000 years ago, Table 4). Taken together, these findings suggest that Painted Buntings are genetically partitioned into interior and coastal populations (Fig. 3).

Studying genetic differentiation between separated populations is important for understanding population divergence and speciation processes, and for defining conservation priorities. Once populations diverge, it is important to understand how much connectivity is maintained through gene flow and determining the intensity and directions of gene flow is critical for species management (Omland et al. 2006). A shallow genealogy that lacks distinct clades, such as seen in my data, may indicate either

that the suggested divergence has occurred too recently for mtDNA to have sorted to monophyly (Baker et al. 2003; Klicka et al. 1999) or that the populations remain connected by gene flow. IM results revealed little to no gene flow into the Atlantic Coast population from the interior or vice versa, an indication that the lack of reciprocal monophyly is due to incomplete lineage sorting, a consequence of a relatively recent divergence. Coalescent analyses placed TMRCA for all haplotypes during the Pleistocene, approximately 320,000 – 64,000 years ago (Table 4), an estimate consistent with one suggested in a previous molecular study that included all members of the *Passerina* clade (Klicka et al. 2001).

Phylogeographic analyses that included all samples revealed a structured haplotype network (Fig. 5), unimodal mismatch distribution, high haplotype diversity, and low nucleotide diversity (Table 1); all indicative of a recent population expansion from an ancestral population with a small N_e (Avise 2000). The estimate of the hypothesized ancestral population (43,000) represents a relatively small ancestral population size when compared to the current estimate of 1,500,000 for the interior population. This scenario of a small ancestral population size, high haplotype and low nucleotide diversity also suggests that the divergence between Atlantic and interior populations of buntings occurred long enough ago to allow for the recovery of haplotype variation by mutation but not so long ago as to allow for an accumulation of large sequence differences (Avise 2000; Rogers and Harpending 1992).

The results presented here demonstrate that genetic differentiation between the Atlantic Coast and interior populations of the Painted Bunting exists despite the relatively shallow evolutionary history of the taxon (Table 1). The structure recovered is not

consistent with the current subspecific geographic limits based on morphological variation (Mearns 1911; Storer 1951). The Atlantic Coast and interior populations that I have defined (see Fig. 3) do correspond with Painted Buntings known to differ with respect to the timing and pattern of molt and migration. All Painted Buntings are short to medium distance Neotropical migrants; however, a portion of the individuals in the interior population migrate to staging areas in southern Arizona and northern Sonora in Mexico to molt before continuing on to their wintering ranges. In contrast, the Atlantic Coast population molt on the breeding grounds prior to fall migration (Thompson 1991b). Additionally, members of the interior population initiate fall migration at least two months later than the Atlantic Coast population (Thompson 1991a).

Evolutionary history and population structure

The IM results suggest that the effective population size of the interior birds (1,500,000) is thirty times greater than that of the Atlantic Coast population (41,000). The low effective population size of the Atlantic Coast birds is in part a reflection of a smaller overall distribution but it may also reflect historical population demographic factors. Populations can experience population bottlenecks in response to challenges in the biotic or physical environment (Avise 2000). It seems probable that a loss of habitat on both breeding and wintering areas, and at critical migratory stopover sites, has played a role in population declines. The Atlantic Coast population, because of its limited and narrow coastal distribution, is likely to have been more severely impacted than the interior population. Urban development and anthropogenic disturbance along the coast and coastal islands and woodland edges has greatly reduced its prime habitat (Sykes and Holzman 2005). The patterns of haplotype and nucleotide diversity support the

hypothesis that the interior population was a center of origin for this species with subsequent dispersal to the Atlantic Coast. Such an interpretation is in agreement with the results of a molecular study of the entire genus (Klicka et al., 2001) in which Mexico was identified as the ancestral area for the *Passerina* sub-clade that includes the Painted Bunting. How this dispersal event occurred is still unknown. It could be that the ancestral population expanded eastward, reaching the coast, with a subsequent range reduction that left the observed gap in their distribution. Alternatively, it is also possible that the Atlantic Coast population was founded via a true founder event, when a group of migrating buntings was blown off course, establishing either a new breeding (or wintering) area.

Highly mobile species are capable of adjusting their migratory pathways (Alderstam and Hedenstrom 1998) and novel migratory routes can arise very rapidly (Able and Belthoff 1998; Berthold 1996). Knowing the extent to which breeding populations are differentiated and how their use of migratory pathways and wintering sites vary, are important for the conservation of migratory birds (Gauthreaux 1996; Haig and Avise 1995). It will also be important to determine levels of connectivity that may occur on the wintering grounds because factors that affect population structure (i.e. gene flow) can conceivably occur at any time during the annual cycle of a migratory bird (Smith et al. 2004). I do not yet know whether Atlantic Coast and interior birds are also isolated during migration and on the wintering grounds. Painted Buntings winter in south Florida (Robertson and Woolfenden 1992), the Bahamas and Cuba (Raffaele et al. 1998), on both coasts of Mexico, and throughout most of Central America (Howell and Webb 1995, Land 1970; Fig. 3). I am currently assessing the genetic connectedness of birds on the

wintering grounds. Individuals migrating from the Atlantic Coast may winter only in south Florida, the Bahamas, and Cuba or, they could continue on to wintering destinations in the Yucatan or beyond (as suggested by Sykes et al. 2007). If the former, Atlantic Coast and interior population segregated on both breeding and wintering grounds, would facilitate a faster divergence of these birds on their own evolutionary trajectories.

Conservation implications

One factor contributing to the overall decline of the Painted Bunting is loss of habitat. On the breeding grounds, through urban development, roads, and agricultural intensification, significant habitat losses have occurred (Lowther et al. 1999; Sykes and Holzman 2005). The effects of this loss are most acute along the Atlantic Coast where this buntings' distribution is limited. Loss of riparian habitats in the southwestern United States and northwest Mexico, used during migration by the interior population, may also be influencing population levels in this species (Lowther et al. 1999; Sykes and Holzman 2005). Wintering habitats in Central America also continue to be degraded. The importance of wintering areas for Nearctic-Neotropical migrants has been widely discussed in the past (Webster et al. 2002).

It is likely that the cage bird trade (wintering grounds) also plays an important role in the decline of the Painted Bunting. The colorful adult male has been in the commercial trade for a very long time, with thousands of live birds being shipped to Europe for sale in the early 19th century (Inigo-Elias et al. 2002). This trade was banned in the United States in the early 20th century, but continues to be legal in other countries (Inigo-Elias et al. 2002). Caged birds are routinely sold in domestic markets in Cuba (Sykes et al. 2007).

An estimated 700 buntings were trapped for the cage bird trade at a single location in Cuba during several days in May 2003 (Sykes et al. 2007). Some estimates suggest that at least 100,000 Painted Buntings were trapped in Mexico between 1984 and 2000. International trade in wild-caught birds was banned in Mexico from 1982 to 1999, but resumed quickly after the ban was lifted. It is estimated that about 6,000 birds per year were exported from Mexico to Europe in 2000 and 2001 (Inigo-Elias et al. 2002). Whether these Mexican exports represent only the more abundant interior form, or also include representatives of the rapidly declining Atlantic Coast form, remains unknown. All of these factors (occurring on breeding and/or wintering grounds) contribute to the decline of Painted Bunting.

Despite uncertainty about the mechanism or timing of divergence of the Painted Bunting, it is apparent that all birds from the interior are more genetically related to each other than to any Atlantic Coast bird. All data support the current recognition of two allopatric and genetically isolated breeding populations in the southern United States. Importantly, my data did not detect any genetic structuring across the putative boundary between the two subspecies of *P. ciris* in Texas and Oklahoma. It may be that genetic differences do separate these two forms within the interior population but the level of resolution provided by mtDNA sequence data is not sufficient to detect them. Further study using different molecular markers is warranted.

The results of this study, taken with the relatively small population size and decreasing population trends, suggest that the Atlantic Coast Painted Bunting should be recognized as an independently evolving taxonomic unit. Relevant criteria for defining evolutionarily significant units (ESUs) for biological conservation have been much

discussed (Moritz 1994). Some authors have argued that ESU designation should be based on significant genetic differentiation at neutral genetic markers (Moritz 1994) while others have suggested using ecological information, as well as genetic differentiation, to delineate ESU's for conservation efforts (Crandall et al. 2000). Ultimately, a comparison and inclusion of multiple sources of data such as molecular markers (mtDNA and nuclear DNA), morphology, behavior, cytology and ecology should permit the most effective way to understand the evolutionary history of a group (Funk and Omland 2003; Rubinoff and Splerling 2004; Bowen et al. 2005). Given my findings of genetic differentiation along with the previously identified information on differential timing and pattern of molt and migration (Thompson 1991a; Thompson 1991b), the interior and Atlantic Coast populations of Painted Buntings should be recognized as distinct ESUs by any definition. Any subsequent conservation efforts or recovery goals should treat these allopatric populations as separate management units. I hope that this study will contribute to the development of conservation strategies that can reverse Painted Bunting population declines.

Chapter 2, Table 1 The genetic diversity within the breeding populations and the values in the columns correspond to sample size (N), number of unique haplotypes (H), number of private haplotypes (Pri.), haplotype diversity (h), nucleotide diversity (π), significance of the mismatch distribution (MM; ns = not significantly different from the expectation under exponential growth), Tajima's D, and Fu's Fs (significant values in bold).

		N	H	Pri.	h	π	MM	D	Fs
Atlantic									
Coast	NC1	10	6	0	0.844	0.002	ns	-0.127	-1.363
	NC2	10	5	0	0.800	0.001	ns	-1.262	-1.320
	SC1	9	5	0	0.861	0.002	ns	0.241	-0.911
	SC2	10	3	0	0.689	0.002	ns	0.927	1.667
	GA1	9	4	0	0.778	0.002	ns	1.612	0.450
	GA2	10	5	0	0.667	0.001	ns	**-1.741**	**-2.260**
	GA3	9	4	0	0.806	0.001	ns	0.497	-0.787
	FL	9	4	0	0.806	0.002	ns	0.881	0.617

Table 1 continued

Interior								
AR	10	7	2	0.867	0.003	ns	**-1.944**	**-2.968**
OK	11	7	4	0.778	0.002	ns	**-1.873**	**-2.442**
LA	10	5	1	0.822	0.002	<0.01	-0.586	-0.815
TX1	9	7	5	0.917	0.002	ns	**-1.823**	**-3.797**
TX2	9	5	1	0.893	0.002	ns	**-1.640**	-1.802
TX3	10	9	7	0.978	0.003	ns	**-1.586**	**-6.320**

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Table 2 Analysis of Molecular Variance (AMOVA)

Group	Source of Variation	% Variation	Φ Statistic	p
P.c. ciris vs. *P.c. pallidior*	Among groups	20	0.20	<0.0001
	Among populations within groups	9.9	0.12	<0.0001
	Within populations	70	0.30	<0.0001
Atlantic Coast vs. Interior	Among groups	28	0.28	<0.0001
	Among populations within groups	1.9	0.03	<0.0001
	Within populations	71	0.29	<0.0001

Table 3 Population pair-wise ΦST values. Significance of ΦST values determined by 1000 random permutations of individuals among populations in a comparison. Values shown in bold significant at $\alpha = 0.05$ after a false discovery rate correction.

	NC1	**NC2**	**SC1**	**SC2**	**FL**	**GA1**	**GA2**	**GA3**	**AR**	**OK**	**LA**	**TX1**	**TX2**	**TX3**
NC1	–													
NC2	0.079	–												
SC1	-0.075	0.077	–											
SC2	-0.071	-0.007	-0.074	–										
FL	-0.030	0.070	-0.023	-0.022	–									
GA1	-0.045	**0.210**	-0.038	0.021	-0.050	–								
GA2	0.190	0.021	**0.193**	0.139	**0.138**	**0.322**	–							
GA3	-0.079	0.122	-0.102	-0.051	-0.031	-0.061	0.211	–						
AR	0.181	**0.398**	0.133	**0.256**	0.232	0.128	**0.482**	0.155	–					
OK	**0.267**	**0.490**	**0.228**	0.352	**0.320**	**0.207**	**0.571**	**0.249**	-0.019	–				
LA	0.156	**0.372**	0.103	0.226	0.208	0.114	**0.456**	0.127	-0.040	0.024	–			
TX1	0.260	**0.493**	0.225	**0.354**	**0.321**	**0.207**	**0.576**	**0.246**	-0.017	0.000	0.011	–		
TX2	**0.188**	**0.434**	0.141	**0.277**	0.049	0.137	**0.532**	0.167	-0.058	-0.015	-0.064	-0.016	–	
TX3	**0.201**	**0.420**	0.166	0.280	0.253	0.140	**0.499**	**0.179**	-0.009	0.002	0.022	-0.011	-0.008	–

Table 4 Estimates of demographic parameters calculated using model parameter estimates from the output of IM analyses. Population parameters as described in text in column 1. Rows correspond to each of the six estimated parameters. Parameters values (column 2) estimated in IM, converted to demographic value (column 3) using formulas provided in text. Bold values have the highest posterior probability and are provided with 95% low and high confidence interval estimates.

Parameter	Parameter Value	Demographic Value
$\Theta_{Atlantic}$	**5.6** (2.4 – 14.4)	**41,000** (18,000 – 106,000)
$\Theta_{Interior}$	**200** (N/A)*	**1,500,000** (N/A)*
$\Theta_{Ancestral}$	**5.8** (0.6 – 33)	**43,000** (4,000 – 230,000)
Years since divergence	**0.6** (0.4 – 1.8)	**38,000** (26,000 – 115,000)
m_1	**0.323** (0 – 2.5)	**0** (0 – 5)
m_2	**0 (N/A)***	**0** (N/A)*
TMRCA	**2.6** (1 – 5)	**166,000** (64,000 – 320,000)

* Posterior distributions of $\Theta_{Interior}$ (effective population size of interior Painted Buntings scaled to the neutral mutation rate) were large across a broad range of values, and unlike the remaining parameter estimates, the tail of the posterior distribution did not approach zero and therefore the 95% HPDs were not calculated. Since the formula for calculating the demographic value of m_2 uses Θ, 95% confidence intervals were not calculated for m_2

.

Fig. 4. Map highlighting the breeding and wintering range of the Painted Bunting (*Passerina ciris*). A solid curved line through eastern Kansas, Oklahoma, and Texas indicates the boundary on the breeding grounds between the recognized ranges of the western subspecies *pallidior,* and the eastern subspecies *ciris*. A 550- km gap separates subspecies *ciris'* two breeding populations. Blue dots indicate sampling locations. Boxes highlight sampling sites included as part of the Atlantic Coast (red) or interior (blue) breeding population. Eight sampling sites (FL, GA1, GA2, GA3, NC1, NC2, SC1, and SC2) are included in the Atlantic Coast population and six sampling sites (LA, AR, OK, TX1, TX2, and TX3) are included in the interior population. Members of the interior breeding population of subspecies *ciris* are from the sampling sites TX2, AR, and LA. All individuals and specific locations are listed in Table 10.

