Natural History Note

A Migratory Divide in the Painted Bunting (*Passerina ciris*)

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abstract: In the painted bunting (*Passerina ciris*), a North American songbird, populations on the Atlantic coast and interior southern United States are known to be allopatric during the breeding season, but efforts to map connectivity with wintering ranges have been largely inconclusive. Using genomic and morphological data from museum specimens and banded birds, we found evidence of three genetically differentiated painted bunting populations with distinct wintering ranges and molt-migration phenologies. In addition to confirming that the Atlantic coast population remains allopatric throughout the annual cycle, we identified an unexpected migratory divide within the interior breeding range. Populations breeding in Louisiana winter on the Yucatán Peninsula and are parapatric with other interior populations that winter in mainland Mexico and Central America. Across the interior breeding range, genetic ancestry is also associated with variation in wing length, suggesting that selection may be promoting morphological divergence in populations with different migration strategies.

Keywords: genomics, phylogeography, migration, ornithology, zoology, population genetics.

Introduction

Migratory divides occur in regions where adjacent populations differ in the timing or route of seasonal migration. Because migratory behaviors have clear fitness impacts and are strongly heritable in several taxa (Helbig 1991; Quinn et al. 2000; Pulido et al. 2001; Zhan et al. 2014), migratory divides are thought to represent a mechanism of lineage divergence in sympatry (Bearhop et al. 2005; Rolshausen et al. 2009). If hybrids between populations differing in migratory behavior attempt an intermediate strategy with fitness lower than that of either parental type, selection is expected to favor the evolution of mechanisms that reduce the probability of breeding across migratory types (Rohwer and Irwin 2011). Recent studies combining genetic and individual tracking data have documented this scenario in a passerine bird (*Catharus ustulatus*; Delmore and Irwin 2014), although reduced hybrid fitness has yet to be rigorously tested in the wild.

Understanding the role of seasonal migration in mediating gene flow among populations is also important in identifying distinct evolutionary and demographic units relevant to conservation and management. Because the level of immigration required to homogenize allele frequencies among populations is much lower than that expected to drive trends in population size (Waples and Gaggiotti 2006), evidence of genetic differentiation is a conservative proxy for demographic independence. Migratory connectivity has long been recognized as a core criterion for delimiting fish stocks (Gillanders 2002; Lipcius et al. 2008; Cadrin et al. 2013), but it has not been widely used in monitoring songbird populations. In part, this flows from our relatively sparse knowledge of variation in migratory behavior within most bird species (Faaborg et al. 2010).

The painted bunting (*Passerina ciris*) is a seasonal migrant to the southern United States with an interior breeding population that stretches across much of Mississippi, Louisiana, Arkansas, Oklahoma, Texas, and northern Mexico and an eastern breeding population that hugs the Atlantic coastline from northern Florida to Virginia (fig. 1). Two subspecies are currently recognized on the basis of similarity in wing length and breast coloration: *P. ciris ciris*, breeding both along the Atlantic coast and in Louisiana and Mississippi, and *P. ciris pallidior*, breeding across the rest of the interior range (Storer 1951). In addition to occupying allopatric breeding ranges, these populations pursue different molt-migration strategies: Atlantic coast populations fly south in September after molting on the breeding grounds, while interior populations depart the breeding grounds in July and molt dur-