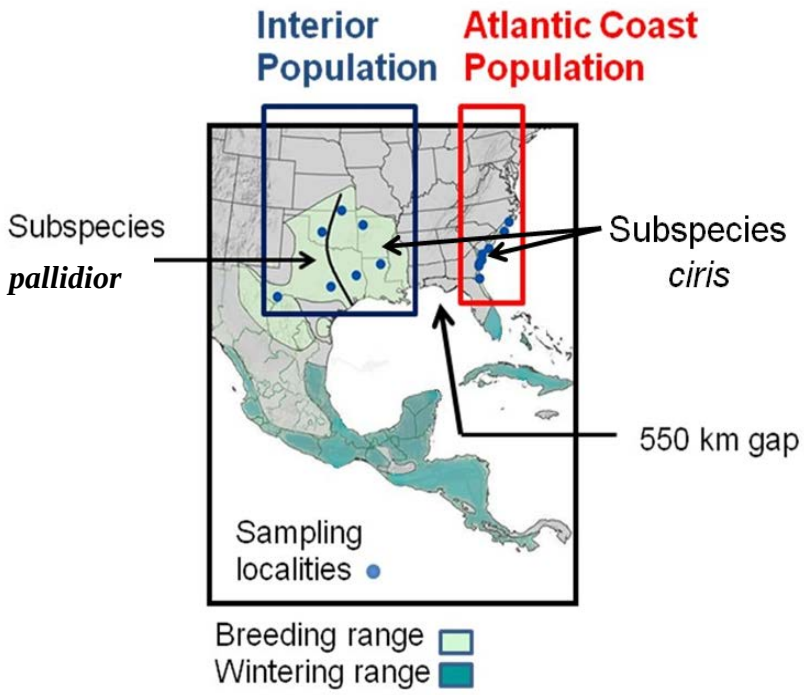


Fig. 5. Median-joining network of Painted Bunting haplotypes (n=138). Circles represent one of the 35 unique haplotypes sized proportionally to the number of individuals sharing the haplotype. Hash marks represent single base pair differences between haplotypes. Small black circles represent median vectors. Circle colors correspond to geographic locations: *P.c. pallidior*, interior (black), *P.c. ciris*, interior (gray), *P.c. ciris*, Atlantic Coast (white).

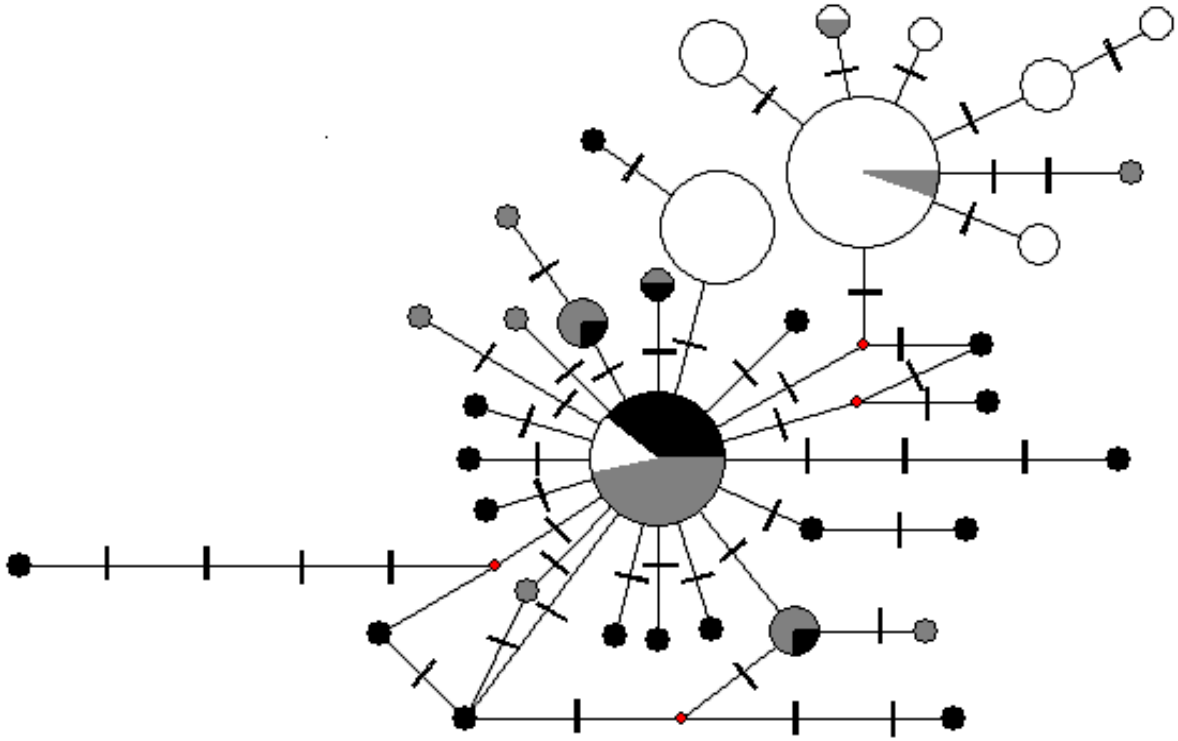


Fig. 6 A mantel test of genetic vs. geographic distance using (a) all Atlantic Coast pairwise Φ_{ST} values, (b) all interior pairwise Φ_{ST} values, and (c) interior minus TX3 pairwise Φ_{ST} values.

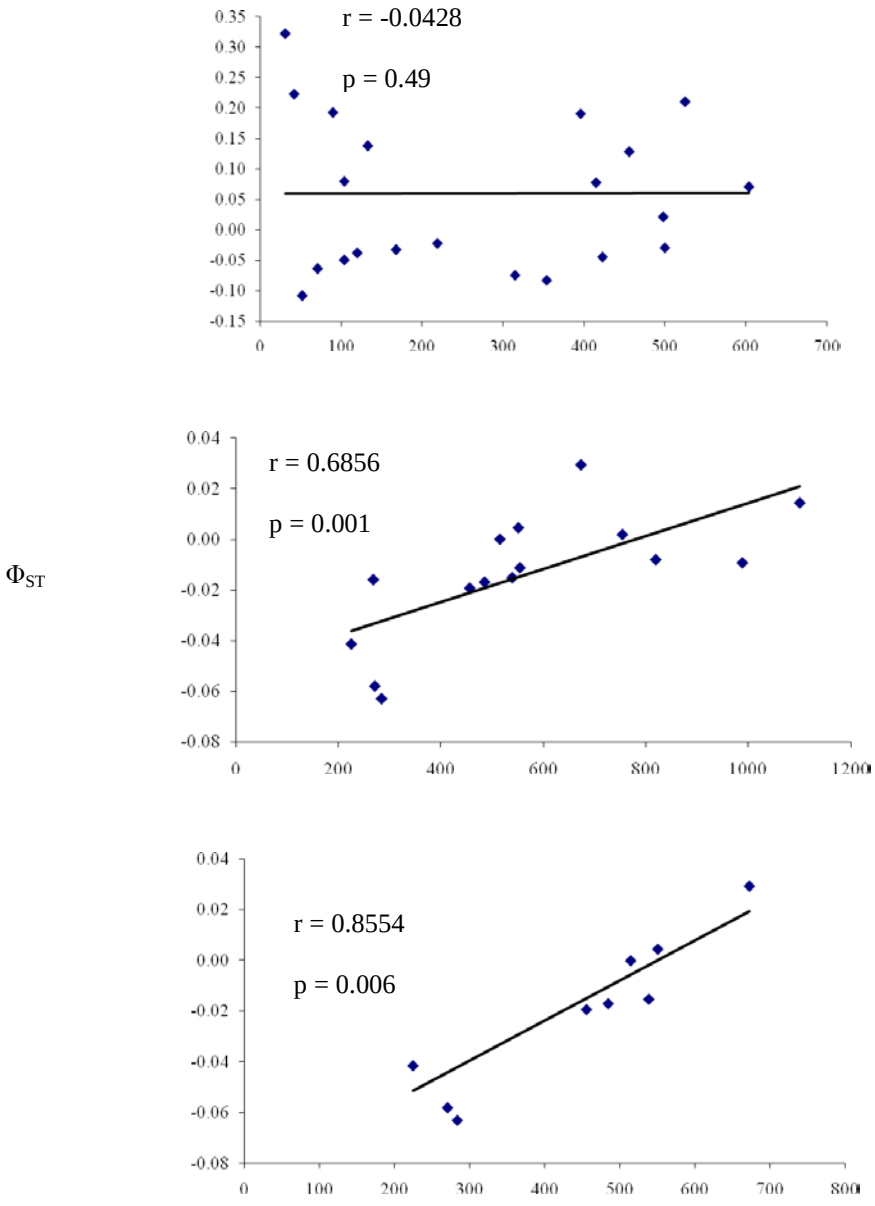
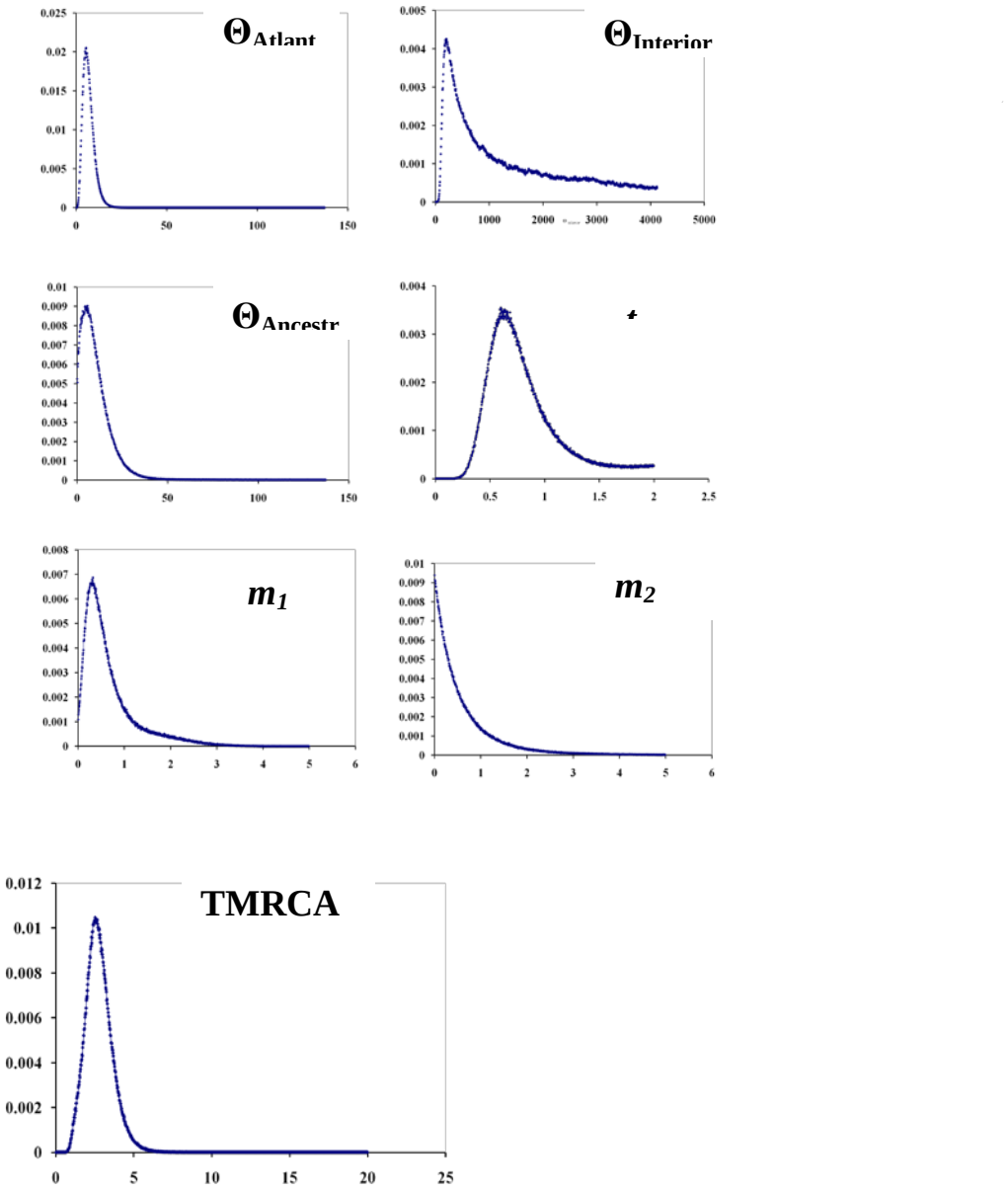


Fig. 7 Posterior distributions of parameter estimates from the IM program scaled to the mutation rate μ. $\theta_{Atlantic}$, $\theta_{Interior}$, and θ_A are effective sizes of the Atlantic, interior, and ancestral populations, $m_1$and m_2 are migration rates; t is the time since population divergence, and TMRCA is the time to most recent common ancestor of the haplotypes.



CHAPTER 3

PHYLOGEOGRAPHY OF THE PAINTED BUNTING (*PASSERINA CIRIS*): IMPLICATIONS FOR CONSERVATION AND EVOLUTION

Abstract

The Painted Bunting (*Passerina ciris*) is a small migratory songbird of conservation concern that breeds in two geographically separate grounds in the southeast and south central United States, and winters in the Florida Keys, the Caribbean, Mexico and portions of Central America. An initial assessment of the breeding grounds suggests that the most significant genetic break within the breeding population is concordant with the 550-km distributional gap that separates the two allopatric breeding populations. For migratory birds, an important aspect of habitat conservation is not only knowledge of the extent to which breeding populations are differentiated but also how these populations vary in their use of migratory pathways and wintering sites. Like many other migratory birds, the Painted Bunting shows differences in molt/migratory strategies between disjunct breeding populations. Birds along the Atlantic Coast molt on the breeding grounds prior to migrating to the wintering grounds, while some birds within the interior population migrate to staging areas in southern Arizona and northern Sonora to molt before continuing on to the wintering grounds. I examined the patterns of connection between breeding and overwintering ranges of this species by evaluating a set of hypotheses related to whether allopatric Atlantic Coast and interior populations use different wintering grounds. Phylogenetic analyses produced a network tree that indicated a separation between the Atlantic Coast breeding and wintering areas (Florida Keys,

Bahamas, and Cuba) and the interior breeding and wintering areas, a result similar to findings of a previous study conducted only on breeding birds.

Introduction

Phylogeography and population structure within the Painted Bunting

The geographical diversity of mitochondrial DNA (mtDNA) haplotypes in populations reflects patterns of historical fragmentation, changes in population size and distribution, and taxon specific dispersal characteristics (Avise 1989), and the use of mtDNA as a marker for phylogeographic studies has provided insights into population histories within the context of evolutionary and biogeographic models (Avise 2000). Molecular population genetics permits description and measurement of how much genetic diversity is present within a species and how much of it is distributed within and among populations. Molecular analyses can be used to describe the present-day geographical distribution of genetic diversity within a species and the phylogenetic relationships among the populations.

The Painted Bunting (*Passerina ciris*) is a small migratory songbird that breeds in the southeast and south central United States, and winters in the Florida Keys, the Caribbean, Mexico and portions of Central America (Fig.8). Two subspecies of the Painted Bunting have been named: *Passerina ciris ciris* and *Passerina ciris pallidior* (Mearns 1911) and the boundary between them runs from east Texas northward between 96° and 97° west longitude (American Ornithologists' Union 1957; Paynter 1970), but may be farther east (Storer 1951; Robbins and Easteria 1992). *Passerina ciris ciris* occurs east of this line and is described as darker red in adult males and darker yellow-green in adult females, and with a smaller mean wing length than that of *P.c. pallidior* (Mearns 1911), which

occurs west of this boundary. *Passerina ciris pallidior* is characterized by “pinker and less orange hue” on red underparts of males (Storer 1951).

The Painted Bunting breeds in two geographically separate Atlantic Coast and interior ranges separated by a 550-km gap at their closet point (Fig. 8). As presently defined, the breeding range of *P. c. ciris* comprises the entire Atlantic Coast breeding population as well as the easternmost portion of the interior population. Along the Atlantic Coast, habitat is primarily shrub, grassland and upland maritime shrub-scrub (American Ornithologists' Union 1998). The breeding range of *P. c. pallidior* comprises the remainder of the interior population. Painted Buntings within the interior breeding population utilize a variety of habitats including open areas with scattered brush and trees, riparian thickets, and, weedy areas (American Ornithologists' Union 1957; Thompson 1991a; American Ornithologists' Union 1998).

Painted Buntings are short to medium distance Neotropical migrants. Neotropical migrants exhibit many different molt/migration strategies. Studies have demonstrated that more than 50% of Neotropical migrant passerine species breeding in western North America migrate to the region of the Mexican Monsoon immediately after breeding, exploiting the seasonal pulse of food generated by the monsoon and undergoing post-breeding molt before migrating to their Neotropical wintering areas (Rohwer et al. 2005; Rohwer et al. 2007; Rohwer et al. 2009). In contrast, more than 95% of Neotropical migrant passerines breeding in eastern North America molt on their breeding grounds before migrating to their Neotropical winter range (Rohwer et al. 2005; Rohwer et al. 2007; Rohwer et al. 2009). The geographically disjunct breeding populations of the Painted Bunting (Atlantic Coast and interior), like many other Neotropical migrants,

exhibit similar patterns of molt and migration. A portion of the interior breeding populations migrate to staging areas in southern Arizona and northern Sonora (an area that is not part of either this bird's breeding or overwintering area) to molt before continuing to wintering ranges, while Atlantic Coast populations' molt on the breeding grounds prior to fall migration (Thompson 1991b).

The conservation of North American migratory songbirds has been the focus of extensive recent research, motivated largely by evidence of long-term decline in populations of numerous species (reviewed by Rappole and Mcdonald 1994; Martin and Finch 1995). Importantly, precipitous declines of the Painted Bunting have made it a bird of considerable conservation concern. Various surveys have indicated dramatic declines in the distribution and abundance of this bird over the past 30 years; the decline in numbers appears to be most severe within the Atlantic Coast breeding population (Hunter et al. 1993; Cox 1996; Sauer et al. 1997; Rich et al. 2004). Factors contributing to the decline are attributed to activities occurring on both the breeding and wintering grounds (Lowther et al. 1999; Inigo-Elias et al. 2002; Sykes and Holzman 2005). Conservation of this species will benefit from an understanding of the major energy-demanding events (breeding, molt, and migration) within its annual cycle. The timing and sequence in which these events occur, and extent to which they overlap with one another, are linked with biological and ecological features of the species themselves (Newton 2008). An initial assessment (Herr et al., 2011) indicates that the most significant genetic break within this species is concordant with 550-km distributional gap that separates the two allopatric breeding populations rather than with recognized subspecies boundaries (Mearns 1911; Storer 1951). The data from this study indicate that the allopatric

breeding populations are recently derived and evolving independently of one another (Herr et al., 2011). Here I incorporate these data with wintering ground data to address alternative questions concerning the migratory patterns that may affect phylogeographic structure and the evolutionary history and conservation of the Painted Bunting.

Relatively few studies have examined the importance of both the seasonal migratory pathway and the timing of molt, as important events in avian speciation (Rohwer and Irwin, in press). Within the Painted Bunting, there is disagreement regarding migratory routes and wintering grounds inhabited by the allopatric breeding populations. Some researchers have suggested that some of the Atlantic Coast breeding populations may cross from Cuba to the Yucatan Peninsula of Mexico during fall migration and possibly winter somewhere in Mexico or Central America (Thompson 1991a; Sykes et al. 2007). Other studies of the Painted Bunting have led to the hypothesis that birds from the Mississippi Valley and the Gulf Coast of Alabama, Mississippi, and Louisiana winter in the Yucatan and that these birds migrate directly across the Gulf of Mexico to their wintering grounds in the Yucatan (Storer 1951; trans-Gulf migration). There is also strong evidence, based on morphological data, that virtually all of the wintering Painted Buntings of Mexico (exclusive of Yucatan) and most of the birds from Central America belong to the populations that breed in Kansas, Oklahoma, and Texas (Storer 1951). In order to address these alternative hypotheses, I will characterize patterns of connection between the populations throughout their annual cycle. Webster et al. (2002) proposed the concept of connectivity for understanding the degree to which breeding individuals from a given demographic unit are shared by various winter locations and vice versa. I will assess the amount of genetic divergence among populations across the distribution,

and the degree of connectivity between the breeding and wintering populations. A pattern of "weak" connectivity suggests that the allopatric breeding birds mix widely on the wintering grounds (Fig. 9a; adapted from Webster et al., 2004), while "strong" connectivity suggests that breeding and wintering grounds are tightly linked (Fig. 9b; adapted from Webster et al. 2004), and lend support to the hypothesis that predicts that the Atlantic Coast breeding populations winter in South Florida, the Bahamas and Cuba and do not cross the Gulf of Mexico into the Yucatan (see Storer 1951).

The molecular analyses will be used to describe the present-day geographical distribution of genetic diversity within the species and the phylogenetic relationships among the populations. A comparison of genetic lineages across the distribution of the Painted Bunting will enable well-informed decisions to be made on how to best conserve and manage declining populations of this migratory bird. The presence of two allopatric breeding populations, along with the utilization of different molt and migration strategies both within the interior breeding populations and between the Atlantic Coast and interior breeding populations, as well as different wintering grounds, could promote further isolation of these populations and may contribute further to the separate evolutionary trajectories of the subspecies.

Specifically, my objectives are to test alternative hypotheses as to the genetic structure between the previously identified Atlantic Coast and interior breeding populations with that of the wintering populations. The allopatric Atlantic Coast and interior populations may overwinter throughout the wintering range of this species, a pattern of "weak" connectivity (Fig. 9a). A pattern of "weak" connectivity may be common in migratory species and may have important consequences at the population level. For example, the

strength of migratory connectivity may affect the ability of migratory species to evolve in response to changing selective pressures, such as those that might result from climate change (Webster et al. 2002). An alternative hypothesis would be a pattern of "strong" connectivity in which the allopatric breeding populations of the Painted Bunting overwinter in separate wintering ranges. Breeding and wintering ranges were compared in five Neotropical migrants and the distribution of eastern and western lineages on the wintering grounds differed among species and ranged from complete segregation to some geographic mixing of eastern and western groups at locations on the wintering ground (Smith et al. 2004). I will address the degree of connectivity between the interior breeding and Mexican/Central American wintering populations and compare that to the Atlantic Coast breeding and Caribbean wintering populations (see Table 5).

Evolutionary History Painted Bunting

In order to address hypotheses regarding the evolutionary history of the Painted Bunting, I will use a combination of analyses that focus on genetic patterns at different temporal scales. I will propose alternative scenarios as to which processes, and the relative timing of the processes, that may have led to the present day distribution of the Painted Bunting. Genetic differentiation within the Painted Bunting may coincide with the geological or climatic changes that have shaped the geographic range of this species, and, as a result, altered the connectivity between the populations over time. The evolution of different migratory strategies involves changes in a complex combination of behavioral, ecological, and life-history traits (Berthold et al. 2003), and from an evolutionary perspective, it will be important to discern where and when these complex combinations of traits evolve. Numerous models can be proposed to explain the interplay

between evolutionary history, patterns of migration, and patterns of molt which all serve to promote longevity and increase reproductive success within the Painted Bunting. The mechanisms by which expansions may occur are not always well known, especially in species that are not restricted to a specific habitat, such as migratory birds (Boulet and Gibbs 2006).

It has been proposed that in birds, migratory species will show less genetic structure along the main migration route than away from it because gene flow is facilitated by migration (Helbig 2003). Support for this hypothesis was found in the northern Yellow Warbler (*Dendroica petechia*, group *aestiva*), a Neotropical migrant with an extremely wide breeding distribution. In this species, gene flow is restricted longitudinally (i.e. along an east-west axis) but not latitudinally (i.e. along the north-south axis) and the latitudinal gene flow axis parallels the migration axis (Boulet and Gibbs 2006). It has also been suggested that latitudinal gene flow may be facilitated by the general wind patterns occurring in North America and by strong flows of maritime tropical air masses in spring, and that migration may have facilitated range expansion during colonization of lands after ice sheet retreat (Gauthreaux Jr. 1980). There are other migratory species that exhibit longitudinal differentiation in North America and the shaping of the east-west differentiation has been attributed to both latitudinal gene flow and the glaciation events of the Pleistocene (Smith et al. 2004). Incorporating information about migratory patterns in general, previous studies of migratory species in North America, and information gained on the degree of connectivity within the Painted Bunting, I will inform models that could explain the evolution of the disjunct breeding and wintering ranges of present day.

In this paper I investigate phylogeography and demographic changes of the Painted Bunting using mtDNA sequence data. I address several questions regarding the genetic structure and evolutionary history of this species. I first describe mtDNA sequence variation within and among local populations throughout their distribution in order to test the hypothesis that the allopatric breeding populations overwinter in separate populations as well (i.e. connectivity). An assessment of the degree of connectivity within the species will play an important role in the development of an effective conservation measure to help reverse the declining populations. Additionally, information gained from these hypotheses will then be used to inform hypotheses as to how different molt/migratory routes may have evolved within this species (as addressed above).

Methods

Sampling and Laboratory Methods

I obtained 67 new samples from 7 sites across the wintering ground distribution of the Painted Bunting to add to the 138 samples available from breeding grounds populations (Herr et al., in press; Appendix I). Total genomic DNA was extracted from tissue, blood and feathers using the DNeasy tissue extraction kit (Qiagen, Valenica, CA). A phylogenetic tree developed using a mitochondrial marker should have a better chance of accurately recovering recent splitting events because its effective population size is one-fourth that of a nuclear gene (Moore 1995). I therefore, sequenced the protein-coding mitochondrial gene NADH dehydrogenase subunit 2 (ND2). I amplified ND2 via polymerase chain reaction (PCR) using the primers L5215 (Hackett 1996) and HTrpc (STRI) in 12.5 μl reactions using the following protocol: denaturation at 94 °C for 10 min, 40 cycles of 94 °C for 30 s, 54 °C for 45 s, and 72 °C for 2 min, followed by 10 min

elongation at 72 °C and 4 °C soak. Products were purified using ExoSAP-IT (USB Corporation, Cambridge, MA) and PCR products were sent to High-Throughput Genomics Unit (University of Washington) for all subsequent steps. Light and heavy strands were aligned in Sequencher 4.9 (GeneCodes Corporation, Ann Arbor, MI). Sequences were translated into amino acids to check for premature stop codons. Complementary strands of each gene were unambiguously aligned using the program Sequencher (Gene Codes Corporation, Ann Arbor, MI). Both light and heavy strands were sequenced for all PCR fragments and no gaps, insertions, or deletions were apparent in the aligned sequence. All sequences were translated without problem into amino acid form.

Phylogenetic Analyses and Population Structure

It is becoming clear that maximizing inferences from any phylogeographic study requires a combination of approaches that examine haplotype relatedness and demographic history, and the use of this combination of approaches has been shown to elucidate geographic structure as well as the evolutionary history producing the structure (Bernatchez 2001; Althoff and Pellmyr 2002; Pfenninger and Posada 2002). The use of multiple approaches has been shown to narrow the range of plausible hypotheses about mechanisms and processes of divergence (Pfenninger and Posada 2002; Morando et al. 2004).

I therefore examined the genetic structure within the Painted Bunting using a series of analyses that focus on genetic patterns at different temporal scales, thereby employing both phylogenetic and population genetic approaches. All analyses were performed using a dataset (n = 205) which included information from both breeding (see Herr et al,

in press) and wintering ground data. The use of a bifurcating tree, may be misleading, especially when the ancestral haplotypes are extant (Althoff and Pellmyr 2002), and the use of a haplotype network may more accurately portray the true evolutionary history of a lineage (Smouse 1998; Posada and Crandall 2001). I therefore used a median-joining network to visualize relationships among haplotypes (program Network 4.1.1.2; Bandelt et al. 1999). Instead of a series of bifurcations, the Network software reconstructs all shortest maximum parsimony trees from a given data set. Median networks provide a useful representation of intraspecific data that are characterized by having few base substitutions between sequences. In contrast to standard tree representation, where only the tips of the tree are labeled, nodes in a median network represent either sampled haplotypes or inferred intermediates. Relationships among the haplotypes was also inferred using a method of statistical parsimony and 95% probability criterion for connections (Templeton et al. 1992) as implemented in the software package TCS, version 1.21 (Clement et al. 2000). TCS was performed as an alternative means of analysis because it can infer ancestral or intermediate haplotypes (as opposed to assuming that these haplotypes are extinct). Statistical parsimony has been demonstrated to exhibit its highest resolving power and significantly out perform traditional phylogenetic approaches when the level of divergence among sequences is low (see: Crandall 1995, 1996; Posada and Crandall 2001). I used the same sequence alignment that I used in the median network analysis described above.

Phylogenetic analyses and population structure

I examined demographic history of populations using the programs Arlequin v. 3.11 (Excoffier et al. 2005) and DnaSP (Rozas et al. 2003) to evaluate departures from

expectations of neutral equilibrium dynamics in the genetic structures of populations, and to analyze patterns of genetic variation within and among populations. Population genetic parameters were calculated for all populations (20) from which we had ≥ 6 individuals (Weir and Cockerham 1984; Excoffier et al. 1992). Genetic diversity within populations was characterized by the number of unique haplotypes per population and the number of private haplotypes per population. Population variability was estimated as haplotype diversity (H) and nucleotide diversity (π). Measures of haplotype and nucleotide diversity are useful in examining the demographic history of a lineage (Grant and Bowen 1998). Centers of origin should be more diverse in haplotype and nucleotide diversity than more recently founded populations (Althoff and Pellmyr 2002), and a large amount of haplotype diversity, but low nucleotide diversity, is consistent with a population bottleneck and rapid population growth (Emerson et al. 2001).

I also performed a number of statistical tests used to estimate past demographic processes such as population expansion. Historical events (i.e. population expansion) can leave a genetic "footprint" that may be detected in sequence data (Ramos-Onsins & Rozas 2002). Analysis of mismatch distribution provide a statistical means to confirm that a proliferation of haplotypes is recent and due to population genetic bottlenecks and/or population expansions (Emerson et al. 2001). Mismatch distributions (i.e. pairwise differences between haplotypes) were generated to test for historical population expansion events within populations by comparing the observed frequency distribution of pairwise nucleotide differences among individuals with distributions expected from a population expansion (Rogers and Harpending 1992). Populations at demographic equilibrium or in decline should exhibit a multimodal distribution of pairwise differences,

whereas populations that have experienced a sudden demographic expansion should display a star-shaped phylogeny and a unimodal distribution (Slatkin and Hudson 1991; Rogers and Harpending 1992). However, mismatch analyses employ a number of assumptions (e.g. random mating, and infinite allele's model) that may not be met in many populations (Wakeley and Hey 1997; Schneider and Excoffier 1999). Because of these limitations, mismatch analyses were coupled with Tajimas's *D* to test for localized population expansion (Tajima 1989) and a test of selective neutrality using Fu's *Fs* test (Fu 1997). Significantly negative *D* or *Fs* values indicate a relative excess of rare haplotype variants, suggesting expansion in population size or population bottleneck; positive values suggest a relative excess of intermediate-frequency alleles, which is expected under a model of population subdivision or balancing selection, coincident with stable population size over time (Tajima 1989; Fu 1997). Critical values for the *D* statistics were determined assuming the beta-distribution as implemented in Arlequin (Excoffier 2005). For the *Fs* tests, $p \leq 0.02$ were assumed to be significant (Excoffier 2005).

I then incorporated population genetic analyses that examine recent population structure. For sequence data, analyses that use both haplotype divergence and the frequency of haplotypes within and among populations should be favored; and even in the case of limited sequence divergence, the distribution of the divergence within and among populations can provide insights into the genetic structure (Excoffier et al. 1992). Analyses, such as analysis of molecular variance (AMOVA) are useful for examining recent geographic structure and permit the testing of *a priori* hierarchical patterns of geographic structure and provide a way to determine the geographic scale of genetic

structure (Excoffier et al. 1992). To estimate population structure at various levels of geographic organization, I used a hierarchical AMOVA in a series of nested procedures. Genetic structure was assessed at three hierarchical scales: within populations, among populations within groups, and among groups. Haplotypic correlation measures, Φ-statistics, were generated with the AMOVA analyses and the following relationships for these statistics are used in the paper: Φ_{CT} = among groups, Φ_{SC}= among populations within groups, and Φ_{ST} = among individuals within populations. This method provides Φ-statistics that are analogous to Wright's F_{ST} (Excoffier et al. 1992). In all analyses a sampling site is equivalent to a population. Significance of variance components was tested using 1000 permutations of the original distance matrix. To identify larger-scale genetic populations, I grouped populations to maximize among group variance (Φ_{CT} values). I performed two nested AMOVAs with sequences grouped by region and then by individual population within each region (i.e. sampling locality) to explore whether significant genetic variation exists at multiple geographic levels. I used an *a priori* expectation of a genetic division between breeding populations (sampling sites) in the interior and Atlantic Coast to form two breeding ground regions (Herr et al., in press). I then separated the wintering birds considering two separate scenarios. As previously stated, it has been suggested that some of the Atlantic Coast breeding populations may cross from Cuba to the Yucatan Peninsula of Mexico during migration and possibly winter somewhere in Mexico or Central America (Thompson 1991a; Sykes et al. 2007). In order to address this hypothesis, I performed the first analysis by including populations from South Florida, Bahamas, Cuba, Cozumel, and the Yucatan (representing 1 wintering group, as suggested in hypothesis) combined with the Atlantic Coast breeding population.