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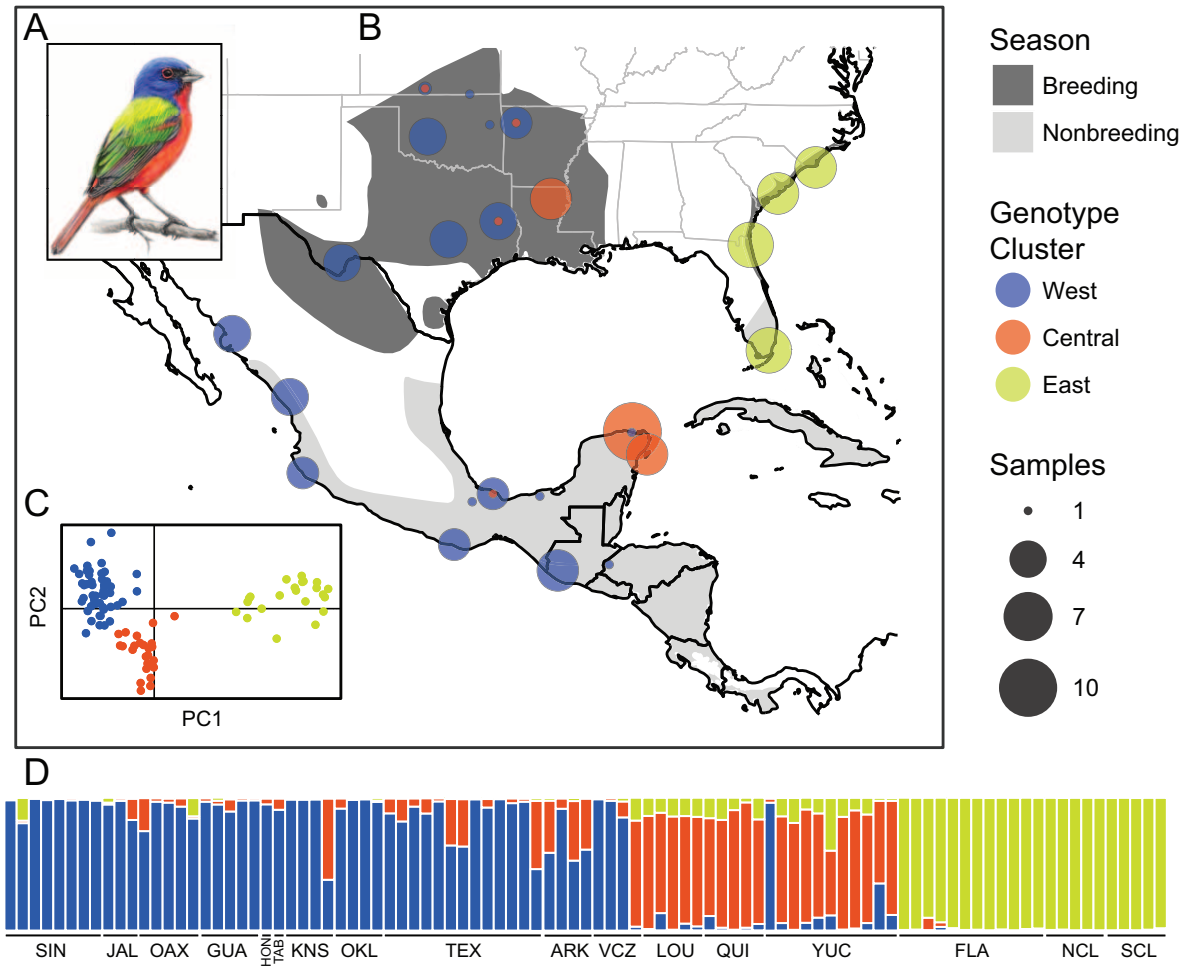

Figure 1: *A*. Male *Passerina ciris*. *B*, Sampling localities, with points colored by *k*-means cluster and scaled to the number of samples. *C*, Sample coordinates and *k*-means clusters on the first two principal-component (PC) axes. *D*, Results from *structure* at $k = 3$, with each vertical bar representing a sample and the colors depicting the proportion of inferred ancestry from each population. Locality abbreviations: SIN (Sinaloa), JAL (Jalisco), OAX (Oaxaca), GUA (Guatemala), HON (Honduras), TAB (Tabasco), KNS (Kansas), OKL (Oklahoma), TEX (Texas), ARK (Arkansas), VCZ (Veracruz), LOU (Louisiana), QUI (Quintana Roo), YUC (Yucatán), FLA (Florida), NCL (North Carolina), SCL (South Carolina).

ing a migratory stopover in northwestern Mexico (Thompson 1991; Contina et al. 2013; Rohwer 2013).

Painted buntings winter across Mexico, Central America, southern Florida, and the northern Caribbean, but connectivity between breeding and wintering ranges remains poorly characterized. While some researchers have suggested that the Atlantic coast population winters exclusively in southern Florida and on islands in the northern Caribbean (Storer 1951; Thompson 1991), others (e.g., Sykes et al. 2007) maintain that eastern birds may also winter in the Yucatán and farther south. Winter destinations of interior migrants are similarly unresolved. On the basis of wing length measurements and a qualitative analysis of breast coloration, Storer (1951) proposed that the birds breeding in Louisiana and Mississippi are trans-Gulf migrants that winter on the Yucatán Peninsula, while birds that breed farther west use a circum-Gulf route to sites elsewhere in Mexico and Central America. In a meta-analysis of specimen collection records, Linck et al. (2016) proposed that most interior buntings migrate in a counterclockwise pattern around Mexico after molting in Sonora and Sinaloa. A phylogeographic study of mitochondrial DNA variation across the species' breeding range showed significant population structure between Atlantic coast and interior populations (Herr et al. 2011); however, genetic data have not yet been used to identify links between breeding and wintering grounds.

Here we use genome-wide DNA sequence data and morphological analyses of museum specimens to infer phylogeographic history and patterns of migratory connectivity in the species. Specifically, we (1) map migratory connectiv-

ity between breeding and wintering grounds, (2) test for morphological variation associated with divergent migratory strategies in the interior breeding range, and (3) estimate divergence times and rates of gene flow among populations. Our results highlight the contrasting roles of seasonal migration in driving both gene flow and genetic differentiation and have significant conservation implications for an iconic but regionally declining songbird.

Methods

Genetic Sampling

We collected a total of 260 blood, tissue, and feather samples from across the breeding and wintering ranges of *Passerina ciris*, including 138 breeding-range samples previously analyzed in Herr et al. (2011). All Atlantic coast samples were blood and feather samples taken during banding studies. Interior populations were represented by 148 frozen tissue samples of vouchered museum specimens held by the Burke Museum of Natural History and Universidad Nacional Autónoma de México.

Whole-genomic DNA was extracted with Qiagen DNEasy extraction kits. We sequenced 1,041 base pairs of the mitochondrial gene NADH dehydrogenase subunit 2 (ND2) from all samples, using the primers and protocol described in Herr et al. (2011). On the basis of fragment size and DNA concentration, 95 samples were selected for reduced-representation library sequencing via the ddRADseq protocol (table A1, available online; Peterson et al. 2012). We used the digestion enzymes Sbf1 and Msp1 and a size-selection window of 415–515 bp. The resulting libraries were sequenced for 100-bp single-end reads on an Illumina HiSeq 2500.

Reads were assembled into sequence alignments with de novo assembly in the program pyRAD v.3-0-65 (Eaton 2014). We set a similarity threshold of 0.88 for clustering reads within and between individuals and a minimum coverage depth of five (per individual) and a maximum of eight low-quality reads per site. To exclude paralogs from the final data set, we filtered out loci with more than five heterozygous sites and those sharing a heterozygous site across more than 60 samples. We define "locus" throughout this note as a cluster of sequence reads putatively representing the same 100-bp region downstream of an Sbf1 cut site. For clustering analyses, we required each locus to be sequenced in at least half of the samples and randomly selected one parsimony-informative single-nucleotide polymorphism (SNP) per locus, using a custom R script (https://github.com/slager/structure_parsimony_informative). Raw sequence data, assembly parameter files, specimen data, and scripts used to conduct all analyses are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.cp40s (Battey et al. 2018).

Genotype Clustering and Population Assignments

We used multivariate ordination and Bayesian coalescent clustering to identify genetically differentiated populations and assign wintering individuals to breeding regions. For multivariate analyses, we first conducted a principal-components analysis (PCA; Pearson 1901), using the covariance matrix of allele frequencies in each sample, and then identified putative genetic clusters, using *k*-means clustering in the R package Adegenet v2.0.1 (Jombart 2008). We then cross validated population assignments with discriminant analysis of principal components (DAPC; Jombart et al. 2010) by randomly selecting half the samples in each *k*-means cluster, conducting a DAPC on these samples, and predicting the group assignments of remaining individuals with the "trained" DAPC model. Cross-validation analyses were repeated 1,000 times for $k = 2$–4 to estimate cluster assignment accuracy.

Bayesian clustering under a coalescent model with admixture was implemented in the program *structure* (Pritchard et al. 2000) with default priors, correlated allele frequencies, and a chain length of 1,000,000. The first 100,000 steps were discarded as burn-in. We replicated *structure* analyses five times for each value of $k = 2$–4, assessed change in marginal likelihood across values of *k*, using STRUCTURE HARVESTER (Earl 2012), and used CLUMPP (Jakobsson and Rosenberg 2007) to take the mean of permuted matrices across replicates after accounting for label switching. We estimated mean F_{st}, using *structure*'s parameterization, which follows Excoffier's (2001) definition except for using a generalized model with separate drift rates for each population (Falush et al. 2003). We developed a custom web app for visualizing *structure* results (https://cjbattey.shinyapps.io/structurePlotter/; Battey 2017) and summarized output of multivariate analyses by using the R packages plyr (Wickham 2015) and ggplot2 (Wickham 2016).