This region was compared with the remaining wintering populations in Mexico and Central America (GUA, OAX, and VER), combined with the interior breeding population (Fig 10a). In the second scenario, I defined a different configuration of the wintering populations. In this analysis, all wintering populations from Mexico and Central America were combined with the interior breeding population to form a group. This group was compared with the Atlantic Coast breeding population and wintering populations from South Florida, the Bahamas, and Cuba (Fig. 10b). The analysis that maximized values of Φ_{CT} after 1000 random permutations of the DNA sequences was assumed to reflect the most probable geographical subdivision (Excoffier et al. 1992), and lend support to or reject a hypothesis that birds from the Atlantic Coast population overwinter in South Florida, the Bahamas, and Cuba, or whether they continue across the Gulf of Mexico to the Yucatan or elsewhere to overwinter (see: Sykes et al. 2007). If the second scenario is true, this would have a large impact on the preservation of genetic diversity within the small, declining Atlantic Coast breeding population. It has been demonstrated that small, isolated populations in particular, are subject to inbreeding and genetic drift and their genetic variation is consequently expected to be low compared to that of larger populations (Karron 1997). Loss of genetic variation is thought, potentially, to lead to a decrease in a species' ability to survive environmental changes and demographic fluctuations (Milligan et al. 1994). Many times it is uncertain as to what constitutes a high or low level of genetic variation within a species; however, the maintenance of at least a constant level of genetic variation is generally considered essential for long-term protection of a taxon (Simberloff 1988).

To further assess genetic structure among groups (as defined by addressing alternative hypotheses and listed in Table 5), a stepping-stone model among the groups was assumed, and AMOVAs were performed on each of the possible connections. These pairwise AMOVAs provided Φ-statistics (pairwise Φ_{CT}) between pairs of groups. To measure the degree of genetic differentiation among populations, pairwise Φ_{ST} values were calculated for all populations of sample sizes greater than or equal to six (Weir and Cockerham 1984). Pairwise Φ_{ST} values were computed in Arlequin (Excoffier 2005) using a distance matrix between haplotypes. A sequential Bonferroni correction (Rice 1989) was applied table-wide.

Results

Phylogenetic analyses

DNA sequencing yielded 1,041 base pairs of ND2 for 205 individuals, 138 from the breeding grounds and 67 from the wintering grounds. No insertions or deletions were present. Of the 1,041 base pairs, 954 were constant with 87 variable sites, 29 of which were parsimony informative.

The median-joining network contained 54 haplotypes, 24 haplotypes belonging exclusively to individuals from one of the breeding ground populations, 19 from an exclusively wintering ground population, and 11 haplotypes shared by both (Fig. 11, Table 6). One of the two most common haplotypes (48 individuals) was also widespread and was comprised of at- least one individual from each population. Forty of the individuals of this haplotype were members of one of the interior breeding or wintering populations. The remaining eight individuals of this haplotype were from Atlantic Coast populations. A second common haplotype (48 individuals) was composed of individuals

found primarily on the Atlantic Coast (43 breeding/wintering individuals), with only five individuals from interior breeding (two), and wintering (three) populations. An additional common haplotype (24 individuals) occurred exclusively in Atlantic Coast populations. Twenty of these were Atlantic Coast breeding individuals, and four belonged to Atlantic Coast wintering individuals. Of the remaining haplotypes, 39 were unique to single individuals. Thirty-four of the haplotypes were from interior breeding or wintering populations, with only five unique haplotypes found among Atlantic Coast breeding or wintering populations. The remaining eleven haplotypes were shared among individuals. There was only one haplotype shared among individuals found on both interior and Atlantic Coast populations (one individual from interior breeding and one individual from Atlantic Coast breeding population). The most divergent haplotypes were found in the interior populations.

The topology of the network generated with TCS (not shown), is consistent with the network generated in Network. Fifty-four haplotypes were again identified. The haplotype identified as the one with the highest outgroup probability was the haplotype that contained 48 individuals, at-least one individual from each of the 22 localities, as seen in the median-joining network produced by the program Network (see Fig. 11, Table 6).

The data on the genetic diversity within populations from the breeding and wintering grounds are presented in Table 7. Mismatch distributions (not shown) for all sampled populations were unimodal and, with the exception of the population from Louisiana ($p<0.01$), did not differ from that expected of an expanding population. Nucleotide diversity was low in all populations and comparable to levels seen in other songbird

studies (see: Milot et al. 2000; Zink et al. 2001; Spellman et al. 2007; Zink et al. 2008), ranging from a low of 0.001 in GA2 to a high of 0.003 in AR (Table 7). Almost all unique haplotypes (private alleles) were restricted to interior populations (breeding/wintering), with each population in the interior having at least one private haplotype (range one-eight). Two populations had very many private alleles, TX3 with seven and GUA with eight. There were no private alleles found on the Atlantic Coast breeding grounds and only two on the Atlantic Coast wintering grounds (FLWN and BAH, each with one). Tajima's *D* values were significant in five of the six interior breeding populations (AR, OK, TX1, TX2, and TX3), and in only one from the Atlantic Coast breeding populations (GA2). GUA also had a significant Tajima's *D* value. Significant Fu's *Fs* values were obtained for four of the same interior breeding populations (AR, OK, TX1, and TX3), with only one from the Atlantic Coast breeding populations (GA2). Fu's *Fs* values were significant in three wintering populations, two from the interior, and one from the Atlantic Coast (YUC, GUA, and FLWN). Fu's *Fs* values for GUA and TX3 were very low (-6.76 and -6.32 respectively). These values, as well as the number of private haplotypes in these two populations, may be an artifact of incomplete sampling on the breeding ground. There were no samples collected from the most southerly portion of the breeding range, in Mexico (see Fig. 8).

Although most of the variation was found within populations in both AMOVA analyses (71% and 70% respectively), a significant portion of the total genetic variance is due to differences among groups (Table 8). Approximately 24% of the variation is explained when Atlantic Coast vs. interior groups are compared (scenario 1, Fig 10a), whereas 28% of the variation is explained when the data were partitioned as suggested in

the second scenario (see Fig. 10b). In pairwise AMOVAs among groups adjacent to each other (Fig.9), significant genetic variation was indicated between Atlantic Coast breeding and interior breeding groups ($\Phi_{CT} = 0.29$, $p < 0.00003$), interior wintering and Atlantic Coast wintering groups ($\Phi_{CT} = 0.21$, $p < 0.02$), interior wintering and Atlantic Coast breeding groups ($\Phi_{CT} = 0.29$, $p < 0.0003$), and interior breeding and Atlantic Coast wintering groups ($\Phi_{CT} = 0.21$, $p < 0.01$). Comparisons of Atlantic Coast breeding and Atlantic Coast wintering groups and interior breeding and interior wintering groups were not significant ($\Phi_{CT} = -0.002$, $p < 0.06$, and $\Phi_{CT} = -0.40$, $p < 0.2$ respectively). Negative values of Φ_{CT} are an artifact of the statistical method and are equivalent to zero (Long 1986; Tansley and Brown 2000; Jonsdottir et al. 2001).

I partitioned the molecular variation in pairwise comparisons of populations into within-population and total-variance components to obtain pairwise Φ_{ST} values (Table 9). The majority of the Φ_{ST} values found to be significant were those comparing Atlantic coast populations (breeding/wintering) to interior populations (breeding/wintering). The highest Φ_{ST} values of 0.607, 0.606, and 0.601 were observed in comparisons of GA2 with OAX, OK, and VER respectively. There were only three significant differences between any pair of interior breeding and wintering populations and only five significant Φ_{ST} values in pairwise comparisons of Atlantic Coast breeding and wintering populations. Three of those comparisons involved the breeding population GA2, with Φ_{ST} values of 0.322 with GA1, 0.138 with FL and 0.193 with SC1.

Discussion

Phylogeography and Population Structure: Painted Bunting

Genetic diversity within populations from across the entire distribution provide evidence of a recent range expansion; including unimodal mismatch distributions, high haplotype diversity, and low nucleotide diversity (Table 7; Rogers and Harpending 1992; Grant and Bowen 1998; Avise 2000). Pairwise AMOVA analyses among geographically separate groups (Fig. 12) indicate significant genetic differentiation between Atlantic Coast and interior regions suggesting that gene flow is limited and support the hypothesis that the allopatric Atlantic Coast and interior breeding populations overwinter in separate areas as well.

The relative abundances of ancestral and derived haplotypes can provide insight into the relative ages of populations (Templeton et al. 1995). Common haplotypes are most likely to be involved in a range expansion or dispersal. With reduced gene flow between the ancestral and colonized populations, derived haplotypes arising within the colonized population will remain geographically confined and should be closely related to one another, separated by few mutational steps (Templeton et al. 1995; Hewitt 1996; Hewitt 2000). The network tree shows a much more complex interior lineage with two centers of differentiation and supports the hypothesis that the Atlantic Coast population is derived from a more ancestral interior population (Fig 11).

Summary statistics also support this conclusion. Members within the interior population have greater haplotype diversity. Private haplotypes are restricted to interior breeding ground populations, another indication that the Atlantic Coast population is a more recently founded population (Table 7). The reduced genetic diversity and low

effective population size within the Atlantic Coast population may have resulted from genetic drift of a small founding population along the Atlantic Coast following dispersal from the interior population. The pattern of lower genetic variation within the Atlantic Coast breeding population is similar to that seen in comparisons of Western and Florida scrub-jays (McDonald et al. 1999) and different forms of the North American Burrowing Owl (Korfanta et al. 2005).

Coalescent analyses indicate that the Painted Bunting diverged from the Varied Bunting during the Pleistocene (~2.2 Mya, see Fig. 2). A previous study conducted on the breeding grounds (Herr et al., in press) demonstrated that the allopatric Atlantic Coast and interior breeding populations diverged ~ 38,000 years ago. These estimates must be interpreted with caution for reasons including assumptions about historical population sizes, and errors associated with the stochastic nature of single locus nucleotide differentiation (Edwards and Beerli 2000). Nonetheless, even allowing for a 10-fold error in my estimate, the divergence in Painted Bunting populations falls within the Pleistocene time period. Our results add to a growing body of evidence implying that the Pleistocene was an important period for intraspecific differentiation (e.g., Avise and Walker 1998). Many studies have highlighted the historical impact of the Pleistocene epoch on phylogeographical patterns of biota throughout the world (Hewitt, 2000; Hewitt, 1996). The Pleistocene epoch was a roughly two million-year period of cyclical glacial advances and retreats that ended about 10,000 years ago. Global temperatures have fluctuated many times between cold and warm conditions during the Quaternary. These major climatic reversals have caused great changes in the distribution of species as evidenced in the fossil record. These range shifts had effects on the distribution of genetic

variation within species (Hewitt, 2004). At the height of the last ice age in North America (Wisconsin) the Laurentide ice sheet extended south of the Great Lakes to approximately 40°N (Hewitt, 2000). Although glaciers never advanced beyond the middle latitudes of the United States, climatic fluctuations associated with these events had considerable effects upon the biota throughout unglaciated regions of North America (Mila et al., 2006; Riddle et al., 2000).

The historical biogeography of the Gulf Coast and the southeastern United States is relatively well studied. Various factors have affected species distributions and population structures in this region. Fluctuations in sea level as a result of glacial and interglacial periods affected habitat availability. During glacial maxima, the Florida peninsula was enlarged due to lower sea levels and was roughly 100 to 150% larger than present day, with most of the increase in size occurring along the Gulf Coast due to a much higher continental shelf (Watts and Hansen, 1994). This newly exposed land was part of a feature that has come to be known as the Gulf Coast Corridor (Emslie, 1998). It is thought to have connected the arid lands of the southwest US and Mexico to Florida. This corridor consisted primarily of dry, open scrubland and savannah although patches of other habitat types such as wetlands and hammocks also occurred (Webb, 1990). During the mid-Pleistocene, this corridor was broken by changes in sea levels as a result of glacial cycles, and the expansion of the Mississippi wetlands resulting in the loss of semiarid habitats along the Gulf Coast (Graham 1999; Webb 1990). Important physiographic features in this region include the Appalachian Mountains, the Mississippi River, and the Apalachicola and Savannah River drainages. These geographic features have been suggested as possible barriers to gene flow in many organisms including

freshwater fish, terrestrial and freshwater tetrapods, coastal vertebrates and invertebrates, as well as many plants (Howes et al., 2006; Joly and Bruneau 2004; Zamudio and Savage, 2003).

Connectivity: breeding and overwintering distributions

There have been relatively few demographic studies, however, that have characterized levels of population connectivity between breeding and overwintering areas of Neotropical migrant songbirds (but see: Smith et al. 2004); limited by the ability to follow a songbird through a complete annual cycle. For example, the strength of connectivity may affect the ability of migratory species to evolve in response to changing selective pressures, such as those that might result from climate change (Webster et al. 2002). Some migratory songbird species show "strong" connectivity patterns. The Black-throated Blue Warbler (*Dendroica caerulescens*) does not have a disjunct breeding range, but most birds wintering on western Caribbean islands come from the northern portion of the species' breeding range, while those on more easterly islands are primarily from southern breeding areas (Rubenstein et al. 2002). Swainson's Thrush (*Catharus ustulatus*) show nearly complete segregation of migratory routes and of over-wintering sites (Ruegg and Smith 2002).

The results obtained from the breeding ground study (Herr et al. in press) indicated a genetic break between the Atlantic Coast and interior populations. I used this information to explore patterns of connectivity between breeding and overwintering ranges for this species. It has been suggested that Atlantic Coast Painted Buntings may cross the Gulf of Mexico from Cuba to the Yucatan Peninsula of Mexico during migration and possibly winter somewhere in Mexico or Central America (Sykes et al.

2007). The present study indicates that this is not the case. Eight of the 15 individuals collected in the Yucatan are part of the most common and widespread haplotype (Table 6). Three others are shared among individuals found in the interior population. There is only one unique haplotype and the remaining three haplotypes are part of the second common haplotype.

My results lend support that these two allopatric breeding populations overwinter in separate locations. Pairwise Φ_{ST} analyses of almost all Atlantic Coast breeding populations are not significantly different from any Atlantic Coast wintering population (as defined in Table 5). Almost all comparisons of Atlantic Coast breeding populations with interior wintering populations are significant (Table 7). Additionally, there was no significant variation of pairwise AMOVAs among groups (Φ_{CT}) when Atlantic Coast breeding and Atlantic Coast wintering, or interior breeding and interior wintering were analyzed. Results of both of these analyses support the hypothesis that the Atlantic Coast breeding population winters primarily in South Florida, the Bahamas and Cuba, while birds from the interior breeding populations' winter in Mexico and Central America.

These patterns suggest that when viewed at a broad scale, connectivity is strong. However, with the use of a single mtDNA marker, I was unable to resolve whether there are also within region patterns of connectivity. There has been some success in combining mtDNA data with other intrinsic markers (e.g. isotope data and genetic data such as amplified fragment length polymorphisms (Bensch et al. 2002; Lovette et al. 2004) to increase resolution in order to address finer scale connectivity of populations, and additional analyses, combining my results with other markers, is warranted.