Mitochondrial DNA

Mitochondrial DNA analyses were conducted to identify the most likely breeding population of wintering samples collected in areas not well represented in ddRAD (double digest restriction site–associated DNA) sequencing. We created 12 hypothetical population assignment schemes based on the results of nuclear SNP clustering, varying only the population assignment of samples from regions without nuclear SNP sequence data (Cuba, Bahamas, Costa Rica, and Nicaragua; data are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.cp40s; Battey et al. 2018). Assignment schemes were compared by conducting an analysis of molecular variance (AMOVA; Excoffier et al. 1992) in the R package poppr (Kamvar et al. 2014) and ranking models by the percentage of total variance explained by the population factor (following Herr et al. 2011). We also inferred

a median-joining haplotype network, using the R package pegas (Paradis 2010) to visualize the distribution of haplotypes among putative populations.

Demographic Modeling

To estimate the timing of population splits and rates of gene flow among populations, we fitted demographic models to the joint site frequency spectrum (SFS) of our nuclear SNP alignment in the program δaδi v1.7 (Gutenkunst et al. 2010). We randomly selected 10 samples from each *k*-means population and called SNPs from this subset in pyRAD. The pyRAD VCF (variant call format) files were then converted to δaδi's input format with a custom R script (https://github.com/cjbattey/vcf2dadi). A single biallelic SNP was randomly selected from each locus, and the final data set was projected to a size of five diploid individuals per population (proj = [10, 10, 10]). This yielded 3,044 SNPs from 4,128 loci.

We fitted two nine-parameter demographic models representing the general phylogeographic history of the group (fig. S1; figs. S1–S7 are available online). In both models a single ancestral population first splits into eastern and western groups, one of which then splits a second time to form the central population. Migration is allowed between eastern + central and western + central populations after the final divergence event. The models differ only in whether eastern or western birds are sister to the central population. We ran 40 optimizations from randomized starting positions for each model, using the optimize_log() function in δaδi, and assessed uncertainty across 100 parametric bootstrap replicates of our original data (sampling each locus with replacement). We ranked models by calculating the difference in Akaike information criterion (AIC; Akaike 1974) of the highest-likelihood parameter set for each model.

To convert model parameters to demographic values, we used the average genome-wide mutation rate of *Geospiza fortis* (3.44×10^{-9} substitutions/site/generation; Nadachowska-Brzyska et al. 2015) and a *Passerina* generation time of 1.63 yr (Weir and Schluter 2008). We estimated the effective sequence length for SNP calling by multiplying the total base pairs in our pyRAD alignment by the fraction of all SNPs incorporated in the SFS after projection.

Morphology

Two previous studies documenting significant range-wide variation in painted bunting wing length concentrated primarily on differences between the allopatric coastal and interior breeding populations (Storer 1951; Thompson 1991). Here we focused on testing for morphological variation associated with the putative migratory divide within the interior breeding population, because variation in migration distance has previously been associated with wing length in both birds and butterflies (Voelker 2001; Altizer and Davis 2010). We measured wing chord and tarsus length (as a proxy for body size) of 56 museum specimens of adult male painted buntings collected in the breeding season (April–June). Wing chord was measured to the nearest 0.5 mm with a metal stop ruler. Tarsus length was measured to the nearest 0.01 mm with digital calipers.

We calculated and mapped mean values for both morphological traits for each unique locality recorded in the Burke Museum specimen database. After initial analyses found that wing chord and tarsus length were not significantly correlated (ordinary least squares regression: $P = .32$, $R^2 = 0.02$, df = 54), we treated these variables as independent. Following Slager et al. (2015), we used a principal-components analysis implemented in R to create a synthetic variable combining wing chord and tarsus length. We conducted ordinary least squares linear regressions to test for significant correlations between specimen longitude and each of wing length, tarsus length, and the first principal-component (PC) axis of wing and tarsus length. Finally, for the 21 specimens with both genomic and morphological data, we used linear regression to test for correlations between morphological traits and the proportion of "central" ancestry inferred by *structure*.

Results

Sequence Assembly

Illumina sequencing returned an average of 721,942 quality-filtered reads per sample. Clustering within individuals identified 36,497 putative loci per sample, with an average depth of coverage of 17. After clustering across individuals and applying paralog and depth-of-coverage filters, we retained an average of 9,010 loci per sample. As in most studies using RADseq-style reduced-representation libraries, we observed a large effect of missing-data filtering on the size of our alignments, ranging from 238 to 25,434 unlinked SNPs for an all-samples-present versus a three-samples-present cutoff (Eaton et al. 2015; Leaché et al. 2015; DaCosta and Sorenson 2016). The final alignment used for clustering analyses included 3,615 unlinked parsimony-informative SNPs from 5,950 loci sequenced in at least 48 of 95 samples.

Genotype Clustering

In *k*-means clustering, the Bayesian information criterion (BIC) showed a single clear shift in slope at $k = 2$, while *structure* returned the highest marginal likelihood at $k = 3$ (fig. S2). Individual population assignments were similar in both methods, with $k = 2$ models splitting breeding individuals between the Atlantic coast and interior breeding ranges and wintering individuals between Florida and Mexico/

Central America (fig. 1). At $k = 3$, both methods cluster a group of breeding birds from Louisiana, eastern Texas, and Arkansas with wintering birds from the Mexican states of Yucatán and Quintana Roo. This "central" population appears to represent the easternmost end of a genetic cline across the interior breeding range, with samples from eastern Texas and western Arkansas falling in intermediate locations in principal-components space and showing relatively high levels of admixture in *structure* analyses. Mean F_{st} in three-population *structure* models was 0.11. Neither clustering method found geographically coherent clusters beyond $k = 3$ (figs. S3, S4).

Discriminant analyses estimated assignment probabilities over 0.99 for all individuals in both two- and three-population models. In cross-validation analyses, DAPC models trained on a random sample of half the individuals in each population correctly predicted the population assignment of an average of 99.7% of the remaining individuals at $k = 2$ and 97.4% at $k = 3$. DAPC cross validation was also surprisingly robust at k values of 4 (85.5%) and 5 (76.2%), suggesting that denser population sampling could reveal further genetic structure in the interior breeding range.

Table 1: ND2 AMOVA results

Variation explained by population (%)	k	Cuba	Bahamas	Central America
32.38	2	E	E	W
30.30	2	W	E	W
30.10	2	E	W	W
28.57	3	E	E	W
28.41	2	W	W	W
28.37	2	E	E	E
26.82	3	C	E	W
25.87	2	W	E	E
25.67	2	E	W	E
25.31	3	E	E	E
23.53	2	W	W	E
23.45	3	C	E	E

Note: Individual assignment schemes for mitochondrial DNA AMOVAs, ranked by the percentage of total variance explained by the population factor. k is the total number of populations in the model. Letters in the last three columns indicate the population assignment for each model: C = central; E = eastern; W = western. ND2 = NADH dehydrogenase subunit 2; AMOVA = analysis of molecular variance.

Mitochondrial DNA

The population assignment scheme that explained the highest percentage of total variance in AMOVA results grouped wintering birds from Cuba and the Bahamas with eastern breeding populations and those from Costa Rica and Nicaragua with western breeding populations (table 1). Models including a central population of Louisiana and Yucatán birds were consistently ranked lower than two-population models, but the highest-ranked three-population model followed the same assignment scheme as the top model overall. All AMOVA results were significant ($P < .01$). Haplotype networks were similar to those inferred with the breeding-season data set of Herr et al. (2011), with the majority of western samples sharing a single common haplotype and most eastern samples sharing one of two alternate haplotypes (fig. S5).

Demographic Modeling

Of the two demographic models we tested in δaδi, the model showing a sister relationship between the western and central populations was better supported; however, the difference in AIC scores between models was just 0.78, providing weak support for this topology (Burnham and Anderson 2004). In both models the internode distance between the first and second divergence events is relatively short (around 17% of the total tree depth), and the migration rate after the last divergence event is much higher between the western and central populations than between the eastern and central populations (table 2; figs. S6, S7). In the highest-likelihood parameter set, eastern and western + central populations diverged approximately 646,000 yr ago, followed by central and western populations approximately 566,000 yr ago. Gene flow is highest between the central and western populations (3–9 migrants/generation) but also significant between the eastern and central populations (1–3 migrants/generation).

Although we observed relatively low uncertainty across bootstrap replicates, exact figures for divergence times and population sizes should be interpreted with some caution, given uncertainty in the generation time and mutation rate. We note that our estimates of both population divergence times and migration rates are significantly higher than those in a previous study of mitochondrial DNA under a two-population model (Herr et al. 2011). Because migration and divergence times have opposing effects on the level of differentiation observed in modern samples, differences in parameter estimates could be caused by the presence of a "likelihood ridge" in which different combinations of these parameters produce similar likelihood scores. Alternatively, different inferences across genetic markers could be caused by biological phenomena, such as selective sweeps in mitochondrial genomes (Meiklejohn et al. 2007).