Conservation implications

Understanding linkages between areas used by animals throughout their life history is critical to their effective conservation since efforts can be directed more appropriately at breeding, wintering, and stopover sites (e.g., Myers et al., 1987). Effective conservation and management of migratory bird species requires an understanding of when, where, and how populations are limited (Sillett and Holmes 2005). Demographic studies indicate that populations of many Neotropical migrant songbirds are limited at least partly by their winter habitats (Sherry and Holmes, 1996). Conservation efforts need to integrate explicitly the effects of local ecological factors over large spatial scales and to integrate effects of winter survival with reproduction and survival at other times of year. My attempt to link breeding and overwintering populations is of conservation relevance. Given my findings of genetic differentiation of Painted Buntings within the breeding and wintering populations, along with the previously identified information on molt and migration (Thompson 1991b), the interior and Atlantic Coast populations should be recognized as distinct. Conservation efforts should treat these allopatric populations as separate lineages. It may be that the interior population should be treated as separate entities as well (as discussed above).

Although there is genetic differentiation between the Atlantic Coast and interior populations, no significant differences exist within the interior populations as demonstrated by the lack of significant population pairwise Φ_{ST} values between interior populations. Differences between the interior populations may have arisen relatively recently, and possibly in the presence of ongoing gene flow. Studies have shown that migratory routes can arise very rapidly (Berthold 1996; Able and Belthoff 1998). The

rapid appearance of a new migratory route may be facilitated by nongenetic factors such as learning or cultural change; however studies on a variety of taxa have demonstrated that genetic differences often underlie migratory differences among populations (Raleigh 1971; Berthold et al. 1992; Dingle 1994; Berthold 1996). Rapid evolutionary change is thought to be driven by strong natural selection (Fraser and Bernatchez 2005) and perhaps facilitated by assortative mating (Webster and Marra 2004).

The genetic similarity between the *P. c. pallidior* and *P.c. ciris* subspecies within the interior breeding distribution contrasts with the phenotypic differences between these forms. The results presented here demonstrate that migratory divergence exists within the interior population and apparently in the face of ongoing gene flow, the specific evolutionary mechanisms responsible are not known. This migratory divergence may be consistent with the migratory divide in interior populations of Painted Buntings that move to the staging area for their fall molts (Thompson 1991b; Young 1991) or perhaps the divide is consistent with the presently defined subspecies boundaries {see Fig. 3; American Ornithologists' Union, 1957). Additionally, sub-specific delineation in bird taxonomy has traditionally been based on recognizable differences in morphological character variation in populations in different geographical regions. The two subspecies of Painted Buntings have been described based on variation in plumage color and wing length and the genetic diversity may be concordant with their morphological separation. The recognized boundary that separates the two subspecies runs through Texas at about 96°/97° west longitude. This is a dividing line between different ecoregions in Texas and these regions have very different environmental characteristics including habitat, amount of rainfall, elevation, and soil conditions. These different environmental or nongenetic

influences may have effected phenotypic change in these birds. Geographic character variation in birds is many times attributed to natural selection for phenotypes that reflect locally adapted genetic differences (James, 1983). This geographical variation may have arisen and may be maintained by local selection pressures acting to maintain adaptive differences in plumage variation (Zink R and Remsen Jr., 1986) or the phenotypic differences may reflect plastic responses to environmental cues however this seems unlikely, as other studies have shown that heritable variation exists in plumage color variation (Theron et al., 2001). In addition, there is circumstantial evidence for geographically varying selection pressures on plumage variation in birds (Price, 1998).

Migratory divides have been suggested as a driver of speciation within birds (Thompson 1991b), but I find no evidence of genetic divergence in the interior breeding populations. The lack of genetic divergence could be due to ongoing gene flow, the relatively young age of this species, or my inability to detect signatures of genetic divergence with the marker utilized in this project. I hypothesize that geographical variation in local selection pressures act to maintain adaptive differences in migratory behavior, molt strategy, and possibly plumage variation. Alternatively, the phenotypic differences may reflect plastic responses to environmental cues (Lessells 2008). This does not seem likely, as other studies have shown that heritable variation exists for migratory behavior (Berthold and Helbig 1992) as well as plumage traits (Theron et al. 2001) in birds. Differences in wing length may be the result of pressures arising from the differing migratory pathways used by the populations. There have been several recent studies involving migratory divides and their relative importance in speciation, hybrid zones, and rapid evolution of morphological traits (Sutherland 1998; Ruegg 2007; Ryan

et al. 2007; Brelsford and Irwin 2009; Rolshausen et al. 2009). For example, a recent establishment of a migratory divide within Central European blackcaps (*Sylvia atricapilla*) has been shown to develop within 30 generations. It was shown that differential migratory orientation facilitated reproductive isolation of sympatric populations. The genetic divergence in sympatry was shown to exceed that of allopatric populations and was associated with phenotypic differences in wing morphology. It was hypothesized that restricted gene flow accelerated the evolution of adaptive phenotypic divergence toward different selection regimes (Rolshausen et al. 2009). There is also evidence that similar adaptive processes can occur in more than 50 bird species that have recently changed their migratory behavior (Sutherland 1998; Fiedler 2003).

The data presented here demonstrate that differences exist between Atlantic Coast and interior populations of the Painted Bunting that show little genetic differentiation from one another at neutral markers and without being reciprocally monophyletic. Similar results have been reported in other migratory taxa (Bensch et al. 1999; Buerkle 1999; Brower and Jeansonne 2004; Davis et al. 2006), an indication that changes in complex ecological traits can occur rapidly and often precede divergence at neutral markers. Differences in morphology, migratory pathway, and molting strategies also exist among populations of the interior birds. It is essential, that further work, aimed at determining the exact location of the migratory divide within the interior, be done. Understanding the dynamics of gene flow between the closely related forms may provide insight into the process of speciation. Hybrid zones have sometimes been correlated with migratory divides and there is evidence suggesting that differences in migration-related traits may promote reproductive isolation (Rohwer and Manning 1990; Helbig 1991; Bensch et al.

1999; Webster et al. 2002; Irwin and Irwin 2004; Bearhop et al. 2005; Webster and Marra 2004). A more comprehensive understanding of the contemporary processes involved in the present day Painted Bunting will allow for a much greater understanding of the evolutionary history of this bird.

Conclusions

In this paper, I have provided evidence about processes affecting the genetic structure and present-day distribution of the Painted Bunting. While my results are statistically significant and geographically explicable, further research, employing additional markers, using stable isotope analyses, or satellite telemetry will refine and augment my conclusions. Additional studies will further augment my conclusions and provide stronger evidence about the forces that have shaped the genetic structure of the Painted Bunting. My data is not sufficient to determine whether the interior populations represent two separate independently evolving entities, or incomplete lineage sorting as hypothesized for the Atlantic Coast and interior populations, or whether the morphological differences remain despite ongoing gene flow. I believe, however, that the data presented here will have important consequences in any conservation strategy aimed at reversing the declines of the Atlantic Coast population.

Ultimately, evolutionary biologists attempt to understand how selection pressures may favor one particular life history over another, in order to provide evolutionary explanations for the diversity of life histories in the living world. This is especially important in order to have an understanding of how life histories might respond to changes in selection pressures, including the changes caused by anthropogenic environmental change. Increased sampling and additional markers will allow for more

accurate hypothesis testing involving molt/migration strategies employed by different populations of the Painted Bunting and provide better solutions in how to protect species in changing climates; especially in light of global warming.

Chapter 3, Table 5 Sampling sites (i.e. populations) included in one of four regional populations as described in paper. (Specific locality and GPS coordinates in Appendix 1).

Interior Breeding Population:
Arkansas (AR)
Louisiana (LA)
Oklahoma (OK)
Texas 1 (TX1)
Texas 2 (TX2)
Texas 3 (TX3)

Interior Wintering Population:
Cozumel (COZ)
Guatemala (GUA)
Oaxaca (OAX)
Veracruz (VER)
Yucatan (YUC)

Atlantic Coast Breeding Population:
Florida (FL)
Georgia 1 (GA1)
Georgia 2 (GA2)
Georgia 3 (GA3)
North Carolina 1 (NC1)
North Carolina 2 (NC2)
South Carolina 1 (SC1)
South Carolina 2 (SC2)

Atlantic Coast Wintering Population:
Florida Wintering (FLWN)
Bahamas (BAH)
Cuba (CUY)

Table 6 Haplotype frequency by geographic location as seen in median-joining network as constructed in Network (Bandelt et al., 1999). Outgroup probabilities as estimated in program TCS (Clement et al., 2000). * indicates haplotype with greatest ancestral probability.

Hap. #	# Ind.	Interior Breeding (*P.c. pallidior*)	Interior Breeding (*P.c. ciris*)	Atlantic Coast Breeding (*P.c. ciris*)	Bahamas Winter	Cozumel Winter	Cuba Winter	Florida Winter	Guatemala Winter	Oaxaca Winter	Veracruz Winter	Yucatan Winter	Haplotype outgroup probabilities as estimated in TCS
1	48	10	14	4	1	1	1	1	5	2	1	8	0.12*
2	48	0	2	34	2	0	5	2	0	0	0	3	0.08
3	24	0	0	20	1	0	3	0	0	0	0	0	0.08
4	10	0	0	7	3	0	0	0	0	0	0	0	0.01
5	5	1	4	0	0	0	0	0	0	0	0	0	0.06
6	5	0	0	5	0	0	0	0	0	0	0	0	0.01
7	5	0	0	3	0	0	0	2	0	0	0	0	0.06
8	5	1	3	0	0	0	0	0	0	0	0	1	0.06
9	3	1	1	0	0	0	0	0	0	0	0	1	0
10	3	0	0	2	1	0	0	0	0	0	0	0	0
11	2	0	1	0	0	0	0	0	0	0	0	1	0.06
12	2	0	1	1	0	0	0	0	0	0	0	0	0
13	2	0	0	2	0	0	0	0	0	0	0	0	0
14	2	0	0	0	0	0	0	0	2	0	0	0	0

15	2	1	0	0	0	0	0	0	0	0	1	0	0
16	1	0	1	0	0	0	0	0	0	0	0	0	0
17	1	0	1	0	0	0	0	0	0	0	0	0	0
18	1	0	0	0	1	0	0	0	0	0	0	0	0
19	1	0	0	0	0	1	0	0	0	0	0	0	0
20	1	0	0	0	0	0	0	1	0	0	0	0	0
21	1	0	0	0	0	0	0	1	0	0	0	0	0
22	1	0	0	0	0	0	0	1	0	0	0	0	0.01
23	1	0	0	0	0	0	0	1	0	0	0	0	0.03
24	1	0	0	0	0	0	0	0	1	0	0	0	0.06
25	1	0	0	0	0	0	0	0	1	0	0	0	0.06
26	1	0	0	0	0	0	0	0	1	0	0	0	0
27	1	0	0	0	0	0	0	0	1	0	0	0	0
28	1	0	0	0	0	0	0	0	1	0	0	0	0
29	1	0	0	0	0	0	0	0	1	0	0	0	0
30	1	0	0	0	0	0	0	0	1	0	0	0	0
31	1	0	0	0	0	0	0	0	1	0	0	0	0
32	1	0	1	0	0	0	0	0	0	0	0	0	0
33	1	0	0	0	0	0	0	0	0	1	0	0	0

34	1	0	0	0	0	0	0	0	0	1	0	0	0
35	1	1	0	0	0	0	0	0	0	0	0	0	0
36	1	1	0	0	0	0	0	0	0	0	0	0	0
37	1	1	0	0	0	0	0	0	0	0	0	0	0
38	1	1	0	0	0	0	0	0	0	0	0	0	0.06
39	1	1	0	0	0	0	0	0	0	0	0	0	0.06
40	1	1	0	0	0	0	0	0	0	0	0	0	0.06
41	1	1	0	0	0	0	0	0	0	0	0	0	0
42	1	1	0	0	0	0	0	0	0	0	0	0	0
43	1	1	0	0	0	0	0	0	0	0	0	0	0
44	1	1	0	0	0	0	0	0	0	0	0	0	0
45	1	1	0	0	0	0	0	0	0	0	0	0	0
46	1	1	0	0	0	0	0	0	0	0	0	0	0
47	1	1	0	0	0	0	0	0	0	0	0	0	0
48	1	1	0	0	0	0	0	0	0	0	0	0	0
49	1	1	0	0	0	0	0	0	0	0	0	0	0
50	1	1	0	0	0	0	0	0	0	0	0	0	0
51	1	0	1	0	0	0	0	0	0	0	0	0	0
52	1	0	0	0	0	0	0	0	0	0	1	0	0

53	1	0	0	0	0	0	0	0	0	0	1	0	0
54	1	0	0	0	0	0	0	0	0	0	0	1	0
Total	205	30	30	78	9	2	9	9	15	4	4	15	0.76

Table 7. Genetic diversity within populations. The values in the columns correspond to sample size (N), number of unique haplotypes (H), number of private haplotypes (Pri.), haplotype diversity (Hd), nucleotide diversity (π), the significance of the mismatch distribution (MM; ns = not significantly different from the expectation under exponential growth), Tajima's *D* (*D*), and Fu's *Fs* (*Fs*). Significant values shown in bold.

BREEDING		**N**	**H**	**Pri.**	**Hd**	***π***	**MM**	***D***	***Fs***
Atlantic Coast	NC1	10	6	0	0.844	0.002	ns	-0.127	-1.363
	NC2	10	5	0	0.800	0.001	ns	-1.262	-1.320
	SC1	9	5	0	0.861	0.002	ns	0.241	0.911
	SC2	10	3	0	0.689	0.002	ns	0.927	1.667
	GA1	9	4	0	0.778	0.002	ns	1.612	0.450
	GA2	10	5	0	0.667	0.001	ns	**-1.741**	**-2.260**
	GA3	9	5	0	0.806	0.001	ns	0.497	-0.787
	FL	9	4	0	0.806	0.002	ns	0.881	0.617
Interior	AR	10	7	2	0.867	0.003	ns	**-1.944**	**-2.968**
	OK	11	7	4	0.778	0.002	ns	**-1.873**	**-2.442**
	LA	10	5	1	0.822	0.002	<0.01	-0.586	-0.815
	TX1	9	7	5	0.917	0.002	ns	**-1.823**	**-3.797**
	TX2	9	5	1	0.893	0.002	ns	**-1.640**	-1.802
	TX3	10	9	7	0.978	0.003	ns	**-1.586**	**-6.320**

WINTERING									
Atlantic Coast	FLWN	9	6	1	0.917	0.002	ns	0.518	**-6.212**
	BAH	9	6	1		.003	ns	-0.345	**-4.774**
	CUY	9	4	0	0.694	0.002	ns	0.497	**-8.726**
Interior	YUC	15	6	1	0.705	0.001	ns	**-1.580**	**-18.839**
	GUA	15	10	8	0.895	0.002	ns	**-1.865**	**-20.673**
	OAX	4	3	2	0.883	0.002	ns	-0.797	-1.513
	VER	4	4	2	1.000	0.002	ns	-0.797	-1.514

Table 8 Analysis of molecular variance (AMOVA). Analyses were performed separately for populations in each group.

Group	Source of Variation	% Variation	Φ Statistic	P
Scenario 1: A	Among Groups	24.3	0.24	< 0.0001
Interior Breeding/GUA, VER, OAX Wintering vs. Atlantic Coast Breeding/FLWN, CUY, BAH, COZ, and YUC Wintering	Among populations within groups	4.9	0.06	< 0.001
	Within populations	70.8	0.29	< 0.0001
Scenario 2: B	Among Groups	27.7	0.28	< 0.0001
Interior Breeding/GUA, VER, OAX, COZ, and YUC Wintering vs. Atlantic Coast Breeding/FLWN, BAH, and CUY Wintering	Among populations within groups	2.4	0.03	< 0.03
	Within populations	69.9	0.30	< 0.0001

Table 9 Population pair-wise Φ_{ST} values. Significance of Φ_{ST} values determined by 1000 random permutations of individuals among populations in a comparison. Values shown in bold significant at $\alpha = 0.05$ after a false discovery rate correction. Table A: Interior breeding vs. Interior wintering. Table B: Atlantic Coast breeding vs. Atlantic Coast wintering. Table C: Interior wintering vs. Atlantic Coast wintering. Table D: Atlantic Coast breeding vs. interior breeding.

Table A

	YUC	GUA	OAX	VER	AR	OK	LA	TX1	TX2	TX3
YUC	*									
GUA	**0.057**	*								
OAX	0.093	0.045	*							
VER	**0.112**	0.021	-0.053	*						
AR	-0.029	0.019	-0.011	0.011	*					
OK	0.036	0.005	0.013	-0.015	-0.022	*				
LA	-0.021	**0.060**	0.067	0.045	-0.037	0.028	*			
TX1	0.034	0.015	0.005	-0.008	-0.016	0.001	0.012	*		
TX2	-0.040	0.017	0.037	0.037	-0.061	-0.017	-0.074	-0.018	*	
TX3	0.032	0.032	-0.044	-0.003	-0.007	0.002	0.024	-0.011	-0.009	*

Table 9B

	NC1	NC2	SC1	SC2	FL	GA1	GA2	GA3	BAH	FLWN	CUY
NC1	*										
NC2	0.079	*									
SC1	-0.075	0.077	*								
SC2	-0.071	-0.007	-0.074	*							
GA1	-0.045	**0.210**	-0.038	0.021	-0.050	*					
GA2	0.190	0.021	**0.193**	0.139	**0.138**	**0.322**	*				
GA3	-0.079	0.122	-0.102	-0.051	-0.031	-0.061	0.211	*			
BAH	-0.042	-0.025	-0.060	-0.082	-0.035	0.017	0.123	-0.020	*		
FLWN	**0.147**	0.302	0.067	0.189	0.183	0.171	0.345	0.079	0.162	*	
CUY	-0.084	0.128	-0.093	-0.055	-0.033	-0.064	0.232	-0.101	-0.022	0.147	*

Table 9C

	YUC	GUA	OAX	VER	BAH	FLWN	CUY
YUC	*						
GUA	**0.057**	*					
OAX	0.093	0.045	*				
VER	**0.112**	0.021	-0.053	*			
BAH	**0.227**	**0.323**	**0.270**	**0.270**	*		
FLWN	**0.270**	**0.333**	**0.311**	**0.311**	0.162	*	
CUY	**0.156**	**0.273**	**0.255**	**0.255**	-0.022	0.147	*

Table 9D

	AR	OK	LA	TX1	TX2	TX3	NC1	NC2	SC1	SC2	FL	GA1	GA2	GA3
AR	*													
OK	-0.022	*												
LA	-0.037	0.028	*											
TX1	-0.017	0.001	0.012	*										
TX2	-0.061	-0.017	-0.074	-0.018	*									
TX3	-0.007	0.002	0.024	-0.011	-0.009	*								
NC1	**0.196**	**0.286**	**0.162**	**0.265**	**0.196**	**0.208**	*							
NC2	**0.416**	**0.521**	**0.386**	**0.493**	**0.451**	**0.432**	0.079	*						
SC1	**0.147**	**0.249**	0.108	**0.228**	0.148	**0.173**	-0.075	0.077	*					
SC2	**0.273**	**0.379**	**0.236**	**0.354**	**0.289**	**0.289**	-0.071	-0.007	-0.074	*				
FL	**0.249**	**0.347**	**0.217**	**0.321**	**0.260**	**0.262**	-0.030	0.070	-0.023	-0.022	*			
GA1	**0.141**	**0.226**	0.120	**0.207**	**0.144**	**0.146**	-0.045	**0.210**	-0.038	0.021	-0.050	*		
GA2	**0.500**	**0.606**	**0.473**	**0.577**	**0.553**	**0.512**	0.190	0.021	**0.193**	0.139	**0.138**	**0.322**	*	
GA3	0.163	**0.261**	0.128	**0.240**	0.166	**0.180**	-0.079	0.122	-0.102	-0.051	-0.031	-0.061	0.211	*

Fig.8 Distribution of the Painted Bunting. Light green indicates breeding range, dark green wintering range. A 550-km gap separates subspecies *ciris'* two breeding populations. Blue dots indicate sampling locations on breeding and wintering grounds. Group defined in text as interior breeding population highlighted dark blue box; group defined as Atlantic Coast breeding population highlighted in red.

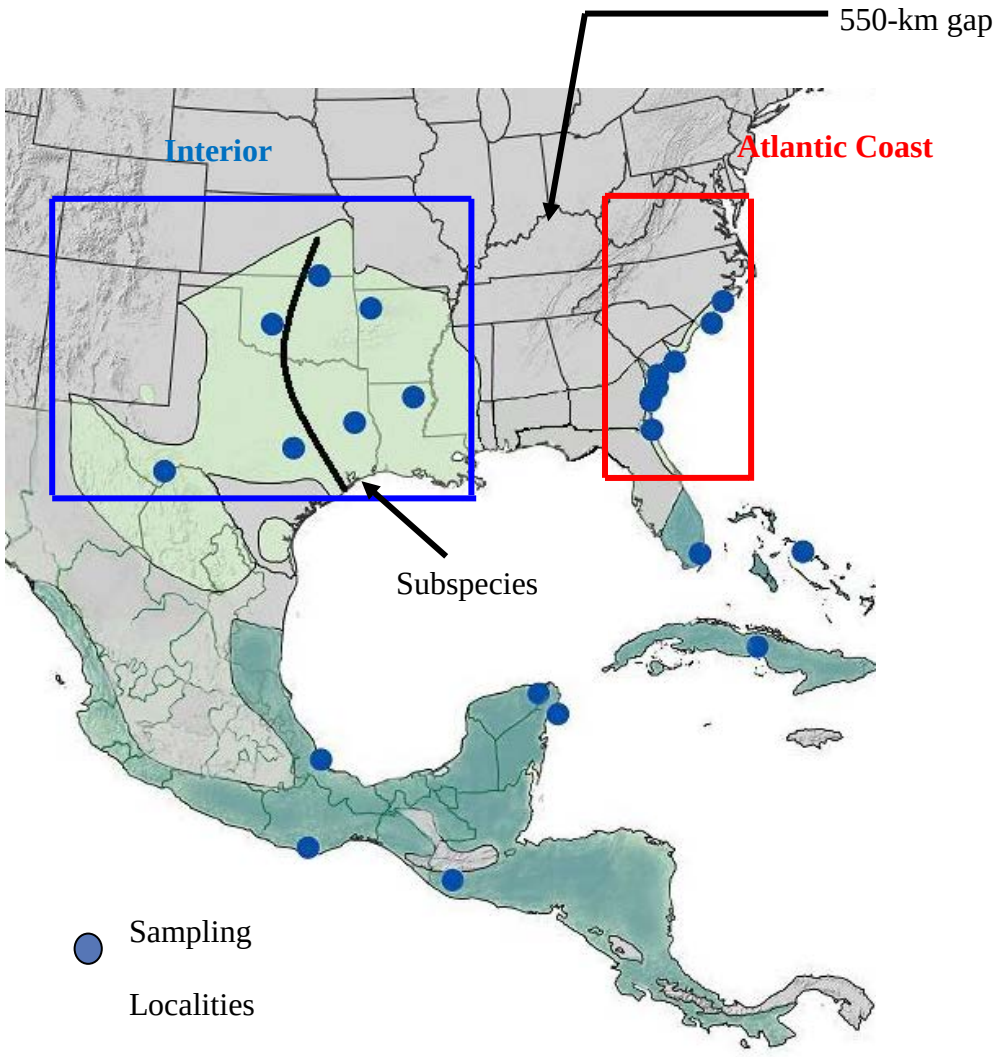


Fig. 9a and b "Weak" connectivity pattern in which allopatric breeding populations (interior and Atlantic Coast) overwinter throughout the wintering range (adapted from Webster and Marra, 2004). "Strong connectivity pattern in which allopatric breeding populations overwinter in specific wintering range (adapted from Webster and Marra, 2004).

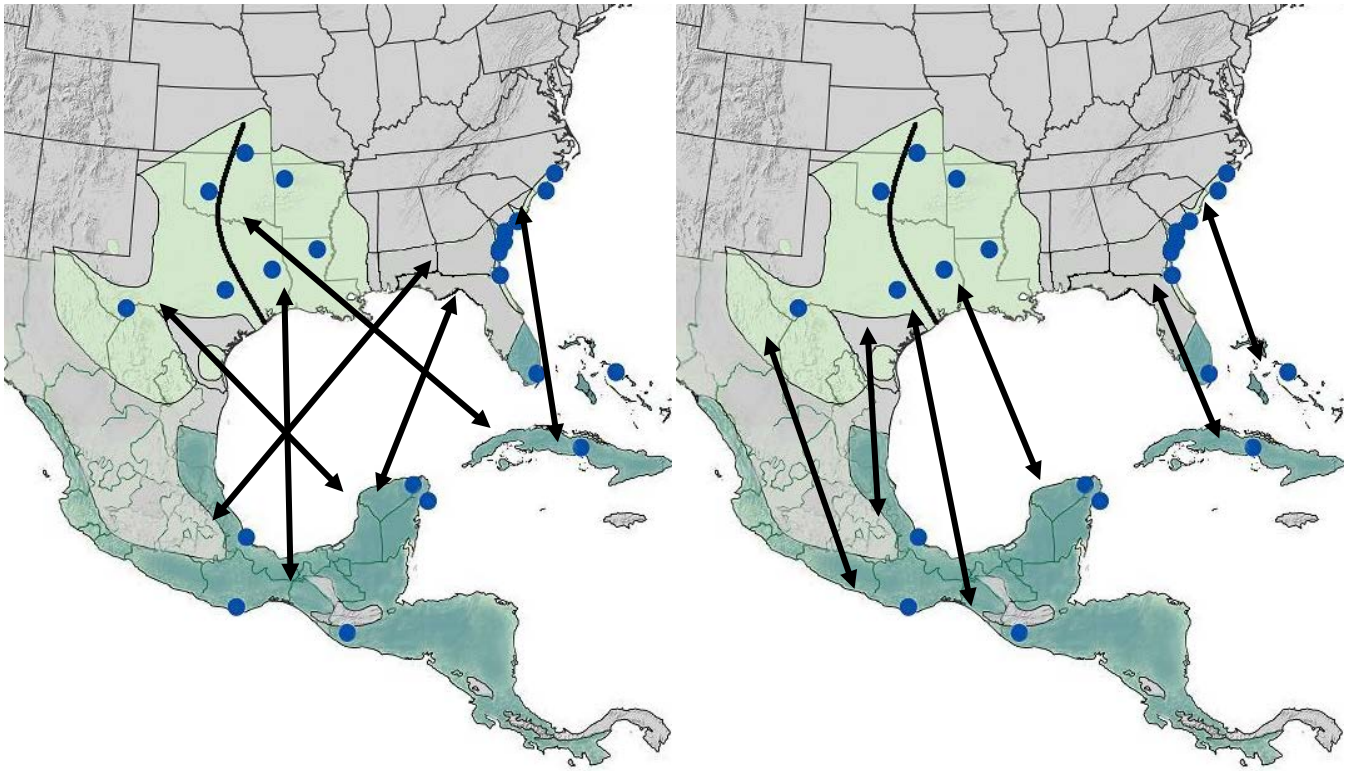


Fig. 10a Scenario 1 Distribution map highlighting groups based on hypothesized wintering ranges (see Sykes et al., 2007). Groups defined as interior breeding population, interior wintering population (GUA, OAX, and VER), Atlantic Coast breeding population, and Atlantic Coast wintering population (FLWN, BAH, CUY, COZ, and YUC).

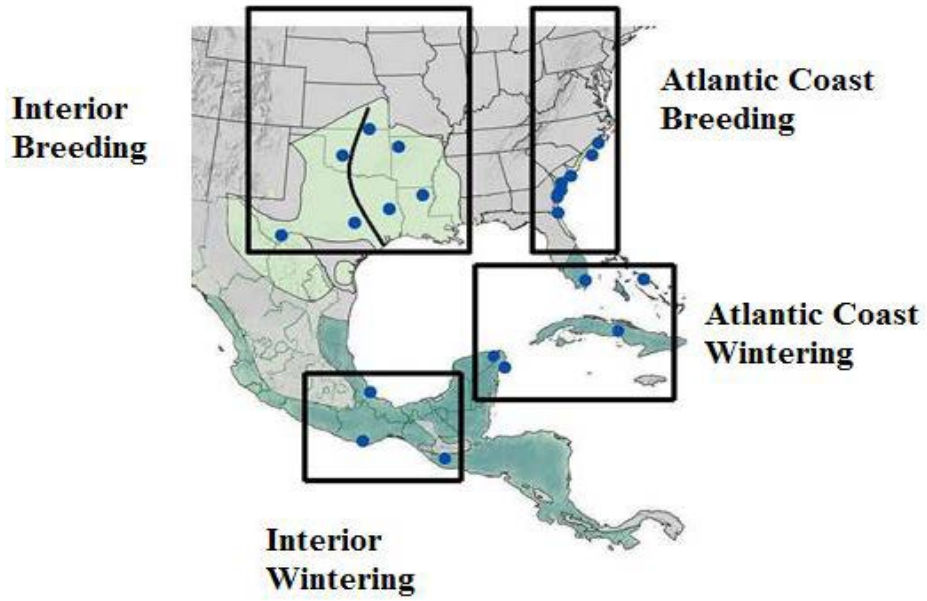


Fig. 10b Scenario 2. Distribution map highlighting groups based on geographic isolation (alternative hypothesis). Groups defined as interior breeding population, Atlantic Coast breeding population, interior wintering population (GUA, OAX, VER, COZ, and YUC), and Atlantic Coast wintering population (FLWN, BAH, and CUY).

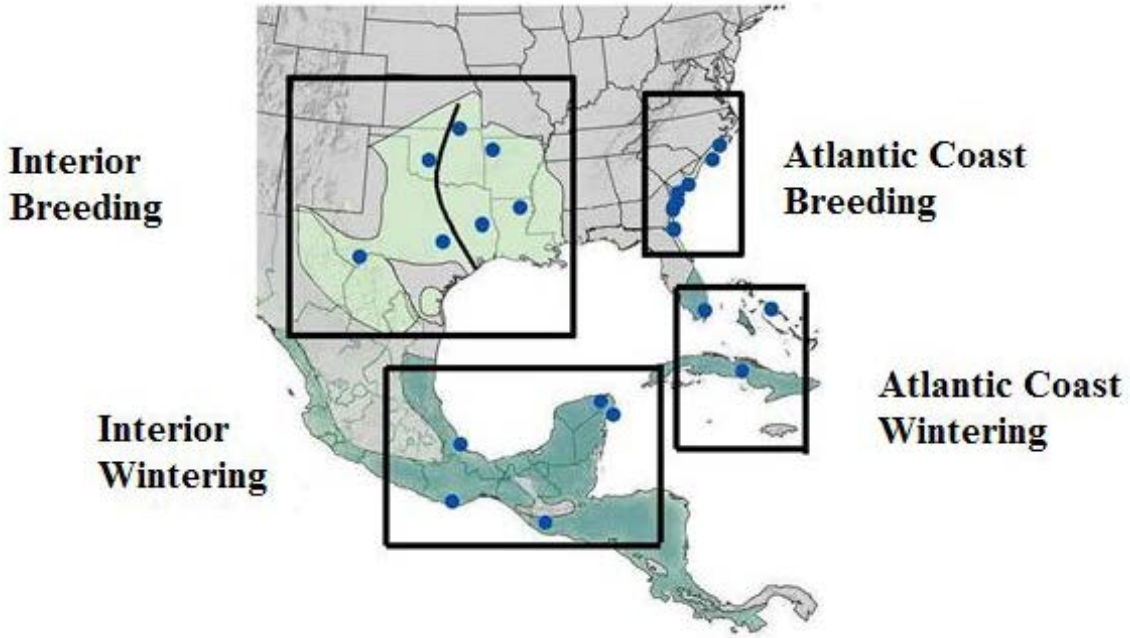


Fig. 11 Median-joining network of Painted Bunting haplotypes (n=205). Circles represent one of the 54 unique haplotypes sized proportionally to the number of individuals sharing the haplotype. Hash marks represent single base pair differences between haplotypes. Small red circles represent median vectors.