Morphology

As in Storer (1951) and Thompson (1991), we observed a cline in wing length across Texas, Oklahoma, Arkansas, and Louisiana (fig. 2), with the shortest-winged birds in Louisi-

Table 2: Demographic model parameter estimates

	((Western, central), eastern)	((Eastern, central), western)
N_{ref}	92,806 (81,759–104,907)	116,157 (102,160–122,264)
N_w	592,365 (529,869–695,108)	572,601 (520,918–678,487)
N_e	43,702 (33,913–56,992)	41,627 (30,303–50,487)
N_c	256,213 (204,475–322,629)	304,896 (261,781–370,534)
T1	566,477 (524,704–606,666)	461,452 (418,442–537,264)
T2	646,705 (605,602–684,570)	680,303 (611,003–742,773)
Nm_wc	7.12 (5.51–8.81)	6.95 (5.61–9.14)
Nm_cw	3.75 (3.08–4.58)	3.57 (2.69–4.08)
Nm_ec	.93 (.87–1.05)	.98 (.90–1.06)
Nm_ce	1.82 (1.43–2.7)	2.00 (1.41–2.39)
LL_model	−620.58 (−660.74 to −607.95)	−620.97 (−659.70 to −612.73)

Note: δaδi parameter estimates and 95% confidence intervals for three-population isolation-with-migration models with western + central or eastern + central populations as sister taxa. Migration parameters (Nm_) are the number of migrants per generation, with the receiving population listed first. "LL_model" is the log likelihood of the model under optimized parameter sets.

ana. Wing chord ($P < .01$, $R^2 = 0.33$, df = 54) and the first PC axis of wing chord and tarsus length ($P < .01$, $R^2 = 0.29$, df = 54), but not tarsus length alone ($P = .07$, $R^2 = 0.04$, df = 54), were significantly correlated with longitude. For the 22 specimens with both genomic and morphological data, wing chord ($P < .01$, $R^2 = 0.38$, df = 20) and wing + tarsus PC1 ($P < .01$, $R^2 = 0.24$, df = 20), but not tarsus length ($P = .67$, $R^2 = 0.01$, df = 20), were also significantly correlated with the proportion of "central" ancestry in *structure* results.

Discussion

Migratory Connectivity

Our study used thousands of genome-wide SNPs, along with mitochondrial DNA (mtDNA) sequences, to produce the first range-wide map of migratory connectivity in painted buntings (fig. 1), yielding two major findings. First, we show that the Atlantic and interior breeding populations maintain allopatry year-round. Clustering analyses failed to group any individuals from the Atlantic coast breeding population with any individuals from wintering localities in Mexico or Central America, a result consistent with previously hypothesized wintering-range limits (Thompson 1991). Second, we found strong signals of a migratory divide that corresponds to the geographic break between *Passerina ciris pallidior* and *P. ciris ciris* in eastern Texas proposed by Storer (1951). These genetically conserved migratory programs may have important implications for the role of seasonal migration in shaping the evolutionary trajectory of populations. While seasonal migration can facilitate gene flow and promote homogenization among geographically segregated populations (Arguedas and Parker 2000), it may also restrict gene flow and increase differentiation as differences in the timing and orientation of these seasonal movements begin to evolve among populations (Baker et al. 1994; Rohwer and Irwin 2011). Our data indicate that the latter is occurring within painted buntings, potentially indicating the presence of incipient species. We believe that this pattern reflects the consequences of extreme site fidelity across both the breeding and wintering ranges of the species.

Even in relatively well-studied taxa such as birds, the difficulty of tracking individuals and populations year-round has impeded research on many aspects of the ecology and evolution of migratory behavior (Webster et al. 2002). Painted buntings are no exception, with previous studies proposing alternate migratory routes but failing to provide conclusive evidence of range-wide patterns. On the basis of similarities in plumage brightness and wing length, Storer (1951, 1982) concluded that individuals breeding in the Mississippi Valley migrated directly across the Gulf of Mexico to winter on the Yucatán Peninsula. Before this study, however, only a small number of mist-net captures on a single barrier island (Simons et al. 2004) and anecdotal observations from ships (e.g., Frazar 1881) or oil platforms (Sullivan et al. 2009) provided support for trans-Gulf migration. Similarly, using banding records and differences in mean wing length between populations, Thompson (1991) proposed that eastern painted buntings winter exclusively in southern Florida and the Caribbean, with western painted buntings wintering across Mexico and Central America. Unfortunately, a geolocator study (Contina et al. 2013) attempting in part to verify Thompson's hypotheses was hindered by low retrieval rates and stochastic individual behavior. By resolving these long-standing questions in painted bunting biology, our work joins Ruegg et al.'s (2014) study of the Wilson's warbler in using genomic data sampled across both