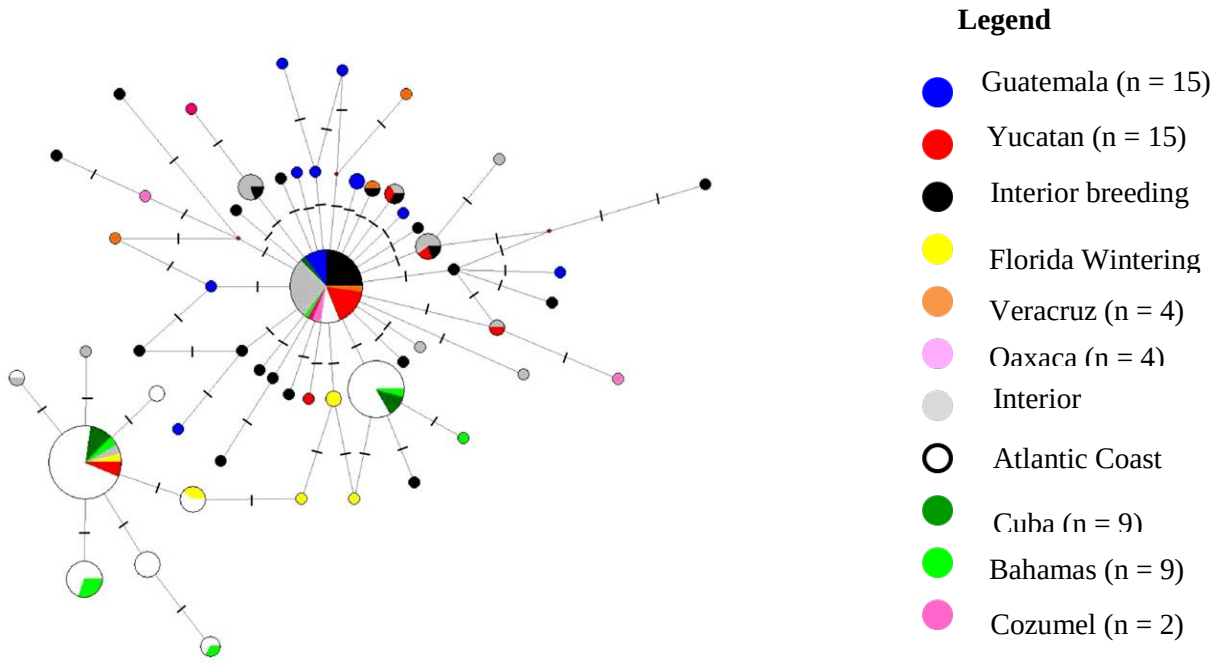


Fig. 12 Distribution of the Painted Buntings year round range; arrows indicate possible stepping-stone dispersal. Pairwise Φ_{CT} statistics performed on six possible stepping-stone scenarios.

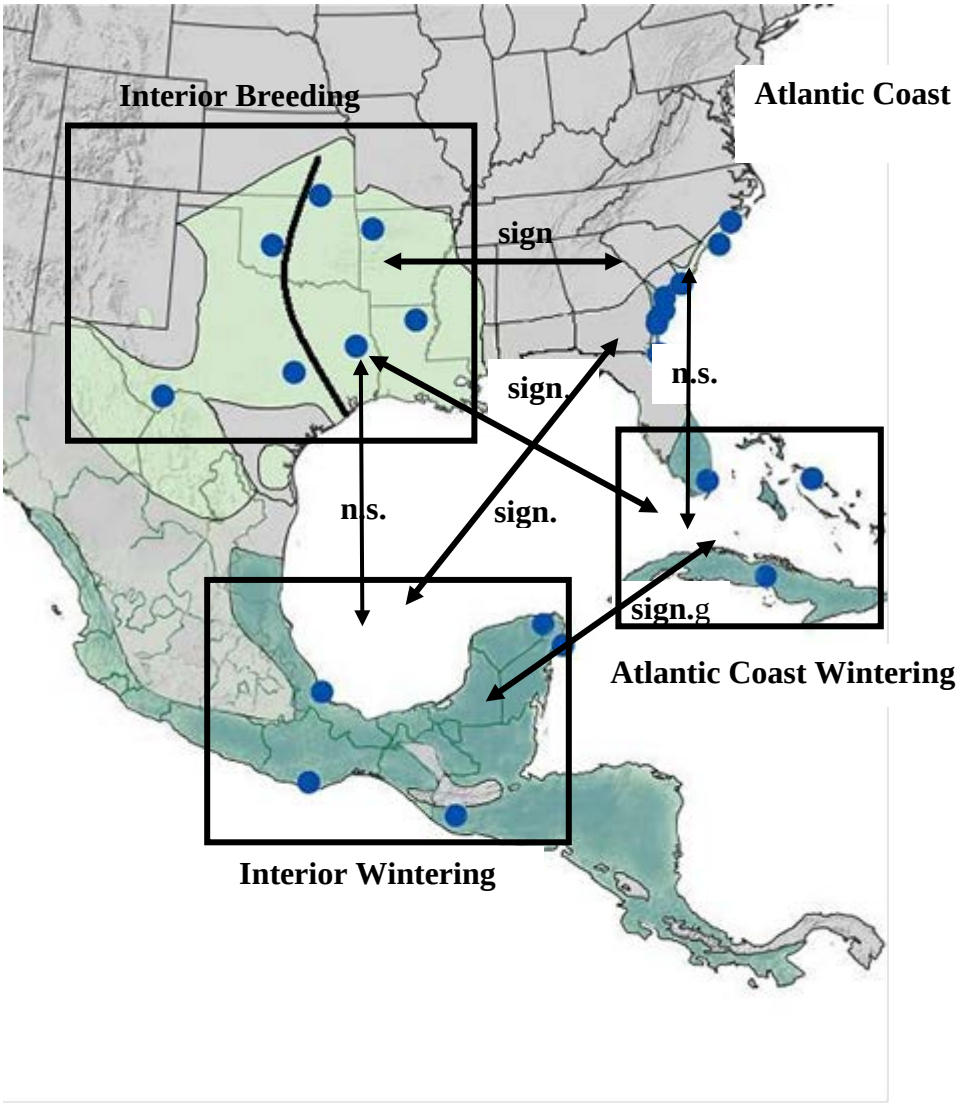


Table 10 Specimen Data

Genus	Species	Catalog[1]/Band Number	Prep Number	County/State Province	County/Parish	Specific Locality[2]	Coordinates in Decimal Degrees
Passerina	*ciris*	MBM 16263	cah163	Arkansas	Hempstead	Rick Evans WMA	35.80, -93.81
Passerina	*ciris*	MBM 16263	cah165	Arkansas	Hempstead	Rick Evans WMA	35.80, -93.81
Passerina	*ciris*	MBM 16265	cah156	Arkansas	Hempstead	Rick Evans WMA	35.80, -93.81
Passerina	*ciris*	MBM 16086	cah148	Arkansas	Hempstead	Rick Evans WMA	35.80, -93.81
Passerina	*ciris*	MBM 16085	cah154	Arkansas	Hempstead	Rick Evans WMA	35.80, -93.81
Passerina	*ciris*	MBM 16080	cah142	Arkansas	Hempstead	Rick Evans WMA	35.80, -93.81
Passerina	*ciris*	MBM16081	cah144	Arkansas	Hempstead	Rick Evans WMA	35.80, -93.81
Passerina	*ciris*	MBM 16082	cah143	Arkansas	Hempstead	Rick Evans WMA	35.80, -93.81
Passerina	*ciris*	MBM 16083	cah147	Arkansas	Hempstead	Rick Evans WMA	35.80, -93.81
Passerina	*ciris*	MBM 16084	cah152	Arkansas	Hempstead	Rick Evans WMA	35.80, -93.81
Passerina	*ciris*	PWRC-JM1	cahjm1	Bahamas		Eleuthera	24.93, -76.17
Passerina	*ciris*	PWRC-JM2	cahjm2	Bahamas		Eleuthera	24.93, -76.17

Passerina	*ciris*	PWRC-JM4	cahjm3	Bahamas		Eleuthera	24.93, -76.17
Passerina	*ciris*	PWRC-JM5	cahjm4	Bahamas		Eleuthera	24.93, -76.17
Passerina	*ciris*	PWRC-JM6	cahjm5	Bahamas		Eleuthera	24.93, -76.17
Passerina	*ciris*	PWRC-JM7	cahjm6	Bahamas		Eleuthera	24.93, -76.17
Passerina	*ciris*	PWRC-JM8	cahjm7	Bahamas		Eleuthera	24.93, -76.17
Passerina	*ciris*	PWRC-YR1	cahjm8	Cuba		Ciego de Avila	21.83, -78.75
Passerina	*ciris*	PWRC-YR2	cahjm9	Cuba		Guayancones	21.90, -78.90
Passerina	*ciris*	PWRC-YR3	cahjm10	Cuba		Ciego de Avila	21.83, -78.75
Passerina	*ciris*	PWRC-YR4	cahjm11	Cuba		Ciego de Avila	21.83, -78.75
Passerina	*ciris*	PWRC-YR5	cahjm12	Cuba		Ciego de Avila	21.83, -78.75
Passerina	*ciris*	PWRC-YR6	cahjm13	Cuba		Guayancones	21.90, -78.90
Passerina	*ciris*	PWRC-YR7	cahjm14	Cuba		Ciego de Avila	21.83, -78.75
Passerina	*ciris*	PWRC-YR8	cahjm15	Cuba		Ciego de Avila	21.83, -78.75
Passerina	*ciris*	PWRC-YR9	cahjm16	Cuba		Ciego de Avila	21.83, -78.75
Passerina	*ciris*	PWRC 2020-52283	52283	Florida	Duvall	1A-FL	30.39, -81.50
Passerina	*ciris*	PWRC 2020-52287	52287	Florida	Duvall	1A-FL	30.39, -81.50

Passerina	*ciris*	PWRC 2020-52307	52307	Florida	Duvall	3B-FL	30.44, -81.44
Passerina	*ciris*	PWRC 2020-52314	52314	Florida	Duvall	3B-FL	30.44, -81.44
Passerina	*ciris*	PWRC 2020-52315	52315	Florida	Duvall	3B-FL	30.44, -81.44
Passerina	*ciris*	PWRC 2020-52327	52327	Florida	Duvall	3C-FL	30.41, -81.43
Passerina	*ciris*	PWRC 2020-52329	52329	Florida	Duvall	3C-FL	30.41, -81.43
Passerina	*ciris*	PWRC 2020-52330	52330	Florida	Duvall	3C-FL	30.41, -81.43
Passerina	*ciris*	PWRC 2020-52335	52335	Florida	Duvall	5B-FL	30.44, -81.47
Passerina	*ciris*	PWRC 2020-52641	52641	Georgia	Chatham	12A-GA3	31.89, -80.97
Passerina	*ciris*	PWRC 2020-52657	52657	Georgia	Chatham	12A-GA3	31.89, -80.97
Passerina	*ciris*	PWRC 2020-52662	52662	Georgia	Chatham	12A-GA3	31.89, -80.97
Passerina	*ciris*	PWRC 2020-52666	52666	Georgia	Chatham	12A-GA3	31.89, -80.97
Passerina	*ciris*	PWRC 2020-52679	52679	Georgia	Chatham	12A-GA3	31.89, -80.97
Passerina	*ciris*	PWRC 2020-52683	52683	Georgia	Chatham	12B-GA3	31.89, -80.97
Passerina	*ciris*	PWRC 2020-52689	52689	Georgia	Chatham	12B-GA3	31.89, -80.97
Passerina	*ciris*	PWRC 2020-52694	52694	Georgia	Chatham	12B-GA3	31.89, -80.97
Passerina	*ciris*	PWRC 2020-52706	52706	Georgia	Chatham	12B-GA3	31.89, -80.97

Passerina	*ciris*	PWRC 2020-52347	52347	Georgia	McIntosh	7A-GA1	31.45, -81.37
Passerina	*ciris*	PWRC 2020-52350	52350	Georgia	McIntosh	7A-GA1	31.45, -81.37
Passerina	*ciris*	PWRC 2020-52354	52354	Georgia	McIntosh	7B-GA1	31.37, -81.40
Passerina	*ciris*	PWRC 2020-52362	52362	Georgia	McIntosh	7B-GA1	31.37, -81.40
Passerina	*ciris*	PWRC 2020-52369	52369	Georgia	McIntosh	7B-GA1	31.37, -81.40
Passerina	*ciris*	PWRC 2020-52371	52371	Georgia	McIntosh	7B-GA1	31.37, -81.40
Passerina	*ciris*	PWRC 2020-52384	52384	Georgia	McIntosh	7B-GA1	31.37, -81.40
Passerina	*ciris*	PWRC 2020-52387	52387	Georgia	McIntosh	7B-GA1	31.37, -81.40
Passerina	*ciris*	PWRC 2020-52388	52388	Georgia	McIntosh	7B-GA1	31.37, -81.40
Passerina	*ciris*	PWRC 2020-52394	52394	Georgia	McIntosh	9A-GA2	31.63, -81.29
Passerina	*ciris*	PWRC 2020-52398	52398	Georgia	McIntosh	9A-GA2	31.63, -81.29
Passerina	*ciris*	PWRC 2020-52399	52399	Georgia	McIntosh	9A-GA2	31.63, -81.29
Passerina	*ciris*	PWRC 2020-52401	52401	Georgia	McIntosh	9A-GA2	31.63, -81.29
Passerina	*ciris*	PWRC 2020-52402	52402	Georgia	McIntosh	9A-GA2	31.63, -81.29
Passerina	*ciris*	PWRC 2020-52410	52410	Georgia	McIntosh	9A-GA2	31.63, -81.29
Passerina	*ciris*	PWRC 2020-52411	52411	Georgia	McIntosh	9A-GA2	31.63, -81.29

Passerina	*ciris*	PWRC 2020-52415	52415	Georgia	McIntosh	9A-GA2	31.63, -81.29
Passerina	*ciris*	PWRC 2020-52426	52426	Georgia	McIntosh	9C-GA2	31.65, -81.27
Passerina	*ciris*	PWRC 2020-52434	52434	Georgia	McIntosh	9C-GA2	31.65, -81.27
Passerina	*ciris*	PWRC 2020-52440	52440	Georgia	McIntosh	9C-GA2	31.65, -81.27
Passerina	*ciris*	MBM-10589	dhb4507	Guatemala		Retalhuleu	14.60, -95.33
Passerina	*ciris*	MBM-10767	dhb4551	Guatemala		Retalhuleu	14.60, -95.33
Passerina	*ciris*	MBM-10875	dhb4620	Guatemala		Retalhuleu	14.60, -95.33
Passerina	*ciris*	MBM-10479	dhb4348	Guatemala		Retalhuleu	14.60, -95.33
Passerina	*ciris*	MBM-10876	jk02015	Guatemala		Retalhuleu	14.60, -95.33
Passerina	*ciris*	MBM-10653	dhb4530	Guatemala		San Marcos	14.60, -95.33
Passerina	*ciris*	MBM-10654	dhb4531	Guatemala		San Marcos	14.60, -95.33
Passerina	*ciris*	MBM-10933	gav2406	Guatemala		San Marcos	14.60, -95.33
Passerina	*ciris*	MBM-10934	gav2405	Guatemala		San Marcos	14.60, -95.33
Passerina	*ciris*	MBM-10935	jk02-087	Guatemala		San Marcos	14.60, -95.33
Passerina	*ciris*	MBM-10503	dhb4521	Guatemala		Quetzaltenango	14.66, -95.33
Passerina	*ciris*	MBM-10504	dbh4524	Guatemala		Quetzaltenango	14.66, -95.33

Passerina	*ciris*	MBM-10588	dhb4503	Guatemala		Retalhuleu	14.66, -95.33
Passerina	*ciris*	MBM-11021	jk02010	Guatemala		Retalhuleu	14.66, -95.33
Passerina	*ciris*	MBM-11022	jk02017	Guatemala		Retalhuleu	14.66, -95.33
Passerina	*ciris*	MBM-193	dbh4349	Guatemala		Retalhuleu	14.66, -95.33
Passerina	*ciris*	MBM-14029	jmd069	Mexico		Oaxaca	15.92, -96.62
Passerina	*ciris*	MBM-14030	dbh5557	Mexico		Oaxaca	15.92, -96.62
Passerina	*ciris*	MBM-14037	dbh5582	Mexico		Oaxaca	15.92, -96.62
Passerina	*ciris*	MBM-14028	dbh5578	Mexico		Oaxaca	15.92, -96.62
Passerina	*ciris*		gls10	Mexico	Quint. Roo	Cozumel	20.43, -86.90
Passerina	*ciris*		gls23	Mexico	Quint. Roo	Cozumel	20.43, -86.90
Passerina	*ciris*		tux67	Mexico	Vera Cruz	Los Tuxtlas	19.17, -96.15
Passerina	*ciris*		tux77	Mexico	Vera Cruz	Los Tuxtlas	19.17, -96.15
Passerina	*ciris*		tux1094	Mexico	Vera Cruz	Los Tuxtlas	19.17, -96.15
Passerina	*ciris*		tux1107	Mexico	Vera Cruz	Los Tuxtlas	19.17, -96.15
Passerina	*ciris*	MBM 20539	BRB 902	Mexico	Yucatan	El Cuyo	21.52, -87.70
Passerina	*ciris*	MBM 20540	BRB 942	Mexico	Yucatan	El Cuyo	21.52, -87.70

Passerina	*ciris*	MBM 20541	BRB 941	Mexico	Yucatan	El Cuyo	21.52, -87.70
Passerina	*ciris*	MBM 20542	BRB 928	Mexico	Yucatan	El Cuyo	21.52, -87.70
Passerina	*ciris*	MBM 20543	BRB 877	Mexico	Yucatan	El Cuyo	21.52, -87.70
Passerina	*ciris*	MBM 20544	BRB 849	Mexico	Yucatan	El Cuyo	21.52, -87.70
Passerina	*ciris*	MBM 20548	BRB 876	Mexico	Yucatan	El Cuyo	21.52, -87.70
Passerina	*ciris*	MBM 20549	BRB 850	Mexico	Yucatan	El Cuyo	21.52, -87.70
Passerina	*ciris*	MBM 20550	BRB 885	Mexico	Yucatan	El Cuyo	21.52, -87.70
Passerina	*ciris*	MBM 20551	BRB 893	Mexico	Yucatan	El Cuyo	21.52, -87.70
Passerina	*ciris*	MBM 20557	BRB 883	Mexico	Yucatan	El Cuyo	21.52, -87.70
Passerina	*ciris*	MBM 20553	BRB 878	Mexico	Yucatan	El Cuyo	21.52, -87.70
Passerina	*ciris*	MBM 14571	jk04545	Kansas	Chautauqua	Hulah WMA	37.02, -96.26
Passerina	*ciris*	MBM 14519	cah097	Louisiana	Ouachita	Russell Sage WMA	32.48, -91.97
Passerina	*ciris*	MBM 14520	cah098	Louisiana	Ouachita	Russell Sage WMA	32.48, -91.97
Passerina	*ciris*	MBM 14521	cah099	Louisiana	Ouachita	Russell Sage WMA	32.48, -91.97
Passerina	*ciris*	MBM 14522	cah100	Louisiana	Ouachita	Russell Sage WMA	32.48, -91.97
Passerina	*ciris*	MBM 14523	jk04540	Louisiana	Ouachita	Russell Sage WMA	32.48, -91.97

Passerina	*ciris*	MBM 16075	cah137	Louisiana	Ouachita	Russell Sage WMA	32.48, -91.97
Passerina	*ciris*	MBM 16076	cah138	Louisiana	Ouachita	Russell Sage WMA	32.48, -91.97
Passerina	*ciris*	MBM 16077	cah139	Louisiana	Ouachita	Russell Sage WMA	32.48, -91.97
Passerina	*ciris*	MBM 16078	cah140	Louisiana	Ouachita	Russell Sage WMA	32.48, -91.97
Passerina	*ciris*	MBM 16079	cah141	Louisiana	Ouachita	Russell Sage WMA	32.48, -91.97
Passerina	*ciris*	PWRC 2020-65622	65622	North Carolina	Brunswick	19C-NC2	33.86, -77.99
Passerina	*ciris*	PWRC 2020-65625	65625	North Carolina	Brunswick	19C-NC2	33.86, -77.99
Passerina	*ciris*	PWRC 2020-65632	65632	North Carolina	Brunswick	19C-NC2	33.86, -77.99
Passerina	*ciris*	PWRC 2020-65637	65637	North Carolina	Brunswick	19C-NC2	33.86, -77.99
Passerina	*ciris*	PWRC 2020-65639	65639	North Carolina	Brunswick	19C-NC2	33.86, -77.99
Passerina	*ciris*	PWRC 2020-65645	65645	North Carolina	Brunswick	19C-NC2	33.86, -77.99
Passerina	*ciris*	PWRC 2020-65648	65648	North Carolina	Brunswick	19A-NC2	33.87, -78.00
Passerina	*ciris*	PWRC 2020-65655	65655	North Carolina	Brunswick	19A-NC2	33.87, -78.00
Passerina	*ciris*	PWRC 2020-65658	65658	North Carolina	Brunswick	19A-NC2	33.87, -78.00
Passerina	*ciris*	PWRC 2020-65586	65586	North Carolina	New Hanover	21B-NC2	34.05, -77.92
Passerina	*ciris*	PWRC 2020-52229	52229	North Carolina	Onslow	24C-NC1	34.57, -77.27

Passerina	*ciris*	PWRC 2020-65593	65593	North Carolina	Onslow	23A-NC1	34.05, -77.92
Passerina	*ciris*	PWRC 2020-52790	52790	North Carolina	Onslow	24B-NC1	34.58, -77.26
Passerina	*ciris*	PWRC 2020-52797	52797	North Carolina	Onslow	24B-NC1	34.58, -77.26
Passerina	*ciris*	PWRC 2020-52800	52800	North Carolina	Onslow	24B-NC1	34.58, -77.26
Passerina	*ciris*	PWRC 2020-52803	52803	North Carolina	Onslow	24B-NC1	34.58, -77.26
Passerina	*ciris*	PWRC 2020-52804	52804	North Carolina	Onslow	24B-NC1	34.58, -77.26
Passerina	*ciris*	PWRC 2020-52808	52808	North Carolina	Onslow	24B-NC1	34.58, -77.26
Passerina	*ciris*	PWRC 2020-52817	52817	North Carolina	Onslow	24B-NC1	34.58, -77.26
Passerina	*ciris*	MBM 14305	jmd369	Oklahoma	Caddo	Fort Cobb WMA	35.21, -98.48
Passerina	*ciris*	MBM 14307	cah090	Oklahoma	Caddo	Fort Cobb WMA	35.21, -98.48
Passerina	*ciris*	MBM 14306	cah091	Oklahoma	Caddo	Fort Cobb WMA	35.21, -98.48
Passerina	*ciris*	MBM 14313	dhb5702	Oklahoma	Caddo	Fort Cobb WMA	35.21, -98.48
Passerina	*ciris*	MBM 14312	dhb5703	Oklahoma	Caddo	Fort Cobb WMA	35.21, -98.48
Passerina	*ciris*	MBM 14308	dhb5713	Oklahoma	Caddo	Fort Cobb WMA	35.21, -98.48
Passerina	*ciris*	MBM 14309	dhb5714	Oklahoma	Caddo	Fort Cobb WMA	35.21, -98.48
Passerina	*ciris*	MBM 14310	dhb5715	Oklahoma	Caddo	Fort Cobb WMA	35.21, -98.48

Passerina	*ciris*	MBM 14311	dhb5716	Oklahoma	Caddo	Fort Cobb WMA	35.21, -98.48
Passerina	*ciris*	MBM 523	cah081	Oklahoma	Caddo	Fort Cobb WMA	35.21, -98.48
Passerina	*ciris*	MBM 14564	jk04554	Oklahoma	Muskogee	Gruber WMA	35.72, -95.20
Passerina	*ciris*	PWRC 2020-65507	65507	South Carolina	Beaufort	15C-SC2	32.35, -80.84
Passerina	*ciris*	PWRC 2020-65510	65510	South Carolina	Beaufort	15C-SC2	32.35, -80.84
Passerina	*ciris*	PWRC 2020-65511	65511	South Carolina	Beaufort	15C-SC2	32.35, -80.84
Passerina	*ciris*	PWRC 2020-65516	65516	South Carolina	Beaufort	15C-SC2	32.35, -80.84
Passerina	*ciris*	PWRC 2020-65520	65520	South Carolina	Beaufort	15C-SC2	32.35, -80.84
Passerina	*ciris*	PWRC 2020-65521	65521	South Carolina	Beaufort	15C-SC2	32.35, -80.84
Passerina	*ciris*	PWRC 2020-65523	65523	South Carolina	Beaufort	15C-SC2	32.35, -80.84
Passerina	*ciris*	PWRC 2020-65524	65524	South Carolina	Beaufort	15C-SC2	32.35, -80.84
Passerina	*ciris*	PWRC 2020-65529	65529	South Carolina	Beaufort	15C-SC2	32.35, -80.84
Passerina	*ciris*	PWRC 2020-65537	65537	South Carolina	Charleston	17C-SC1	32.72, -79.99
Passerina	*ciris*	PWRC 2020-65545	65545	South Carolina	Charleston	17C-SC1	32.72, -79.99
Passerina	*ciris*	PWRC 2020-65549	65549	South Carolina	Charleston	17C-SC1	32.72, -79.99
Passerina	*ciris*	PWRC 2020-65556	65556	South Carolina	Charleston	17C-SC1	32.72, -79.99

Passerina	*ciris*	PWRC 2020-65557	65557	South Carolina	Charleston	17C-SC1	32.72, -79.99
Passerina	*ciris*	PWRC 2020-65564	65564	South Carolina	Charleston	17C-SC1	32.72, -79.99
Passerina	*ciris*	PWRC 2020-52467	52467	South Carolina	Jasper	13C-SC1	32.08, -80.96
Passerina	*ciris*	PWRC 2020-52477	52477	South Carolina	Jasper	13C-SC1	32.08, -80.96
Passerina	*ciris*	PWRC 2020-52478	52478	South Carolina	Jasper	13C-SC1	32.08, -80.96
Passerina	*ciris*	PWRC 2020-52499	52499	South Carolina	Jasper	13A-SC1	32.09, -81.03
Passerina	*ciris*	MBM 14548	mm133	Texas	Brewster	Black Gap WMA	29.59, -103.00
Passerina	*ciris*	MBM 14685	mm141	Texas	Brewster	Black Gap WMA	29.59, -103.00
Passerina	*ciris*	MBM 16091	cah150	Texas	Brewster	Black Gap WMA	29.59, -103.00
Passerina	*ciris*	MBM 16268	cah159	Texas	Brewster	Black Gap WMA	29.59, -103.00
Passerina	*ciris*	MBM 16434	cah170	Texas	Brewster	Black Gap WMA	29.59, -103.00
Passerina	*ciris*	MBM 16601	bts05071	Texas	Brewster	Black Gap WMA	29.59, -103.00
Passerina	*ciris*	MBM 16795	cah177	Texas	Brewster	Black Gap WMA	29.59, -103.00
Passerina	*ciris*	MBM 862	cah155	Texas	Brewster	Black Gap WMA	29.59, -103.00
Passerina	*ciris*	MBM 16271	cah160	Texas	Brewster	Black Gap WMA	29.59, -103.00
Passerina	*ciris*	MBM 863	cah157	Texas	Brewster	Black Gap WMA	29.59, -103.00

Passerina	*ciris*	MBM 14870	jsb111	Texas	Nacogdoches	Alazon Bayou WMA	31.48, -94.75
Passerina	*ciris*	MBM 14871	jsb113	Texas	Nacogdoches	Alazon Bayou WMA	31.48, -94.75
Passerina	*ciris*	MBM 14686	cah105	Texas	Nacogdoches	Alazon Bayou WMA	31.48, -94.75
Passerina	*ciris*	MBM 16088	cah146	Texas	Nacogdoches	Alazon Bayou WMA	31.48, -94.75
Passerina	*ciris*	MBM 16089	cah149	Texas	Nacogdoches	Alazon Bayou WMA	31.48, -94.75
Passerina	*ciris*	MBM 16090	cah153	Texas	Nacogdoches	Alazon Bayou WMA	31.48, -94.75
Passerina	*ciris*	MBM 16087	cah145	Texas	Nacogdoches	Alazon Bayou WMA	31.48, -94.75
Passerina	*ciris*	MBM 16273	cah162	Texas	Nacogdoches	Alazon Bayou WMA	31.48, -94.75
Passerina	*ciris*	MBM 16272	cah161	Texas	Nacogdoches	Alazon Bayou WMA	31.48, -94.75
Passerina	*ciris*	MBM 14683	cah108	Texas	Williamson	Granger WMA	30.66, -97.38
Passerina	*ciris*	MBM 14517	cah086	Texas	Williamson	Granger WMA	30.66, -97.38
Passerina	*ciris*	MBM 14515	cah087	Texas	Williamson	Granger WMA	30.66, -97.38
Passerina	*ciris*	MBM 14516	dhb5734	Texas	Williamson	Granger WMA	30.66, -97.38
Passerina	*ciris*	MBM 14566	jk04518	Texas	Williamson	Granger WMA	30.66, -97.38
Passerina	*ciris*	MBM 14567	jk04519	Texas	Williamson	Granger WMA	30.66, -97.38
Passerina	*ciris*	MBM 14688	jk04561	Texas	Williamson	Granger WMA	30.66, -97.38

Passerina	*ciris*	MBM 14689	jk04562	Texas	Williamson	Granger WMA	30.66, -97.38
Passerina	*ciris*	MBM 14687	jk04563	Texas	Williamson	Granger WMA	30.66, -97.38

[1]MBM = Marjorie Barrick Museum of Natural History, PWRC = Patuxent Wildlife Research Center

[2]WMA = Wildlife Management Area

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VITA

Graduate College
University of Nevada, Las Vegas

Connie A. Herr

Degrees:

Doctor of Philosophy Candidate, Biology, 2011
University of Nevada, Las Vegas.

Master of Science, Biology, 1991
University of Nevada, Las Vegas.

Bachelor of Science, Biology, 1988
University of Nevada, Las Vegas.

Special Honors and Awards:

1986. Outstanding Student in Biology Award, UNLV
1986. UNLV Alumni Association Graduate Scholarship, UNLV, $1,000
1986. UNLV Alumni Association Certificate, UNLV
1990. UNLV Alumni Association Graduate Scholarship, UNLV, $1,000
1991. Bradley/Dill Award in Environmental Biology, UNLV, $500
1991. Outstanding Thesis Award, UNLV
2005. Graduate and Professional Student Association, UNLV, $500
2006. Graduate and Professional Student Association, UNLV, $750
2007. Graduate and Professional Student Association, UNLV, $750

Publications:

C. Herr et al. Phylogeography of a vanishing North American songbird: the Painted Bunting (*Passerina ciris*). (in press).

S.D. Smith, **C.A. Herr**, K.L. Leary & J.M. Piorkowski (1995). Soil-plant water relations in a Mojave Desert mixed shrub community: a comparison of three geomorphic surfaces. Journal of Arid Environments, 29: 339-351.

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Dissertation Examination Committee:

Committee Chairperson, Brett R. Riddle, Ph.D.
Committee Member, Lloyd R. Stark, Ph.D.
Committee Member, Daniel B. Thompson, Ph.D.
Graduate Faculty Representative, Mark Buttner, Ph.D.