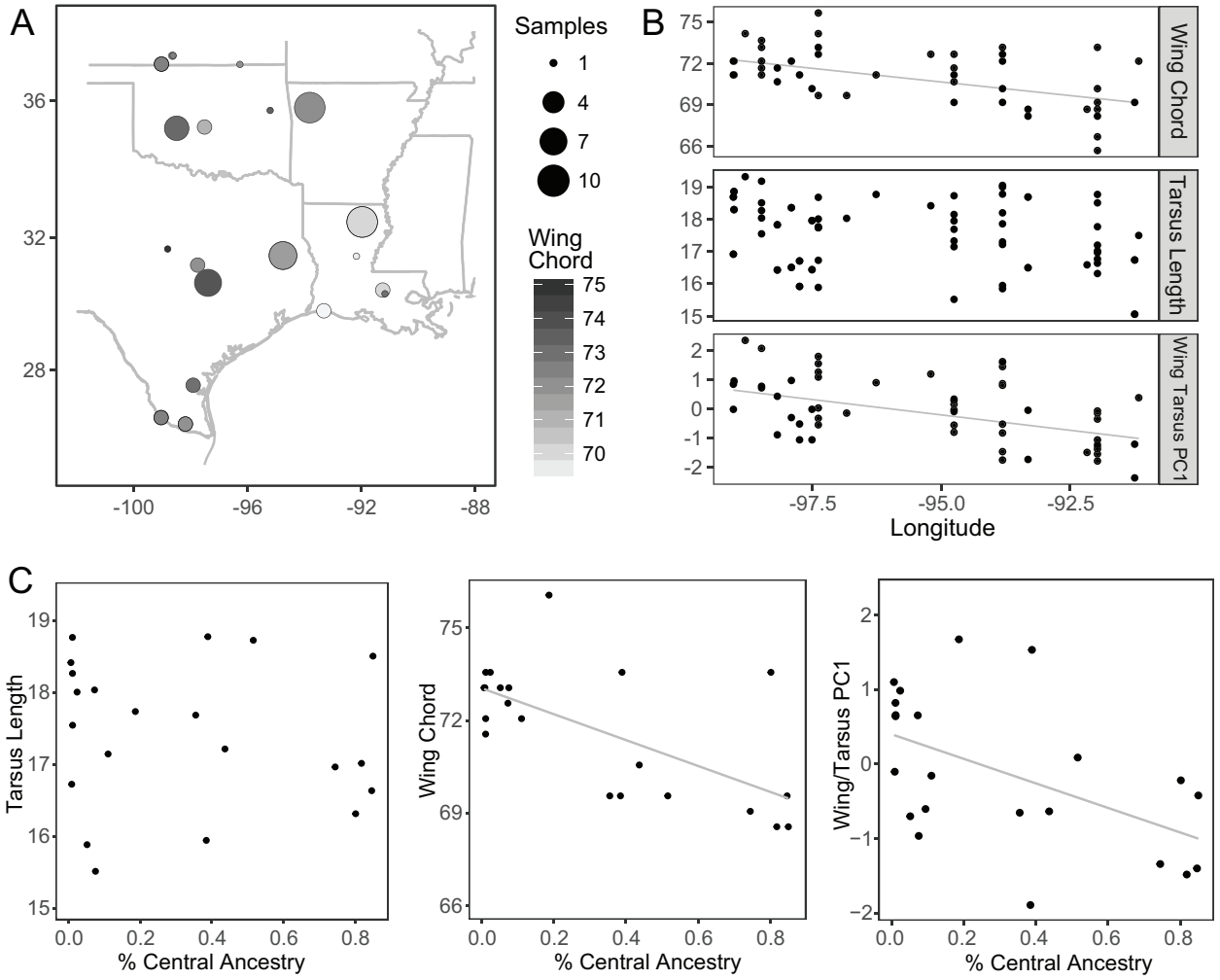

Figure 2: *A*, Map of specimen localities, with points scaled by sample size and shaded according to mean wing chord. *B*, Linear regression of morphology as a function of longitude, with significant correlations shown as gray lines. *C*, Linear regression of morphology as a function of the proportion of "central" ancestry in three-population *structure* runs. PC1 = principal-component axis 1.

breeding and wintering ranges to address recalcitrant questions in the natural history of avian migration.

Phylogeography

The discordance between the longitude of the painted bunting subspecies boundary (recognized on the basis of morphology) and the longitudinal limits of allopatric Atlantic and interior breeding populations has long vexed ornithologists (e.g., Storer 1951; Thompson 1991; Herr et al. 2011). Are current subspecies range limits an accurate reflection of phylogeographic structure and demographic independence, or do plumage and wing length characteristics reveal a hidden history of assortative mating? Our research corroborates both hypotheses. While our results broadly match the sole previous study of genetic variation within *P. ciris* based on an mtDNA marker (Herr et al. 2011), the increased resolution afforded by genome-wide SNP data also reveals a previously undiagnosed genetic cluster consistent with morphological work by Storer (1951) and Thompson (1991; fig. 1).

Niche modeling suggests that the east-west gap in the painted bunting distribution is well within the potential climatic envelope of the species (Shipley et al. 2013). Thus, we suggest that the contemporary distribution and genetic structure of the painted bunting are likely the result of one of three scenarios: (1) adaptive morphological evolution related to migratory distance (as inferred from variation in wing length), which may have been followed by the extinction of an intermediate portion of the ancestral range, potentially due to the fitness costs of trans-Gulf migration; (2) slow population ex-

pansion out of separate late Pleistocene refugia (Johnson et al. 2004); or (3) a jump dispersal event (likely wind aided) in which a subset of interior trans-Gulf migrants reached the Atlantic coast (or Cuba), while retaining their the primary (north-south) migratory axis (Greenberg and Marra 2005).

Implications for Conservation

The Atlantic coast population of painted buntings is a charismatic taxon restricted to a narrow strip of habitat heavily affected by agricultural and residential development and has consequently attracted substantial conservation efforts. The species as a whole is listed as "near threatened" on the International Union for the Conservation of Nature (IUCN) red list and is considered a "species of special concern" in the US Fish and Wildlife Service's Migratory Bird Program Strategic Plan (US Fish and Wildlife Service 2008). These designations are based primarily on data from the Breeding Bird Survey (BBS) finding that eastern populations have declined at an average rate of 1.17%/yr (95% confidence interval [CI] 3.12% to −0.08%/yr; Sauer et al. 2017), although trends in the region are not well supported because relatively few BBS routes are located in suitable habitat. In the west, BBS trends are highly variable, with populations apparently stable or expanding across northern Texas, Arkansas, and Oklahoma while declining in Louisiana and Mississippi.

In contrast to earlier hypotheses of population structure in the species, which split buntings into eastern and western subspecies in eastern Texas (Storer 1951) or into two populations separated by the breeding-range gap in the southeastern United States (Thompson 1991; Herr et al. 2011), our analysis identified three populations (fig. 1). Reexamined in this framework, BBS survey data suggest that western populations are healthy and expanding in the north while central and eastern populations are declining. In Louisiana, BBS data identifies a well-supported decline of 1.85%/yr (95% CI 2.95%–0.95%/yr)—a faster rate than in the east, where most conservation attention has focused. Eastern populations, meanwhile, have both the lowest effective population size and the lowest levels of gene flow with other populations. Although we are agnostic as to Thompson's (1991) hypothesis that they represent a separate species, the eastern population would likely qualify as a distinct population segment in the context of Endangered Species Act listing criteria (US Fish and Wildlife Service et al. 1996) if abundance declines significantly in the future. However, populations in the Mississippi alluvial valley region are also genetically differentiated from other painted buntings, show a better-supported trend of population declines than eastern birds (mean 1.70%/yr [95% CI 2.86%– 0.59%/yr]), and should be monitored as a distinct demographic unit in analyses of survey data for the species.

Conclusions

We documented a migratory divide in the painted bunting, using mitochondrial DNA, genome-wide SNPs, and morphological analyses. Breeding populations from Louisiana largely migrate to the Yucatán Peninsula, while those from central Texas and Oklahoma migrate first to molting grounds in western Mexico and then to southern Mexico and Central America. Genetic data indicate that the Atlantic coast breeding population is allopatric from interior populations year-round, wintering only in southern Florida, the Bahamas, and Cuba. These populations have a deep history of divergence with gene flow, with all three splitting approximately 500,000–700,000 yr ago but continuing to exchange an average of 1–9 migrants per generation after divergence. The genetic cline west of the Mississippi is also associated with variation in wing length, suggesting that selection may be promoting morphological divergence in populations with different migration strategies.

It is remarkable that basic life-history traits of this charismatic and relatively well-studied species remain to be discovered; this points to an ongoing need for natural-history observations to drive advances in both conservation and evolutionary biology. In this case our results support monitoring of the putatively declining central and eastern populations as separate demographic and evolutionary units for conservation purposes. This species is also a promising system for further studies of the genetic mechanisms underlying variation in molt and migration in songbirds. Painted buntings were historically a common caged pet in the United States and have reportedly been bred in captivity (Greene 1883), making them a potentially tractable system for conducting controlled crosses. Because Atlantic coast and interior breeding populations differ in the timing and location of molt in addition to migration (before and during fall migration, respectively), future studies in this system could provide insight into the mechanisms underlying temporal variation in the full annual cycle of passerine birds.

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